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Objectives :

1. To stimulate research on various aspects of Food Science, Technology and Engineering.
2. To provide a forum for the exchange, discussion and dissemination of current developments in the field of Food Science, Technology and Engineering.
3. To promote the profession of Food Science, Technology and Engineering.

The ultimate object is to serve humanity through better food.

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C O N T E N T S

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Updated Methods for Detection of Adulterants and Contaminants in Edible Oils and Fats : A Critical Evaluation

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Adulterated and contaminated edible oils and fats pose severe health hazard. A number of methods are available to detect these extraneous compounds in the oils and fats, but the details of these are scattered widely in the literature. These updated methods are brought together and described briefly along with their principles, sensitivities and limitations. Reliable methods for detection of adulterants and contaminants in edible oils and fats have assumed critical importance in recent years, due to increase in deliberate or accidental adulteration.

Keywords : Edible oils and fats, Contaminants, Adulterants, Detection methods and their principles, Sensitivities, Limitations.

Adulteration has been a problem in oils and fats trade. It is often deliberate and sometimes accidental. The classic edible oils like groundnut oil, sesame oil, safflower oil, mustard oil and coconut oil are adulterated with the imported edible oils, sold at subsidized prices, or readily available cheaper indigenous oils. There have been instances of adulteration of *vanaspati* (hydrogenated vegetable oil) with animal fats, like beef tallow (Krishnamurthy et al. 1983). The problem becomes more serious, when the edible oils are adulterated with inedible oils such as castor oil, argemone oil and mineral oil. Besides these, contaminants like polyaromatic hydrocarbons (PAH) in solvent-extracted oils and fats, and tricresyl phosphate, an additive in lubricating oils and diesel, gets into the edible oils through the use of diesel tankers and lubricating oil drums for transportation of edible oils. These contaminants pose serious health implications to the consumers (Rossell 1986; Krishnamurthy et al. 1985a).

To a certain extent, it is possible to identify an oil or fat by its physico-chemical characteristics. The quality standards for edible oils under the general statutory rules of our country have been prescribed (PFA 1992). Some of the classical characteristic tests like Bellier turbidity test for groundnut oil, Baudouin test for sesame oil, Halphen's test for cottonseed oil, Reichert test for milk fat, Polenske value for coconut oil and Hexabromide test for linseed oil, have their own intrinsic merits, and are used much more extensively than the sophisticated instrumental methods of analysis (Williams 1966; Hamilton and Rossell 1986). However, the utility of gas-liquid chromatography needs a special mention, as it is the method of choice for the determination of fatty acid composition (Hammond 1986). Prior to the development of gas chromatography, much of the information on fatty acids composition of an oil or fat was used to be obtained by repeated vacuum distillation and fractional crystallization - a single analysis that can now be done in minutes, was taking weeks. Fatty acid composition (codex ranges) for edible oils is tabulated by Padely et al (1986).

Information on the methods for detection of adulterants and contaminants in oils and fats is scattered in the literature, while some of the techniques are confined to individual laboratories. A number of methods have been accepted as standard methods by organisations such as International Union of Pure and Applied Chemistry (IUPAC), Association of Official Analytical Chemists (AOAC) and American Oil Chemists Society (AOCS), while some of the methods have been developed recently and subjected to evaluation trials under collaborative studies. In the present communication, an effort has been made to present the available methods for identification of adulterants and contaminants in a form of critical review. These updated methods for quality assurance of oils and fats have been described, along with the principles involved. Such an exercise may prove to be of interest, not only to the food analysts, but also to the trade and consumers for assessing the quality of edible oils.

Sampling

Adherence to standard sampling methods is of importance for accurate estimation. The International

Organisation for Standardization has developed ISO:5555 - Animal and Vegetable Fats and Oils sampling methods. These have been adopted by BIS (1964) and other Standards Organisations. Sampling techniques for oils and fats have been reviewed by Rossell (1986).

Moisture and impurity

Tests for moisture and impurity in oils and fats are sometimes referred as 'MANDI' (M for moisture and I for impurity) test. Moisture determination in oils by oven drying (103 ± 2°C) methods also estimate other volatile components. Highly unsaturated oils, such as fish oils or linseed oils (drying oils), undergo oxidation under the test conditions and consequently, an increase in weight rather than weight loss may be observed. In these cases, vacuum oven at 80°C is preferred (Rossell 1986). Method, which depends on chemical reaction, such as Karl Fischer method, the original method with improvement (Griffin and Wilcox 1940), estimates only water content. In entrainment distillation method (Bidwell and Sterling 1925), water in the sample is distilled out as an azeotropic mixture with toluene or benzene or xylene. Refined oils should have moisture less than 0.1%, while raw vegetable oils may have moisture in the range 0.1 to 0.3%.

Impurities in oils and fats, generally, refer to those substances, which remain insoluble, and can be filtered off, when the oil or fat is dissolved at 10% concentration in a solvent, such as diethyl ether or petroleum ether. In reporting data, the solvent is to be specified (Cocks and Rede 1966).

Detection of groundnut oil

Bellier turbidity-temperature (BTT) test, is relied upon for identification of groundnut oil. The oils containing long chain saturated fatty acids give a precipitate at a particular temperature, which is specific for the oil, when their alcoholic soap solution is treated with dilute acetic acid solution and 70% ethyl alcohol (AOAC 1984). Groundnut oil is characteristic in containing not less than 4.8 g higher saturated fatty acids, viz. arachidic, behenic and lignoceric acids per 100 g oil and has a highest BTT value of 39-41°C (Krishnamurthy et al. 1985b).

The procedure involves complete saponification of 1 ml of the filtered sample of oil in a 100 ml flat-bottom conical flask (preferably with a long neck), along with 5 ml of 1.5 N alcoholic potash solution, by heating over a boiling water-bath and using an air condenser (about 1.3 meters long) to avoid loss of alcohol, as far as possible. Complete saponification usually takes about 10 min. and the flask is swirled several times during saponification. After cooling, 0.1 ml of phenolphthalein indicator is added and the mixture is neutralized precisely by carefully adding dilute acetic acid (1+2), which is followed by an addition of an extra amount of accurately measured 0.4 ml. To this mixture is added 50 ml of 70% alcohol and mixed. A thermometer (0 to 60°C, reading to 0.5°C, accurately calibrated) is fixed into the flask, with the aid of a velvet cork in such a way that the bulb of the thermometer is immersed in the liquid, but does not touch the bottom of the flask. The flask is heated gently over the water-bath, until the temperature reaches 50°C, and the solution is clear. The flask is allowed to cool in air with frequent shaking until the temperature falls gradually to 40°C (in case of pure groundnut oil, turbidity appears at 39 to 41°C). Then, the flask is cooled with constant shaking and by occasional immersion in a cooling bath maintained at 15°C (±1°C), so that the temperature drop is roughly at the rate of 2°C per min. The temperature at which the first distinct turbidity appears is noted, and it is the turbidity temperature. This turbidity temperature is confirmed by a further cooling, which would result in deposition of the precipitate.

The precipitate is dissolved by gently heating the contents to 50°C over water-bath, and cooled again as described above for second determination of the turbidity temperature. The mean of the two values is taken as the true turbidity temperature. These duplicate values shall agree within ±0.5°C. It is essential that stirring should be continuous and also moderate, while the contents are being cooled in the cooling bath. Violent shaking or agitation should be avoided, as it affects the result adversely.

Detection of sesame oil

Baudouin and/or modified villavecchia tests are generally relied upon, for detection and identification of sesame oil. The development of pink colour due to reaction of sesame oil constituents, namely, sesamolin-sesamol with hydrochloric acid and furfural solution is characteristic of sesame oil (AOCS 1975; Rossell 1986).

The oil or melted fat (5 ml) is taken in a 25 ml measuring cylinder (or test tube), provided with a glass stopper, along with 5 ml of hydrochloric acid and 0.4 ml of 2% furfural solution, prepared with freshly distilled ethyl alcohol. The container

is glass stoppered, shaken vigorously for two min, and the mixture is allowed to separate. The development of a pink or red colour in the lower acid layer indicates presence of sesame oil. The result is confirmed by adding 5 ml of water and shaking again. If the colour in acid layer persists, then sesame oil is present; but it is absent if the colour disappears. There is a legal requirement for vanaspati (hydrogenated vegetable oil) that it shall contain 5% sesame oil as tracer. This is to enable detection of hydrogenated vegetable oils in milk fat (ghee).

Detection of cottonseed oil

Halphen's test is generally followed as an identity and detection test for cottonseed oil. The development of red colour, on heating the oil with a solution of sulphur in carbon disulphide and amyl alcohol, indicates the presence of cottonseed oil. The positive results are also given by hempseed oil, Kapok and other related oils containing cyclopropenoid fatty acids (IUPAC 1979; AOCS 1975; Williams 1966). Cottonseed oil heated to 250°C may give negative results, due to loss of cyclopropenoid group (Fisher and Schuller 1981; Triebold and Aurand 1967).

About 5 ml of the oil or melted fat is taken in a test tube, along with an equal volume of 1% (w/v) sulphur solution prepared in carbon disulphide, and then mixed with an equal volume of amyl alcohol. The mixture is shaken thoroughly, and heated gently on a water bath (70° to 80°C) for a few min, with occasional shaking, until the carbon disulphide has boiled off, and the sample stops foaming. The tube is placed in an oil-bath or a saturated brine-bath maintained at 110-115°C and held for 2.5 h. A red colour at the end of this period, indicates the presence of cottonseed oil.

Detection of palmolein in groundnut oil

The cloud point is that temperature at which a cloud is induced in the sample under the conditions of this test. The cloud is caused by the first stage of crystallization (AOCS 1975).

Sample (60 to 75 g) is heated to 130°C, immediately before making the test. About 45 ml of the heated fat is poured into a wide mouth bottle (100 ml capacity), and the bottle is placed in an ice-cold water bath. The bottle is then cooled in the water bath with stirring, while the thermometer is used to record the temperature. When the sample has reached a temperature of about 10°C above the cloud point, the stirring is done steadily and rapidly in a circular motion, so as to prevent super-cooling and solidification of fat crystals on the sides or bottom of the bottle. From this point on, the thermometer is not removed from the sample, since its removal may introduce air bubbles, which will interfere with the test. The test bottle is maintained in such a position that the upper levels of the sample in the bottle, and the water in the bath are about the same. The bottle is removed from the bath and the temperature is noted. The cloud point is that temperature at which that portion of the thermometer (which was kept immersed in the oil) is no longer visible, when viewed horizontally through the bottle. It is observed that the presence of palmolein over 10% in groundnut oil readily gives the cloud at a temperature higher than that of groundnut oil, due to the presence of palmitic glycerides in higher amounts in palmolein/palm oil.

Detection of rice bran oil in other edible vegetable oils

It involves the isolation of oryzanol in rice bran oil using 30% aqueous potassium hydroxide solution and the detection on thin-layer chromatoplate (Seetharamaiah and Prabhakar 1986; Nasirulla et al. 1992).

The oil (20 ml) is taken in a 100 ml capacity separating funnel along with equal volume of 30% aqueous potassium hydroxide solution. The contents are shaken gently, but constantly for 10 min and allowed to stand for about 45 min to separate the alkali layer. The alkali layer is drawn and neutralized with hydrochloric acid solution, using blue litmus paper. This salt solution is extracted with diethyl ether (20 ml x 3 times). The combined diethyl ether extract is, then, washed with distilled water, and dried over anhydrous sodium sulphate. The solvent is evaporated on hot water bath, the residue is dissolved in chloroform, spotted on TLC plate, and the plate is developed in benzene-acetic acid mixture (100:1, v/v). The solvent front is allowed to move to a distance of 15 cm and the spots visualized in iodine chamber. Appearance of a spot between R_f 0.7 and 0.75, characteristic to rice bran oil, indicates the presence of rice bran oil. The method can detect rice bran oil in other edible vegetable oils up to the minimum of 5% level. This spot was not obtained in the other commonly available edible oils tested.

Detection of linseed oil

In the hexabromide test, the oil in chloroform is treated with bromine, and then with alcohol and

ether. The formation of a precipitate of hexabromide, which is insoluble in ether in cold conditions indicates the presence of linseed oil (Williams 1966).

The oil (1 ml) is added into a boiling tube and dissolved in 5 ml chloroform. About one ml of bromine is added drop-wise, till the mixture becomes deep red in colour. The test tube is, then, cooled in an ice water bath. About 1.5 ml of ethyl alcohol is added drop-wise, while shaking the mixture, until the precipitate, which is first formed, gets just dissolved. Then, 10 ml of diethyl ether is added, the contents mixed, and the tube is placed in the ice water bath for 30 min. Appearance of precipitate indicates the presence of linseed oil.

Formation of hexabromides, insoluble in cold ether, has been observed in low erucic rapeseed oil, mustard oil and mahua oil, when the tubes were kept in ice water bath over a prolonged period exceeding 30 min. The results obtained in such cases have to be viewed with caution. Experiments conducted with these oils, with or without added linseed oil, have shown that hexabromides insoluble in cold ether are formed within 20 min, if linseed oil is present even at 1% level. Any hexabromide insoluble in cold ether, formed after 20 min, need not be taken as an evidence for the presence of linseed oil (Krishnamurthy, unpublished data).

Detection of animal fat in vegetable fat

Microscopic examination of fat crystals : Animal fats, such as beef tallow and lard have been shown to contain trisaturated glycerides. On crystallization, these glycerides exhibit a characteristic crystal appearance, when viewed under microscope (Williams 1966).

Melted fat samples (about 2 g) is taken in test tubes and mixed with 10 ml diethyl ether. The tubes are plugged with cotton and allowed to stand for 3-4 h. at 20°C (slow crystallization gives bigger crystals). In certain cases, it is preferable to first crystallize with a stronger solution of fat from a mixture of ether and ethyl alcohol (1:1). In such cases, the crystals are separated by filtration and re-crystallized in ether. Formation of crystals at the bottom of test tubes indicates presence of beef tallow. For further confirmation, the crystals are placed on a drop of glycerine, taken on a microscopic slide. The crystals are covered immediately with cover glass and examined under x 160 and finally x 400 magnifications. The typical characteristic appearance of beef tallow crystals is the fan-like tufts, the ends of which are more or less pointed. Lard crystals are of chisel shaped. Hydrogenated fats deposit as smaller sized and amorphous crystals. The size and shape of the crystals depend upon the strength of solution, amount of fat taken and time allowed for crystallization.

Separation of cholesterol by reversed phase thin-layer chromatography : A preliminary separation of total sterols from the unsaponifiable matter can be achieved on silica gel-G thin layer chromatography. Subsequently, the sterols are separated by reversed phase chromatography on Kieselghur-G, using liquid paraffin as stationary phase and aqueous acetone saturated with liquid paraffin as the mobile phase (de Souza and Nes 1969).

For the separation of total sterols from unsaponifiable matter, the unsaponifiable matter from the fat sample is extracted, as per the method described elsewhere. The ether is evaporated and the residue dissolved in 5 ml of chloroform. Glass plates of 20 x 20 cm are coated with 0.5 mm thick silica Gel-G, air-dried and activated at 110°C for 2 h. The plates are cooled to room temperature, and spotted with unsaponifiable matter, along with the standard cholesterol. The plates are developed in diethyl ether : petroleum ether (1:1) solvent system, and are removed, when the solvent front reaches 14 cm height (it takes about 30 min.). The plates are air-dried and exposed to iodine vapours for a while. Total sterols spot, corresponding to standard spot of cholesterol, appears as brown coloured spot. The spots are marked and scraped off, with stainless steel blade into a test tube. The sterols are, then, extracted from it, using chloroform. From this, cholesterol is separated by reversed phase thin-layer chromatography.

For preparing equilibrated aqueous acetone with liquid paraffin, 300 ml of 4:1 ratio of acetone : water is taken in a separating funnel and 30 ml of liquid paraffin (heavy grade) is added. The content is shaken well and kept for 18 h at room temperature for equilibration. The lower layer of liquid paraffin is separated and diluted to 5% v/v level with petroleum ether. This is, then, used for treating Kieselghur-G coated thin-layer chromatographic plates. The upper acetone-water mixture serves as solvent system to develop the paraffin treated plates.

For the preparation of the plates for reversed phase TLC, glass plates of 20 x 20 cm are coated to 0.5 mm thick layers with Kieselghur-G and water (1:2) slurry. After air-drying, the plates are activated

at 110°C for 1 h and cooled to room temperature in a desiccator. For treatment of TLC plates with liquid paraffin, the plates are dipped carefully by holding horizontally for a few seconds in a tray containing 5% liquid paraffin solution in petroleum ether, prepared as above. The plates are, then, air-dried.

The sterols in chloroform, isolated from unsaponifiable matter by a preliminary separation on silica gel-G thin-layer chromatography, are then spotted on paraffin treated plates along with standard cholesterol. The plates are developed, using solvent system of acetone : water (4:1), which was earlier equilibrated with paraffin. After the solvent front has ascended to a height of 15 cm, the plates are removed and air dried. These are sprayed with p-anisaldehyde reagent (1.5 g p-anisaldehyde and 1.5 ml conc. sulphuric acid in 27 ml ethyl alcohol), followed by heating at 110°C for 5 min. The sterol spots appear as blue spots on pale pink background. Cholesterol appears at R_f 0.48, distinctly separated from other closely related sterols.

Detection of animal fat in vegetable fat based on the presence of unusual fatty acids in animal fats by gas liquid chromatography : For preparing fatty acid methyl esters, melted fat (30 to 50 mg, 1 drop) is taken in a glass-stoppered test tube, and 1 ml dichloromethane/benzene is added, followed by 2 ml of 1% sodium methoxide solution (1 g sodium dissolved in 100 ml of anhydrous methanol). The test tube is held at 60°C for 10 min, cooled and 0.1 ml of glacial acetic acid is added, followed by 5 ml of distilled water and 5 ml petroleum ether (40°-60°C). The contents are mixed, and the layers are allowed to separate. About 2 ml of the upper layer, containing the methyl esters, is taken in a small tube and concentrated by passing nitrogen gas, before injecting to gas chromatograph. Gas chromatograph with flame ionisation detector and stainless steel column of 10 ft, packed with 15% diethylene glycol succinate on chromosorb W (80-100 mesh) or any other intermediate polar stationary phase column, is employed. The column temperature is maintained at 185°C, while the flow rate of carrier gas nitrogen is kept at 2.8 kg/cm² (25 ml/min) and chart speed at 1 cm/min.

The fatty acid composition of animal fat (beef tallow) and *vanaspati* adulterated with animal fat show the presence of odd chain fatty acids and branched chain fatty acids, namely 15:0, 15:1, 17:0 and 17:1. These fatty acids are absent in vegetable fats. On the basis of this fact, it is possible to detect the presence of animal fat (beef tallow) in vegetable fats (Krishnamurthy et al. 1983; Krishnamurthy and Nagaraja 1987; Pearson 1981).

Detection of mineral oil in edible oils

Petroleum ether, as a developing solvent, separates non-polar mineral oils from glycerides on silica gel-G TLC (Rossell 1986; Mani and Lakshminarayana 1964).

A simple method of TLC using micro-slides, without involving TLC applicator, is described. Two glass slides are held together (face to face) and dipped in a slurry of silica gel-G (45 g) in a mixture of chloroform and methanol (80 + 20). The slides are withdrawn, separated from each other and allowed to dry in air for 10 min. Solution of oil in chloroform (10 µl of 1.0% solution) is applied, using a capillary tube. The slide is placed in a beaker/jar containing about 5 ml petroleum ether. The beaker/jar is covered with a watch glass and the solvent is allowed to travel for 6 cm from the origin (about 4 min). The slide is removed, dried in air, sprayed with 2, 7-dichloro fluorescin solution (0.2%) in ethyl alcohol and viewed under UV light. Occurrence of a yellow fluorescent spot at the solvent front indicates the presence of mineral oil. Alternatively, glass plates (10x20 cm or 20x20 cm), coated with silica gel and activated by heating in an oven at 100°C for 1 h, can be used. A standard, containing white oil or liquid paraffin (1%) in a sample of vegetable oil under test, may be prepared and tested simultaneously as reference.

Detection of castor oil in edible oils

'Triricinolein', a characteristic and predominant triglyceride component of castor oil, is separated on silica gel TLC and visualized by iodine vapours (Lakshminarayana and Mani 1964).

The oil sample (5 µl) is applied as 1% solution in chloroform on activated silica gel-G coated glass plate, and is placed in a chamber containing a solvent consisting of petroleum ether-diethyl ether-acetic acid (60:40:1.5, v/v). The chamber is, then, covered with a glass plate. The solvent is allowed to travel up to a height of 10 cm. The plate is removed from the tank, dried in air, and placed in the visualization tank containing iodine vapour. A characteristic spot with an R_f value of about 0.25 shows the presence of castor oil. A prepared sample of an oil, containing 1% added castor oil, may be run along side, for identification of the spot. This method has a sensitivity of 1% and is specific for castor oil, due to the presence of glycerides

composed of ricinoleic acid (hydroxyoleic acid). However, rancid or oxidized groundnut oil interferes in the detection. Hence, care should be taken, when applying the TLC test to rancid oils and interpretation of result. In such cases, the rancid oil requires to be purified by refining (Nasirullah et al. 1982, 1983).

TLC method for detection of castor oil and its differentiation from rancid oils : The suspected rancid oil (5 ml) is taken in a round bottom flask along with activated charcoal (2 g). The content is mixed thoroughly and heated on boiling water bath for about 30 min with constant shaking. The bleached oil is filtered on a filter paper (Whatman No.1) to separate the charcoal. The filtered oil is now passed through a mini column packed with neutral alumina (10 g) and eluted using hexane (50 ml). This bleached and neutralized oil is spotted on the TLC plate, following the earlier TLC procedure given for the detection of castor oil, along with castor oil for reference purposes.

Detection of argemone oil in edible oils

Argemone (*Argemone maxicana* l.), yellow poppy, is a wild herb, which grows as a weed in mustard field and bears capsules full of brown-black seeds and often, it is a crop contaminant. Because of its resemblance with black mustard, it is also used as an adulterant. The oil is reported to cause glaucoma, dropsy and sometimes total blindness due to the presence of alkaloids, namely, sanguanarine and its related compounds.

The hydrochloric acid extract of the oil sample, containing argemone oil, when subjected to TLC, for the separation of alkaloid, gives fluorescent spots under UV light.

Suspected oil (5 ml) is dissolved in diethyl ether (5 ml) in a stoppered conical flask, and concentrated hydrochloric acid (5 ml) is added to it. The contents are shaken vigorously for about 10 min. The extraction flask is warmed on a hot water bath at 40°C for about 10 min. The contents are transferred to a separating funnel, and the acid layer is collected in a 10 ml beaker. The beaker is placed on a boiling water bath, and the acid is evaporated till dryness. The residue is dissolved in 1 ml of chloroform and acetic acid mixture (90:10, v/v) and spotted on TLC plate, with the help of spotting capillary. The plate is developed in (a) butanol : acetic acid : water (70:20:10, v/v); or (b) hexane : acetone mixture (60:40, v/v). The solvent front is allowed to move upto a distance of 10 cm. A bright yellow fluorescent spot (R_f 0.8 in solvent system 'a', R_f 0.45 in solvent system 'b') observed under UV light, indicates the presence of argemone oil. The spot gives blue fluorescence under UV light, if plate is sprayed with 20% aqueous sodium hydroxide solution. The method is very sensitive, and detects argemone oil at 50 ppm level. A comparative study of the available methods has been made by Nasirulla et al. (1984).

Detection of *karanja (Pongamia glabra)* oil in edible oils

The alkaloids in the *karanja* oil, viz., glabrin, karanjin, karanjone, pongaglabrone and pongamol are extracted, using concentrated hydrochloric acid, separated on TLC plate and visualised under ultra-violet light (Nasirullah et al. 1992).

The suspected oil (20 ml) is taken in a 100 ml capacity separating funnel, along with 10 ml concentrated hydrochloric acid. The contents are shaken gently, for 15 min and then the separating funnel is kept on a stand for about 30 min to allow the separation of acid layer. The acid layer is drawn out in a glass beaker. The beaker is kept on a boiling water bath for evaporating hydrochloric acid to dryness. The residue is dissolved in 0.5 ml of chloroform, and the chloroform solution is spotted on a pre-activated TLC plate, with the aid of spotting capillary. The plate is developed in a solvent system consisting of petroleum ether : diethyl ether : acetic acid (60:40:1, v/v) for 20 min.

The plate is removed, dried at room temperature and viewed under ultra violet lamp. Appearance of three bluish green spots at R_f 0.34, 0.22 and 0.17 confirms the presence of *karanja* oil. The method is sensitive to detect 0.01 % of *karanja* oil in the edible oils.

It is worthwhile mentioning that the development of a yellow colour in the acid layer, during the extraction of alkaloids, gives the clue for the presence of *karanja* oil. The palm and argemone oils do not interfere in the detection method. The TLC can be performed directly on chloroform solution of the oil for detection at levels greater than 1.0% of *karanja* oil in edible oils.

Detection of hydrocyanic acid in edible oils

Hydrocyanic acid is present as an impurity in synthetic allyl isothiocyanate, which is commonly used to enhance the flavour of poor quality mustard oil. Such artificially flavoured mustard oil poses health implications.

The oil (15 to 30 ml, depending on its hydrocyanic acid content) is poured into a 250 ml

capacity conical flask, along with about 50 ml of water, 15 ml of 10% solution of tartaric acid and mixed well. The flask is stoppered with a velvet cork, from which hangs a picric acid paper (about 75 mm long), previously wetted with a drop of 5% sodium carbonate solution. The flask is placed on a hot water bath by the side of the steam vent, and not directly on the steam. In the presence of hydrocyanic acid, the picric acid paper acquires red colour within 10 min. Pink or light reddish hue which may, at times, appear at the periphery of the picric acid paper on prolonged heating of the contents is to be ignored (Nita 1990).

Detection of *kusum* seed (*Schleichera trijuga Sapindaceae*) oil

Kusum oil, also known as macassar oil, contains cyanolipids. This oil is a common adulterant to mustard oil. The detection involves liberation of hydrocyanic acid by heating the suspected oil and absorbing it in potassium hydroxide solution. Presence of *kusum* oil is tested by treating this potassium hydroxide solution with ferric chloride solution to get blue colour (Dutt 1951).

About 50 ml of the suspected oil is heated in a distillation flask and the receiver is connected to a flask containing 5 ml potassium hydroxide solution (2 N). Air is passed through the distillation flask during heating for 30 min, as a carrier to hydrocyanic acid, to bubble through the alkali solution in the receiving flask. The alkali solution with the absorbed hydrocyanic acid is, then, treated with a few drops of ferrous sulphate (2% in water), acidified with a few drops of dilute hydrochloric acid and heated in water bath for 5 min. The filtrate of this solution is treated with few drops of ferric chloride solution (20%, in water). Formation of blue colouration is indicative of the presence of *kusum* seed oil.

Detection of Teaseed oil - *Cammellia sinensis* - *Theaceae*

The unsaponifiable matter of teaseed oil contains theasin (0.2 to 0.3%), which gives the characteristic Fitelson colour reaction test - a modified Leibermann - Burchard reaction (Williams 1966).

The test is performed by taking 0.8 ml acetic anhydride, 1.5 ml chloroform and 0.2 ml concentrated sulphuric acid in a test tube. To this, about 0.5 ml of suspected oil is added. The test tube maintained at 5°C in an ice water bath for 5 min and 10 ml of ice cold ether is added and mixed well. Presence of teaseed oil is indicated by formation of red colour, which reaches a maximum and then fades away.

Detection of tricresyl phosphates and tri-o-cresyl phosphate in edible oils

Tricresyl phosphate in contaminated edible oils is extracted using acetonitrile and detected by thin layer chromatography as well as gas liquid chromatography (Krishnamurthy et al. 1985a). The following reagents are required :

i) developing solvent system consisting of iso-octane : ethyl acetate (90:10) (developing chamber is lined with filter paper); ii) spray reagent, also called Gibb's reagent, is 0.5% solution of 2, 6-dichloroquinone chlorimide in absolute ethyl alcohol, and iii) tricresyl phosphate (TCP) and tri-O-cresyl phosphate (TOCP) are prepared by dissolving individually in ethyl alcohol (1 mg/ml concentration).

For extraction of the contaminants, the oil sample (10 ml), containing about 50 µg TCP or TOCP is taken into separating funnel, along with 50 ml of petroleum ether (40-60°C), which is added to dissolve the oil. Then, 10 ml of acetonitrile, previously equilibrated with petroleum ether, is added. The contents are shaken vigorously and allowed to stand for 10 min. The lower acetonitrile layer is collected in a beaker, and the solvent is evaporated on hot water bath. The residue is dissolved in about 1 ml ethyl or methyl alcohol.

Thin layer chromatography is carried out by spotting about1.0 µl (about 5 µg TOCP) solution. The TLC plate is developed in glass chamber containing iso-octane : ethyl acetate (90:10) for about 45 min. to a height of 10 cm. The plate is removed and dried in air, sprayed with Gibb's reagent and heated in 100°C oven for about 15 min. The characteristic blue violet spot at R_f 0.27, corresponding to TCP and TOCP, is observed.

Detection of coal tar soluble colours in edible oils

The petroleum ether solution of oil sample gives different shades of colour with different concentrations of hydrochloric acid in presence of coal tar oil soluble colour in the oil (Cocks and Rede 1966).

To 5 ml of oil sample in test tubes, 15 ml of petroleum ether is added to each tube, followed by 5 ml of hydrochloric acid solution of different concentrations to different tubes. The change in the colour, indicating the presence of coal tar oil soluble colours in the oil sample, is noticed.

Isolation and confirmation of oil soluble colours: The oil sample in hexane is treated with silica gel to absorb the colours. After eluting the oil with hexane, the colour absorbed by silica gel is recovered by eluting with diethyl ether. Identification of colours is made by silica gel G thin layer chromatography of the diethyl ether solution (Pearson 1981).

About 5 ml of oil sample is taken in a glass stoppered conical flask. Twenty-five ml of hexane, followed by 10 g silica gel (column chromatography grade) and 2 g anhydrous sodium sulphate, are added. The mixture is stirred well, kept aside for 5 min, and the solvent is decanted. Once again, 25 ml of hexane is added, stirred well and the solvent decanted. Likewise, hexane (25 ml) is added 3-4 times to the flask by draining out the solvent each time, to remove almost all the oil leaving behind the silica gel in the flask.

The colouring matter absorbed by silica gel is eluted in the flask by shaking with diethyl ether for 2 to 3 times, using 20 ml solvent each time. The diethyl ether extract is collected in a beaker. The solvent is evaporated on a hot water bath. The concentrated ether extract is spotted, using capillary tubes, on an activated TLC plate, and the plate, is developed in a tank containing solvent mixture (benzene-hexane-acetic acid, 60:40:1, v/v). The plate is removed, when the solvent layer has reached 12 to 15 cm height and dried at room temperature. On heating the plate at 100°C in an oven for 1 h, natural colours like carotenes would fade away, thereby leaving oil soluble coal tar colours.

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RAPID COMMUNICATION *J. Food Sci. Technol., 1993, Vol 30, No.4, 239-240*

A New Protocol for Rapid Sample Preparation for Spectrophotometric Estimation of Carbendazim Residues in Apple, Tomato and Mushroom

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A new protocol for rapid sample preparation has been developed for improved estimation of carbendazim residues (1-10 ppm) in apple, tomato and mushroom by spectrophotometric method. It involves extraction with ethyl acetate, clean-up by partitioning with dilute hydrochloric acid and estimation of carbendazim at 280 nm.

Keywords : New protocol for sample preparation, Carbendazim residue estimation, Spectrophotometric method, Apple, Tomato, Mushroom.

Carbendazim (2 methoxy carbomyl benzimidazole), a popular systemic fungicide, is used extensively on apple, tomato and mushroom against fungal pathogens (Nene and Thapliyal 1979). Methods for its estimation have been reviewed (Baker and Hoodless 1974; Re et al. 1980). Liquid chromatography has been the most common technique employed for its estimation (Kirkland et al. 1973; Gilvydis and Walters 1990). However, it is expensive and requires same clean-up, as that in the spectrophotometric method. The clean-up of extracts is based upon its partitioning in the aqueous phase at low pH and the organic phase at neutral pH. Older technique advocates use of chloroform as solvent for extraction, which besides being hazardous, can not be used in sample containing sugar (BASF 1973, personal communication). Other methods, using dilute solution of sodium hydroxide for adjusting pH (Pifarre and Vayreda 1987), are time-consuming and found to give inconsistent results. Extraction on blending with polar organic solvents leads to tedious emulsions. A new protocol for rapid sample preparation for a simple spectrophotometric method of estimating carbendazim residues in apple, tomato and mushroom has been developed and reported in the present communication. It overcomes all the above mentioned lacunae.

A 100 g sample (apple, tomato or mushroom) with equal amount of anhydrous sodium sulphate and 2.5 ml of concentrated ammonia was blended with 200 ml ethyl acetate at high speed for 3 min. Sodium sulphate was used to prevent emulsion formation during blending. The upper organic layer was decanted and filtered over anhydrous sodium sulphate. The residue left, was again extracted with 100 ml ethyl acetate and filtered in a Buchner funnel under suction. The combined filtrate was reduced to about 80 ml under vacuum at 40-45°C. The volume of the extract was made up to 160 ml in a measuring cylinder, from which 40 ml (equivalent to 25 g crop material) was taken in a separating funnel, and was partitioned twice with 10 ml each of 0.1 N HCl, by shaking vigorously for 2 min each time. The lower aqueous phases were pooled in another separating funnel along with 40 ml ethyl acetate and saturated with 80% sodium bicarbonate solution (about 30 ml) for neutralizing HCl. It was shaken vigorously for 2 min, and the lower aqueous phase was rejected. The organic phase was washed with water and then partitioned with 10 ml of 0.1 N HCl, which was freshly saturated with ethyl acetate by shaking vigorously for 1 min. The absorbance of 0.1 N HCl was recorded in a spectrophotometer at 280 nm against 0.1 N HCl, which was freshly saturated with ethyl acetate. Calibration curve (1-10 ppm) from freshly prepared stock solution of 100 ppm carbendazim in 0.1 N HCl is shown in Fig. 1. The sensitivity was 0.09 absorbance of 1 ppm carbendazim solution.

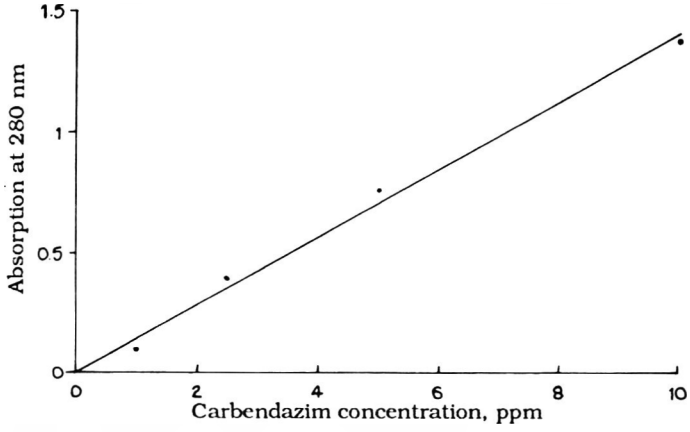

Fig. 1. Calibration curve for carbendazim

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TABLE 1. RECOVERY OF CARBENDAZIM FROM SPIKED APPLE, TOMATO AND MUSHROOM

Spiked, ppm	Amount added, µg	Amount recovered, µg			Mean	% recovery
		R_1	R_2	R_3		
Apple*						
1.0	25.0	18.94	19.59	18.29	18.94	75.56
2.5	62.5	60.27	56.38	63.07	59.90	95.85
5.0	125.0	119.59	118.94	114.08	117.53	94.02
10.0	250.0	248.28	234.67	241.64	241.53	96.61
Tomato*						
1.0	25.0	20.07	16.99	19.75	18.93	75.74
2.5	62.5	55.24	62.21	66.43	61.29	94.29
5.0	125.0	116.67	115.38	124.13	118.72	94.98
10.0	250.0	223.16	232.89	222.30	226.11	90.44
Mushroom*						
1.0	25.0	19.10	18.13	19.26	18.83	75.32
2.5	62.5	56.87	59.30	58.65	58.27	93.23
5.0	125.0	119.27	123.32	127.37	123.32	98.65
10.0	250.0	240.83	234.51	233.21	236.18	94.47

R_1, R_2, R_3 are replications. * Unspiked = Absorbance zero

All chemicals used were of highest purity available and the water used was from Milli-Q system (Milli-RO Plus 10 and Milli-Q Plus, M/s Millipore Intertech, USA). Ethyl acetate was distilled before use.

Recoveries of carbendazim from control extracts of apple, tomato and mushroom, spiked at 1.0-10 ppm with freshly prepared stock solution of carbendazim, are given in Table 1. There was no absorbance in any of the three substrate blanks including 'Red delicious' and 'Golden delicious' apples. It is stressed that the high absorbance in tomato blanks may be encountered, and these could be due to (a) low concentration of ammonia in the extract before blending and (b) inadequate washing of ethyl acetate with water before partitioning. In the present method, slight pale colour is visible in HCl phase, after first partitioning. However, it disappears on second partitioning, and the absorbance becomes zero at 280 nm.

It is interesting to note that the high recoveries at low concentrations are due to the interference by plant co-extractives. In contrast, the low recoveries at low concentrations are due to fixed dissociation of carbendazim on processing, which becomes significant at low concentrations.

The whole process takes 30 min for completion. The absorbance of carbendazim gets reduced on storing overnight in 0.1 N HCl. Second partitioning of neutral aqueous phase in ethyl acetate did not improve recovery (checked without crop material), while the spiking at lower than 1 ppm did not give good recoveries. The maximum residue limit (MRL) of carbendazim for three substrates is 5 mg/kg (Anon 1992). The technique, therefore, is fairly sensitive (1-10 ppm) for monitoring residues of carbendazim in market samples on routine basis. Possibly, the technique can be employed for estimating carbendazim residues in other substrates as well.

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Spoilage Organisms of Canned Acidified Mango Pulp and Their Relevance to Thermal Processing of Acid Foods

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Swelling due to gas formation in commercially canned 'Alphonso' mango pulp (pH 4.3) was caused by *Bacillus licheniformis* (having thermal resistance i.e., D_{100} = 1.25 min at pH 4.2 and D_{100}= 3.12 min at pH 7.0) and *Clostridium sporogenes* (D_{100}=6.8 min at pH 4.5 and $D_{121.1}$=0.51 min at pH 7.0). The metabiosis of the former increased the pH, thereby creating conditions favourable for the growth of the latter. These findings indicate that products having a pH higher than 4.0, but lower than 4.6, should be given thermal process, adequate to destroy *B. licheniformis*, except when *B. coagulans* spoilage is likely to occur.

Keywords : Thermal processing, Mango pulp, Acid foods, *Bacillus licheniformis*, *Clostridium sporogenes*.

Thermal process schedule for low-acid products (pH>4.6) should ensure destruction of *Clostridium botulinum* capable of producing exotoxin, consumption of which may prove fatal. Thermal process schedules for drumstick, okra, elephant yam and potato have been reported (Saikia and Ranganna 1992). For acid products having pH 4.6 and lower, the process schedule is based on the destruction of spore forming species other than *Clostridium botulinum*. Important organisms, which are likely to cause spoilage in acid foods having pH as low as 3.6 and 4.1 and above are (i) *Cl. pasteurium* among the anaerobes, and (ii) *B. coagulans* among the aerobes.

Thermal process schedule evolved on the basis of the inactivation of heat resistant enzyme systems, such as peroxidase and pectinesterase (PE), which cause undesirable changes, have been found to render the canned fruits microbiologically safe (Nath and Ranganna 1977, 1980). Nath et al. (1983) found that the cold point shifted from the geometric centre towards the periphery with an increase in the filling temperature, and accordingly, the processing time decreased in the canning of fruit pulps. Based on this observation, Siddalingu et al. (1983) evolved thermal process for the canning of acidified mango pulp, and the processors are making use of the same for the canning of the fruit pulps. The process involves manual acidification of the pulp to pH 4.0 or lower, heating the acidified pulp to 95°C and above in steam jacketted kettles or heat exchangers, filling the hot pulp into A10 (603x700) cans at temperatures not less than 85°C, sealing, inverting the sealed cans and holding for 5 to 10 min before cooling.

During the 1987 and 1988 seasons, it was found that a few cans from a particular batch of 'Alphonso' mango pulp canned in A10 cans bulged, after a few days. The present investigations were carried out to identify the organisms responsible for spoilage and determine their heat resistance. The importance of isolates in thermal processing of acid foods, besides mango pulp, is discussed in this paper.

Materials and Methods

Isolation and identification of spoilage organisms: Swollen A10 cans of 'Alphonso' mango pulp, supplied by a factory, were turned upside down 5 or 6 times for uniform mixing of the contents. The cans were opened aseptically. Nutrient broth and reinforced clostridial medium, inoculated with the pulp from spoiled cans, were incubated at 38°C. Growth was indicated by the formation of an initial pellicle, and subsequent turbidity in the sub-cultured tubes in the case of aerobes, and by the formation of gas and turbidity in the case of anaerobes. Media and reagents required for identification of aerobic and anaerobic isolates were prepared by following the procedures described by National Canners' Association (NCA 1968), Sneath (1986), and Holdeman et al. (1977). The aerobic and anaerobic isolates were identified according to the procedure given by Sneath (1986), and Cato et al. (1986), respectively. Test for toxin production was done according to the procedures given by NCA (1968) and Holdeman et al. (1977).

Preparation of spore suspension : The spores of aerobic and anaerobic isolates were produced on nutrient agar containing 0.1% soluble starch and 10 ppm $MnSO_4$ according to the procedure of NCA

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(1968) and on beef heart broth (Ranganna 1986), respectively. The harvested spores were counted by MPN technique using 3 tubes (NCA 1968).

Mango pulp was diluted with water in the ratio of 1:4, to get juice. The pH was adjusted to 4.2, and soluble solids content to 10%. Nine ml of the juice was taken in each vial of 20 ml capacity. The vials were plugged with rubber insert, pre-fractured aluminium cap was crimped on to the vial, sterilised at 121°C for 15 min, cooled and stored in refrigerator at 4°C. When required, 1 ml of the spore suspension was injected to each bottle. Spore concentration ranged from 2,30,000 to 7,50,000/ml in different studies. Similar suspensions were prepared in neutral phosphate buffer.

Determination of D value : Decimal reduction time (D) was determined using capillary tubes. Prior to determination, spores were activated by heating at 70°C for 10 min. Six tubes were used for heating at each time interval at a particular temperature. Heating was done in a temperature controlled glycerol bath. After heating, the contents were sub-cultured in the tubes, containing the nutrient broth in the case of aerobic organism, and into beef heart broth in the case of anaerobes. The anaerobic tubes were plugged with sterile sealing mixture and incubated at 37°C for 14 days, as described by Ranganna (1986). Uncorrected and corrected D values were calculated by the fraction negative method of Stumbo et al. (1950).

Identification of fruits and vegetables congenial for growth of B. licheniformis : The fresh fruits and vegetables were prepared in the same way as for canning, and pulped. Each pulp was taken into 3 tubes, one was used as a control. The pH was adjusted to 4.2 in the second and, to 4.5 in the third, using citric acid or alkali. The tubes were sterilised by autoclaving at 121°C for 15 min, cooled and inoculated with spores of *B. licheniformis* isolated from mango. To determine the possibilities of spoilage in the canned products, banana, papaya, jack fruit, guava, sapota and tomato pulps were inoculated with spores of *B. licheniformis* (7.5×10^6) and/or *Cl. sporogenes* (2.4×10^5) to each A1 Tall can (301x411). The pulps, after heating, were filled hot (92°C) into cans in small portions, inoculated with spores of organisms, sealed when full, kept inverted for 10 min, cooled and incubated at 37°C.

Results and Discussion

Commercially canned A10 cans of 'Alphonso' mango pulp, which had bulged during storage were free of seam defects, and the pH was 4.3. Except for bulging of the cans due to gas formation, the colour, consistency and odour of the pulp were similar to those of normal cans. Microscopic examination of cans during 1987, and 1988 seasons indicated the presence of aerobic organisms, which produced tiny bubbles of gas in nutrient broth, but contained no anaerobes, yeasts or moulds. During 1989 season, two cans of mango pulp, which had bulged after long storage, were provided by the factory. The pH was 4.3. No change had occurred in sensory characteristics of the product, though the cans had bulged. The bacteriological examinations showed that the product contained one aerobic and another anaerobic rod-shaped bacteria.

Identification of the isolates : The aerobic isolate, a rod-shaped, Gram-positive, spore-forming facultative aerobe, which produced catalase, did not either reduce sulphate to sulphide or produce mycelium. These features are characteristic of the genus *Bacillus*. The morphological, cultural and biochemical characteristics of the isolate were similar to those of *B. licheniformis*.

The anaerobic isolate was a rod-shaped, Gram-positive, catalase negative, gas forming, obligate anaerobic spore former, indicating that it belonged to the genus *Clostridium*. The cultural, morphological and biochemical characteristics were similar to those reported for *Cl. botulinum* types ABF (proteolytic strains) and *Cl sporogenes*. The isolate did not produce any toxin, thereby indicating that the anaerobic organism isolated from mango was *Cl. sporogenes*. Thermal resistance data, discussed later, lent support to this point.

Investigations carried out to identify the microorganisms responsible for the swelling of canned 'Alphonso' mango pulp showed that it was caused by the facultative anaerobic spore former, *B licheniformis*, and the obligate anaerobic spore former, *Cl. sporogenes*. Margalith and Shoenfeld (1962) isolated thermophilic form of *B. licheniformis*, which caused gaseous spoilage in cans of banana puree. *B. licheniformis* has also been isolated from about 30% of the home canned tomatoes examined by Fields et al. (1977), who reported that many isolates elevated the pH of tomato serum. Alian et al. (1986) found *B. licheniformis* to be the most prevalent organism among the 7 species of *Bacillus* isolated from fresh juice of tomato. Out of 5 strains of *B. licheniformis* investigated by Montville and Sapers (1981), three strains could initiate growth in tomato and puree at a pH of 4.2, but not at 4.0, one had a lower limit of pH 4.4, while the

fifth could not initiate growth at pH 4.4.

The growth of *Cl. botulinum* in acid foods is not due to the ability of the organism to grow under the acidic conditions of the food (pH ≤ 4.6), but rather to the raise in pH above the inhibitory levels (Ito and Chen 1978). *B. licheniformis* could elevate the pH, and deplete oxygen even in an aerobic acidic model system, thereby allowing growth and toxin production by *Cl. botulinum* (Montville 1982). Besides *B. licheniformis*, moulds and one particular strain of *B. coagulans* can give rise to conditions favourable to the growth of *Cl. botulinum* (Odlaug and Pflug 1978, 1979; Anderson 1984).

Cl. sporogenes coexisting with *B. licheniformis* and their metabiosis seems to have not been reported hitherto. *B. licheniformis*, in conformity with the earlier findings, raised the pH of the nutrient broth from 5.0 to 8.5,6.0 to 9.0 and 6.5 to 9.5 after 30 days of incubation at 37°C. *Cl. sporogenes* did not grow below 4.5 in reinforced clostridial medium or beef heart broth. *B. licheniformis* spores added to hot mango pulp at 90°C (pH 4.0) in A10 cans, just before sealing caused no spoilage, even after prolonged incubation for 6 months. In model system, collection of a small amount of gas could be seen at pH ≥ 4.3 (Table 1). *Cl. sporogenes* caused no spoilage of mango pulp over the pH range of 4.0 to 5.0. When both the organisms were present, gas formation as well as slight raise in pH could be seen at pH 4.5 and 5.0 (Table 1).

Resistance studies of bacterial spores to acid have been reviewed by Blocher and Busta (1983). Spores of *Cl. sporogenes* strain PA 3679 are not able to germinate at pH ≤ 4.8, and as seen in the present study, at pH ≤ 5.0. *B. licheniformis* is able to grow at pH 4.2 and above. Considering the extent of gas formation, resulting in hard swells in 1986 and 1987 seasons, the spoilage could not have occurred, had not *B. licheniformis* elevated the pH, and possibly depleted the residual oxygen on the surface layer of mango pulp in the can, thus resulting in an environment favourable to the growth of *Cl. sporogenes* to cause spoilage. Results shown in Table 1 confirm the same. The pH elevated by the activity of *B. licheniformis* could have been reduced by the acid formed from glucose and other sugars by *Cl. sporogenes* (Cato et al. 1986).

TABLE 1. CHANGES DURING THE INCUBATION FOR 6 MONTHS OF MANGO PULP INOCULATED WITH *B. LICHENIFORMIS* AND/OR *CL. SPOROGENES*.

B. licheniformis			*Cl. sporogenes*			*B. licheniformis* ± *Cl. sporogenes*		
Initial pH	Final pH	Production of gas bubble*	Initial pH	Final pH	Production of gas	Initial pH	Final pH	Production of gas
4.0	4.0	- ve	4.0	4.0	- ve	4.0	4.0	- ve
4.3	4.3	+ ve	4.3	4.3	- ve	4.3	4.5	- ve
4.5	4.6	+ ve	4.5	4.4	- ve	4.5	4.7	+ ve
5.0	5.9	+ ve	5.0	4.7	- ve	5.0	5.1	+ ve

* *B. licheniformis* formed only a gas bubble in durham tubes.

D and z values of B. licheniformis *and* Cl. sporogenes: The values of D and z of *B. licheniformis* and *Cl. sporogenes* found in this study are given in Table 2.

TABLE 2. D AND z VALUES OF *CL. SPOROGENES* AND *B. LICHENIFORMIS* ISOLATED FROM MANGO PULP

Organism	Medium	pH	Spore concentration	D Value (min)
Cl. sporogenes	Mango	4.5	24×10^4	$D_{100} = 6.80$
	N.P.B.	7.0	24×10^4	$D_{121.1} = 0.51$
B. licheniformis	Mango	4.2	23×10^4	$D_{100} = 1.25$*
	Mango	4.5	15×10^3	$D_{100} = 1.20$
	N.P.B.	7.0		$D_{100} = 3.12$

* z = 11.45°C : N.P.B. Neutral phosphate buffer.

The D values of the former found in this study are somewhat lower than the reported values (Marglith and Shoenfeld 1962; Montville and Sapers 1981; Montville 1982; and Russel 1982). Commercially canned mango and other tropical fruit pulps packed in India have pH less than 4.0. The limiting factor in the hot fill-hold-cool process for mango and other pulps evolved in this laboratory is that the pH should not exceed 4.0. Banana, guava, orange and papaya, besides a variety of vegetables, supported the growth of *B. licheniformis*

at pH 4.5. Only in papaya and banana, the growth was initiated at pH 4.2, as observed from the evolution of gas bubbles. D values of *B. licheniformis* in fruits and vegetables, which support the growth of organism are given in Table 3.

TABLE 3. D VALUE AT 100°C OF *B. LICHENIFORMIS* IN FRUIT AND VEGETABLE PULPS WHICH SUPPORT THE GROWTH OF THE ORGANISM

Product	pH	D value (min)
N.P.B.	7.0	3.1 ± 0.2
Papaya	4.2	2.2 ± 0.2
	4.5	2.5 ± 0.5
Jack fruit	4.2	2.2 ± 0.5
	4.5	3.8 ± 0.5
Guava	4.2	3.3 ± 0.3
	4.5	4.1 ± 0.2
Peas	4.2	2.5 ± 0.1
	4.5	2.5 ± 0.1
French beans	4.2	2.2 ± 0.2
	4.5	2.6 ± 0.2
Ivy gourd (*Kundri*)	4.2	2.2 ± 0.2
	4.5	2.3 ± 0.3
Cauliflower	4.2	2.1 ± 0.9
	4.5	2.8 ± 0.4
Carrot	4.2	2.2 ± 0.2
	4.5	2.5 ± 0.2

N.P.B. : Neutral phosphate buffer

B. coagulans grows with increasing probability at temperatures higher than 30°C and pH higher than 4.1, and causes flat-sour spoilage in tomato products characterised by lowering of pH by 0.2-1.0 pH units (Fields et al. 1977). An exception to this general understanding has been a strain isolated by Anderson (1984), which was found to grow well in juice having an initial pH of 4.5 (but poor at pH 4.2 or 4.3), and elevated the pH from 4.5 to 5.0 by the end of 6 days (Fields et al. 1977).

Survival of high heat resistant Clostridial spores in acid foods : The D_{95} value of *B. licheniformis* in tomato juice at pH 4.5 was 5.1 (z = 14.9°C) (Montville and Sapers 1981), while the D_{95} values in mango pulp found in this study was 3.42 min (z = 11.45°C). Anderson et al. (1949) reported a value of $F_{100}^{10.8}$=21.5 min for *B. coagulans* in tomato juice (pH 4.4-4.5), while Nath (1978) reported $F_{100}^{7.8}$=65.3 min at pH 4.5. In any case, the D or F values of *B. coagulans* are higher than the values of *B. licheniformis*.

The D value of *Cl. botulinum* decreases with pH. *Cl. botulinum* type A strain isolated from tomato juice had an extrapolated D value of 18 min at 100°C at pH 4.2 (Xezones and Hutchings 1965). *Cl. sporogenes* behaved similarly (Camerson et al. 1980). The D value of *Cl. sporogenes* strain isolated from 'Alphonso' mango pulp at pH 4.5 was 6.8 min at 100°C, but yet significantly higher than that of *B. licheniformis*, and considerably higher than organisms of interest in the region of acid foods.

Spores of high heat resistant *Cl. botulinum* and other organisms known to cause spoilage in low-acid foods remain dormant in heat processed acid foods for long periods. Their germination and growth occur only when the pH in the environment is elevated. Among bacteria and moulds reported to elevate pH, *B. licheniformis* is more heat resistant. The lowest pH at which *Cl. botulinum* and *Cl. sporogenes* are able to grow is pH > 4.8. Allowing a margin of 0.2 units, 4.6 is taken as the demarcation pH for low-acid foods requiring safe thermal process with respect to *Cl. botulinum* from the public health point of view. Applying the same analogy, since *B. licheniformis* is able to grow at pH ≥ 4.2 and elevate the pH, all products having pH higher than 4.0, but lower than pH 4.6 should be given thermal process, adequate enough to destroy *B. licheniformis* with the probable exception of tomato products, which requires inactivation of *B. coagulans*. The heat treatment to inactivate these organisms would also inactivate the toxigenic strains of *Cl. butyricum* and *Cl. barati* (Odlaug and Pflug 1979).

Conclusion

In products having a pH 4.0 or lower, the process schedule evolved on the basis of thermal inactiviation of enzymes, which has hitherto proved adequate under commercial processing conditions, and rendered the canned product microbiologically safe could be continued, and there is no reason to alter the same. The products having pH higher than 4.0, but lower than pH 4.6, should be given thermal process, adequate to destroy *B. licheniformis* with the exception, where *B. coagulans* spoilage (as in tomato) is likely to occur.

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Anthocyanin of *Karwand* (*Carissa carandas*) and Studies on Its Stability in Model Systems

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Anthocyanin from *Karwand* (*Carissa carandas*) is characterized, and its stability is determined in model systems. Based on the chromatographic data and spectral studies, the pigment is identified as cyanidin - 3 - rhamnoglucoside. The data on the stability of the pigment in two model systems showed progressive loss of anthocyanin. The loss was higher at 30°C than at 5°C. A potential exists for the use of *Karwand* anthocyanin as a natural colouring agent for products requiring milder processing treatment and low temperature storage.

Keywords : *Karwand*, Anthocyanin, Extraction, Stability, Model system.

The *Karwand* (*Carissa carandas*), a small edible fruit of purplish red colour, is found almost all over India. It is especially cultivated in Konkan and other North Indian States. The fruit's utility, at present, is restricted to the manufacture of *Karwand* juice, besides being eaten afresh.

There is a growing worldwide interest in developing colour additives for foods. Earlier studies on *Phalsa* fruit and Avocado peel led to the identification of 2 anthocyanin pigments which give a pleasing red colour (Khurdiya and Anand 1981; Prabha et al. 1980). In the present studies, the *Karwand* anthocyanin extract was tested for its stability in a model system for determining its suitability as a more stable natural colour.

Materials and Methods

A sample of 500 g of fresh and ripe fruit peel was blended with about 100 ml 1% methanolic HCl for 5 min (the acid stabilizes the pigment). In order to facilitate complete extraction, the macerate was stored overnight at 5°C, and then centrifuged, and the clear supernatant was decanted. The residue was repeatedly washed with the solvent for extraction of the left-over pigments. The pooled extract was concentrated in a rotary vacuum evaporator at 30°C, and was subjected to ascending paper chromatography using Whatman filter paper No. 3 in butanol : acetic acid : water - 4:1:5 (B.A.W.); formic acid : HCl : water - 5:2:3 (Formic) and acetic acid : HCl : water : 30 : 3 : 10 (Forestal), as solvent systems for determining R_f values of anthocyanin.

The spot from the chromatogram, involving the use of B.A.W. solvent system, was eluted out in 0.01% methanolic-HCl, and the UV-visible spectrum was recorded using a Bausch and Lomb spectronic 2000 spectrophotometer in the range of 270-600 nm. Acid hydrolysis of the eluted component was carried out to separate aglycone and sugar moieties. The sugar was identified by comparison with standard sugar, and the UV-visible spectrum of the aglycone moiety was recorded and its bathochromic shift noted.

Total anthocyanins from fresh and post-processed *Karwand* fruit were extracted (AOAC 1984) and determined by the method described by Fuleki and Francis (1968). The ascorbic acid content was detected by the 2, 6- dichlorophenol indophenol titration method. Processing of the fresh fruit was carried out after bottling in 40°C Brix sugar solution by a) autoclaving at 3 lbs for 12 min and b) immersion in boiling water for 15 min.

Preparation of colourant : The pigment was extracted from the pomace with ethanol, containing 0.03% HCl at the ratio of 5 : 1 (solvent:pomace). The resulting extract was concentrated by rotary vacuum evaporation and absorbed on an IRC cation exchange resin (Ion Exchange, India) for purification. The pigment was eluted out with 0.0001% HCl in ethanol and concentrated to provide the colourant.

The stability of the concentrate was studied in two model systems. In one case, a sugar-citrate solution (12° Brix) of pH 3.5, to which 10% of the colour concentrate was added. This was stored at 5 and 30°C for a period of 20 days, and the anthocyanin content was determined every alternate day. In another case, the yoghurt was prepared by acidifying milk with 2% lactic acid, to which 1%, 1.5% and 2% of the colour concentrates were added. The mixtures were stored at 5°C for 7 days and the anthocyanin contents measured.

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The colourant was added at different concentrations in the model systems to test whether varying concentrations played any role in the stability of the colourant pigment.

Results and Discussion

The R_f values of *Karwand* (*Carissa carandas*) anthocyanin in the three solvent systems, i.e. B.A.W., formic and forestal, were found to be 3500, 4500 and 7400, respectively. The R_f values in B.A.W. and forestal are in close agreement, with those of cyanidin 3-rhamnoglucoside, as reported by Harborne (1958). The anthocyanin gave a bathochromic shift upon addition of 5% ethanolic $AlCl_3$ (2-3 drops), thereby indicating the presence of orthophenolic groups (Gessiman et al. 1953, Gessiman and Jurd 1955) in their aglycone (anthocyanidin portion). The A 440/A max percentage value of 31 indicated 3-glycosides (Harborne 1958). The sugar moieties were found to be glucose and rhamnose. The maximum values of anthocyanidin (529.3) and the anthocyanin component (527.1) are in close agreement, with those of the reported maxima for cyanidin (Harborne 1958). The component was, thus, a cyanidin derivative with sugars, rhamnose and glucose substituted at the 3 position. Based on the above data, the component can be assigned the structure as shown in Fig 1.

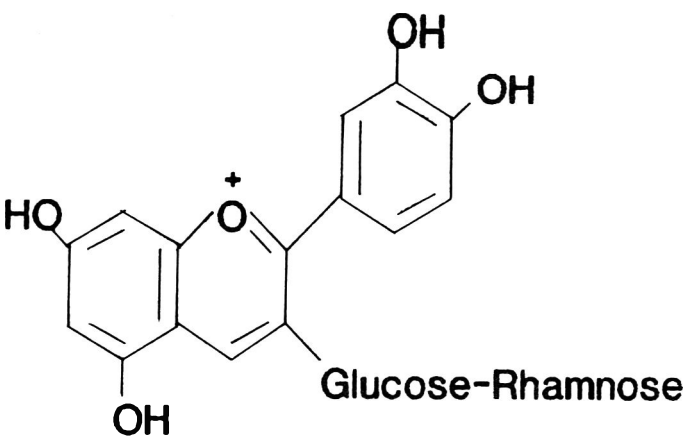

Fig 1. Cyanidin 3-rhamnoglucoside

Table 1 shows the stability characteristics of the anthocyanin and ascorbic acid present in *Karwand* to different processing treatments. The processing of the *Karwand* fruit resulted in loss of anthocyanins and ascorbic acid. Several factors, either individually or in combination, might have played an important role in the destruction of anthocyanin and ascorbic acid. For example, heat and heating time are the major factors responsible for anthocyanin destruction (Meschter 1953; Adams and Ongley 1972). It was observed that, when a product was given a short time high temperature processing treatment, it had more anthocyanin retention, as compared to that in the products kept at a lower temperature for a longer period. Nebesky et al. (1969) found that the oxygen and temperature were the most specific accelerating agents in the degradation of pigments in most fruits. Retention of anthocyanin pigments was greater, when canned under nitrogen than those under air (Daravingas and Cain 1965).

TABLE 1. STABILITY CHARACTERISTICS OF *KARWAND* ANTHOCYANINS AND ASCORBIC ACID TO PROCESSING TREATMENT.

Karwand material	Total anthocyanin, mg/100 g fruit	Ascorbic acid mg/100 ml fruit juice
Fresh	21.55	105.00
Process 'A'*	15.90	54.95
Process 'B'#	17.38	65.94

* Bottled in 40° Brix sugar solution and autoclaved at 3 lbs for 12 min.

\# Bottled in 40° Brix sugar solution and immersed in boiling water for 15 min.

Beattie et al. (1943) and Pederson et al. (1947) observed parallel losses of anthocyanin and ascorbic acid during various processing treatments. Sugar and sugar degradation products, i.e. furfural and hydroxymethyl furfural, accelerate the degradation of pigment and oxygen aggravated the destructive effects of sugar. Although the effect of different parameters on the loss of anthocyanins was studied individually, there has been no attempt to make a comparative study to check pigment stability, when many factors were present in combinations. *Karwand* anthocyanins can, therefore, be stated to be relatively stable to the processing treatment used in the present studies, as the degradation was in the range of 19-26%, which was much lower than that of 63-78% in case of plum anthocyanin under same treatment.

Data in Tables 2 and 3 on the stability of *Karwand* anthocyanins in the model systems indicate a progressive loss in anthocyanin content, which was more, when the model system was stored at 30°C. The increase in colourant concentration (in case of yoghurt) made no significant difference, as pigment retention at all concentrations was almost the same. Though no pasteurization treatment was given and packaging of the model systems (beverage and yoghurt) could not be termed excellent, no visible microbial growth could be seen at the end of the stipulated period.

TABLE 2. STABILITY CHARACTERISTICS OF *KARWAND* ANTHOCYANIN COLOURANT IN SUGAR-CITRATE SOLUTION OF pH 3.5 AND 10% COLOURANT CONCENTRATION FOR 20 DAYS AT 5°C AND 30°C.

Days	Pigment content mg/100 ml	
	5°C	30°C
0	0.51	0.51
2	0.50	0.44
4	0.50	0.43
6	0.48	0.40
8	0.47	0.39
10	0.46	0.38
12	0.45	0.38
14	0.45	0.37
16	0.44	0.36
18	0.44	0.35
20	0.43	0.34

TABLE 3. STABILITY CHARACTERISTICS OF *KARWAND* ANTHOCYANIN PIGMENT CONCENTRATE IN YOGHURT (MILK ACIDIFIED WITH 2% LACTIC ACID) AT pH 3.8 FOR 7 DAYS AT 5°C.

Pigment concentrate, %	Anthocyanin, mg/100 ml on days			
	0	2	4	7
1	0.46	0.44	0.43	0.40
1.5	0.92	0.87	0.84	0.79
2	1.29	1.21	1.18	1.11

The results obtained were comparable to those for cyanidin 3-glucoside from blueberries and enocyanin (colourant prepared from grapes) in a buffered sugar-citrate model system at pH 2.8 for the same period (Teh and Francis 1988). The *Karwand* anthocyanin was more stable than the pigment obtained from *Jambul*, under similar test conditions (Iyer 1992).

It is known that anthocyanin pigments are more stable at low pH values (Meshter 1953). Hence, the *Karwand* anthocyanin pigment may be termed stable and could be used as a colourant in food products requiring mild processing treatments, and could preferably be stored under low temperatures.

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Comparative Hypocholesterolemic Activities of Oryzanol, Curcumin and Ferulic Acid in Rats

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The hypocholesterolemic effect of oryzanol, a mixture of ferulic acid esters of sterols and triterpenols, isolated from rice bran oil was compared with that of curcumin (diferuloyl methane, the yellow pigment of turmeric) and ferulic acid. Feeding 0.5% oryzanol, 0.15% curcumin or 75 mg% ferulic acid in the 1% cholesterol containing diet (HCD) for 7 weeks caused a significant decrease in serum total cholesterol as well as (LDL + VLDL) cholesterol and an increase in HDL cholesterol. Serum lipoprotein (LDL+VLDL) concentration was also decreased. The ratio of LDL-cholesterol to HDL-cholesterol which was 24.9 on the HCD was decreased by 40% by oryzanol, 21% by curcumin and 24% by ferulic acid. Oryzanol and curcumin lowered liver cholesterol levels, whereas ferulic acid was not effective. Oryzanol was a better hypocholesterolemic agent than curcumin or ferulic acid.

Keywords : Oryzanol, Curcumin, Ferulic acid, Hypocholesterolemic activity, Rat feeding.

Oryzanol, a mixture of ferulic acid esters of sterols and triterpenols, is present in rice bran oil (Seetharamaiah and Prabhakar 1986). Several workers (Shinomiya et al. 1983; Nakayama et al. 1986, 1987; Seetharamaiah and Chandrasekhara 1988) have demonstrated that oryzanol is a hypocholesterolemic agent in experimental animals. It has been recently reported that the effect of oryzanol is mainly due to inhibition of absorption of dietary cholesterol and increased faecal excretion of bile acids (Seetharamaiah and Chandrasekhara 1990). The hypocholesterolemic activity of β-sitosterol, one of the components of oryzanol, has been attributed mainly to its effect on the absorption of exogenous cholesterol (Grundy and Mok 1977). Another major component of oryzanol, namely 24-methylene cycloartanol, has also been demonstrated to be a cholesterol lowering compound (Kribuchi et al. 1983). In sucrose- induced hypertriglyceridemic condition in rats, ferulic acid caused a decrease in liver cholesterol and an increase in serum HDL-cholesterol (Srinivasan and Satyanarayana 1988). However, there is no study regarding the effect of ferulic acid in experimental animals fed a high cholesterol diet. Curcumin, the yellow coloured pigment of turmeric is mainly composed of diferuloyl methane and is also known to be effective in lowering cholesterol levels (Subba Rao et al. 1970). The present paper reports the comparative hypocholesterolemic efficacy of oryzanol with that of curcumin and ferulic acid.

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Materials and Methods

Chemicals : Oryzanol was isolated from rice bran oil, as reported elsewhere (Seetharamaiah and Prabhakar 1986). Curcumin was procured from Flavours and Essences Pvt. Ltd., Mysore, India. Ferulic acid and sodium tauroglycocholate (mixture of bile salts) were purchased from Sigma Chemicals Co., USA. Cholesterol was from Sisco Research Lab., Bombay, India. All other chemicals and solvents were of analytical grade, while the solvents were distilled before use.

Animals : Male albino rats of the 'Wistar' strain, weighing 60-70 g, were divided into 5 groups of 6 rats each and kept in individual cages with free access to water and diet. They were fed the following diets for 7 weeks:1) control diet (Seetharamaiah and Chandrasekhara 1988), 2) control + 1% cholesterol + 0.15% bile salts (high cholesterol diet HCD), 3) HCD + 0.5% oryzanol, 4) HCD + 0.15% curcumin, 5) HCD + 75 mg% ferulic acid. After 7 weeks, the rats were fasted for 18 h and sacrificed under ether anaesthesia. Blood was collected by cardiac puncture. The liver was excised, washed with ice-cold isotonic saline and weighed. Serum and liver samples were stored at -20°C until used for analysis.

Lipid analysis : Serum and liver lipids were extracted (Folch et al. 1957) with chloroform-methanol (2:1, v/v). Cholesterol (Searcy and Bergquist 1960) triglycerides (Fletcher 1968) and phospholipids (Marinetti 1962) were estimated by standard methods. Serum HDL-cholesterol was determined in the supernatant after treatment with heparin-manganese reagent (Warnick and Albers 1978). The

precipitate was extracted with chloroform-methanol (2:1, v/v) before using for LDL (including VLDL) cholesterol estimation. Serum lipoproteins were separated by polyacrylamide gel electrophoresis and quantitated by scanning at 620 nm (Beckman spectrophotometer, Model 26), as described elsewhere (Seetharamaiah and Chandrasekhara 1988). Results are expressed as mean ± SEM, and differences between the group means were evaluated for significance by Student's 't' test (Snedecor and Cochran 1967).

respectively. Serum triglycerides and phospholipids of rats fed the different experimental diets were not different from those fed HCD only.

Cholesterol feeding significantly decreased HDL-cholesterol and markedly increased LDL-cholesterol concentrations (Table 2). In animals fed the test compounds, LDL-cholesterol concentrations were significantly lower than those in the HCD fed group. Whereas HDL-cholesterol, showed a tendency to increase (statistically not significant) in rats on the HCD + oryzanol diet, the other two compounds did

TABLE 1. EFFECT OF ORYZANOL, CURCUMIN AND FERULIC ACID ON SERUM LIPIDS OF RATS

Group	Diet	Serum cholesterol, mg/dl			Cholesterol ester / Free cholesterol	Serum triglycerides mg/dl	Serum phospholipids mg/dl
		Total	Free	Ester			
1	Control	65.1±3.0	24.5±1.0	40.6±2.3	1.66±0.06	87.9±8.0	160.6±8.1
2	HCD	311.3 ± 19.7[a]	152.5±6.5[a]	158.9±13.7[a]	1.03±0.05[a]	29.0±3.2[a]	186.9±10.8
3	HCD + 0.5% oryzanol	248.9±10.9[c]	115.8±4.2[a]	133.1±6.3	1.15±0.04[a]	36.0±3.6	175.6±7.9
4	HCD + 0.15% curcumin	250.0±18.2[c]	114.0±5.8[b]	136.0±13.1	1.25±0.14[a]	30.4±3.9	172.5±11.5
5	HCD + 75 mg% ferulic acid	233.9 ± 15.5[c]	113.1±5.3[a]	120.8±12.3	1.06±0.07	31.4±1.1	166.3±13.2

Values are mean ± SEM of 6 rats. [a]p<0.001; [b]p<0.005; [c]p<0.05. Comparisons are between groups 2 and 1, 2 and 3, 2 and 4 and 2 and 5.

Results and Discussion

For comparing the hypocholesterolemic activities of oryzanol, curcumin and ferulic acid, these were fed to rats at doses comparable with regard to ferulic acid content, i.e. 0.5% oryzanol, 0.15% curcumin and 75 mg% ferulic acid in the diet.

The effect of oryzanol, curcumin and ferulic acid on serum lipids is indicated in Table 1. All the test compounds significantly decreased serum total and free cholesterol levels. The decreases in serum total cholesterol in rats fed oryzanol, curcumin and ferulic acid were 20%, 20% and 25%, not alter HDL-cholesterol levels. The ratio of LDL-cholesterol to HDL-cholesterol which was increased to 24.9 by cholesterol feeding was decreased to 15.0, 19.6 and 18.8 in rats fed oryzanol, curcumin and ferulic acid, respectively.

The relative percentages of serum lipoproteins are shown in Fig. 1. The decrease in HDL caused by cholesterol feeding was partly reversed in rats fed oryzanol, curcumin and ferulic acid. The increase in HDL was more in the group fed oryzanol than in the other two groups. The increase in LDL was significantly lower in rats fed with oryzanol, curcumin

TABLE 2. EFFECT OF ORYZANOL, CURCUMIN AND FERULIC ACID ON SERUM LIPOPROTEIN CHOLESTEROL LEVELS IN RATS

Group	Diet	Cholesterol (mg/dl)		LDL cholesterol / HDL cholesterol
		HDL	LDL+VLDL	
1	Control	29.9±1.5	35.2±2.1	1.18±0.07
2	HCD	12.4±1.0[a]	299.4±20.1[a]	24.88±2.38[a]
3	HCD + 0.5% oryzanol	16.0±1.5	232.9±9.1[b]	15.04±1.21[b]
4	HCD + 0.15% curcumin	12.6±1.3	236.0±17.8[c]	19.62±2.24
5	HCD + 75 mg% ferulic acid	11.9±1.0	222.0±15.6[b]	18.82±0.69[c]

Values are mean ± SEM of 6 rats. [a]p<0.0001; [b]p<0.005; [c]p<0.05. Comparisons are between groups 2 and 1, 2 and 3, 2 and 4 and 2 and 5.

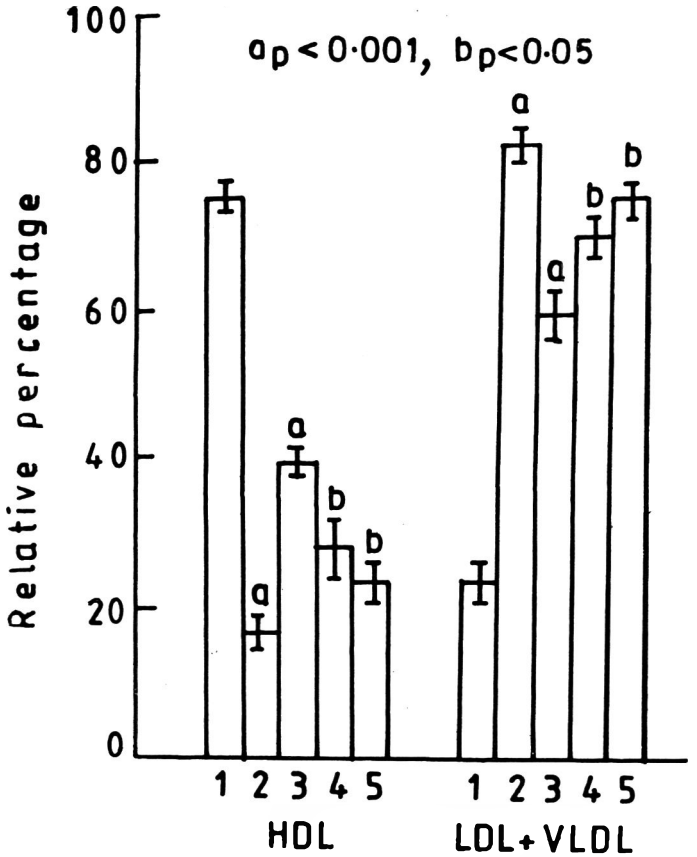

Fig. 1. Influence of oryzanol, curcumin and ferulic acid on serum lipoproteins of rats. 1: Control, 2:HCD, 3 : HCD + 0.5% oryzanol, 4:HCD +0.15% curcumin, 5: HCD + 75 mg% ferulic acid. Values are mean ±SEM of 6 rats. Comparisons between groups 2 and 1, 2 and 3, 2 and 4 and 2 and 5.

Plasma lipoproteins play an important role in the overall metabolism of cholesterol. HDL facilitates the removal of cholesterol from peripheral tissues to the liver for elimination (Miller and Miller 1975; Glomset 1968). It has been shown that during hypercholesterolemia, plasma LDL and LDL-cholesterol will increase and HDL and HDL-cholesterol will decrease (Jencks et al. 1956; Lasser et al. 1973). Many hypocholesteremic agents like B-sitosterol and diosgenin (Cayen and Dvormic 1979), nicotinic acid (Grundy et al. 1981), mevinolin (Grundy and Bilheimer 1984) have been used in patients with coronary heart disease or animals rendered hypercholesterolemic and they have been shown to correct the imbalance in serum lipoprotein levels. Similar to such agents, oryzanol partly corrected this imbalance of lipoproteins by increasing HDL and decreasing LDL. On the basis of the influence on serum and liver cholesterol levels taken together and of the concentrations of serum lipoproteins, it is concluded that oryzanol is more potent than curcumin and ferulic acid.

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TABLE 3. EFFECT OF ORYZANOL, CURCUMIN AND FERULIC ACID ON RAT LIVER LIPIDS

Group	Diet	Cholesterol, mg/g			Cholesterol ester	Triglycerides	Phospho-lipids
		Total	Free	Ester	Free cholesterol	mg/g	mg/g
1.	Control	3.05±0.13	1.31±0.04	1.71±0.12	1.30±0.09	6.4±0.7	20.7±1.2
2.	HCD	107.7±4.8[a]	26.1±0.8	81.5±4.6[a]	3.08±0.18[a]	18.8±1.2[a]	22.0±2.0
3.	HCD + 0.5% oryzanol	79.2±5.7[a]	24.7±1.0	56.2±5.6[b]	2.30±0.24[b]	15.3±1.2[b]	16.3±0.7[b]
4.	HCD + 0.15% curcumin	90.1±4.5[b]	27.7±1.0	63.2±6.5[b]	2.29±0.30	14.6±1.4[b]	18.0±2.0
5.	HCD + 75 mg% ferulic acid	109.2±6.4	27.4±1.2	82.0±5.4	3.06±0.24	15.3±1.5	17.0±1.0[b]

Values are mean ± SEM of 6 rats. [a]p<0.001; [b]p<0.05. Comparisons are between groups 2 and 1, 2 and 3, 2 and 4 and 2 and 5.

and ferulic acid.

The effect of oryzanol, curcumin and ferulic acid on liver lipids is indicated in Table 3. Oryzanol and curcumin significantly decreased liver total cholesterol, esterified cholesterol and triglycerides. Liver total cholesterol was reduced by 26% and 16% in the groups fed oryzanol and curcumin, respectively, but was not influenced by ferulic acid.

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Locust Bean Pods and Seeds : Some Physical Properties of Relevance to Dehulling and Seed Processing

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Studies on some of the properties of locust bean pods and seeds, relevant to dehulling, indicated that the pods have major diameter ranging from 76 to 277 as against from 8 to 12 mm for the seeds. The seed thickness ranged from 5.75 to 7 mm. An average sphericity of 67 and roundness of 65 are the characteristics that allow for some rolling of the seeds as well as sliding on its flat surface. The seed is unable to float in water, but the pod can.

Keywords : Locust bean, Physical properties, Dehulling, Processing.

Locust bean (*Parkia biglobosa*) seed grows on a common leguminous tree known as African locust bean tree in the Savannah zones of Nigeria. The plant produces brownish seeds, which are arranged in pods. When processed, the seeds constitute an important condiment that adds taste and flavour to soup. The processed cake, known as *Iru* is used widely in the South-Western and Middle-belt zones. Traditionally, the seeds are processed by boiling for about 8 h to soften the testa, washing to separate testa from cotyledons, feet mashing, sieving in running water, boiling the cotyledons until a low moisture content is achieved, and fermentation of the resultant mash. Mechanisation of the production of the cake has generated much interest in recent times. This is because, the traditional methods of processing is both tedious and complicated. This makes the processing of large quantities quite difficult.

It is necessary to determine the physical properties of agricultural products, because this helps in designing appropriate machinery and systems for processing and storage. Several researchers (Oje and Ugbor 1991; Sheperd and Bhardwaj 1986; Dutta et al 1988; Fortes 1980; Nelson 1980; Makanjuola 1972) have described the size of crops by measuring their three principal dimensions - major, intermediate and minor diameters. Static coefficients of friction have been determined for different surfaces, such as galvanized steel and plywood (Oje and Ugbor 1991; Fraser 1980; Sheperd and Bhardwaj 1986). Lawal (1989) measured the angle of repose for various grains. Mohsenin (1970) described various methods for measuring crushing force. These methods include the use of automatic recording universal hardness testing machine.

The objective of this study was to determine some of the properties of locust bean seed, at safe storage moisture content, namely; sphericity, roundness, size, volume, surface area, density, static coefficient of friction against different materials, angle of repose, and hardness. Size, volume, surface area, and density were also determined for the pods.

Materials and Methods

Several kg of locust bean pods were obtained from different sources. The material was sun-dried, as is normally done by local producers. The seeds were obtained by hand-peeling the pods and extracting the seeds. The safe storage moisture level was visually estimated by comparing the seed colour and appearance to normal seeds in the market.

One hundred seeds and equal number of pods were randomly selected from the sample. Measurements of dimensions on three mutually perpendicular axes were made, namely; major, intermediate and minor diameters. These dimensions were measured with a vernier calliper for the pods and a micrometer reading to 0.01 mm for the seeds. Sphericity and roundness (Curray 1951) were determined only for the seeds, since the pods are essentially flat. Each seed was placed in its natural resting position on a sheet of graph paper. A sharp thin pencil was used to carefully trace the edges of the seed. The projected area and the diameter of various circles, inscribing and circumscribing the projected areas, were measured (Oje and Ugbor 1991).

The surface areas of both pod and seed were determined by coating the surface with paint and contact printing on a light sensitive flexible paper (Oje and Ugbor 1991). The seed traces were magnified photographically, and the surface edge traces on the paper were then pencil-traced on graph paper. The surface area was measured by

counting the squares within the traced marks. In the case of seeds, the area so obtained was divided by the magnification factor to give the true surface area. The volume, and hence, density of each seed, was determined by the water displacement method, as described by Dutta et al (1980). The seed was dropped into a can filled with water. The water displaced was collected and weighed. The weight of water displaced was used to calculate the volume of water, and hence, the volume of the seed.

The static coefficient of friction (Lawton 1980) for the seeds was determined with respect to four structural surfaces, namely; plywood with its grain parallel and perpendicular to the direction of motion, galvanized steel and glass. A topless and bottomless box of 150x100x40 mm dimensions was filled with the seeds and placed on an adjustable tilting surface. One end of this surface with the box resting on it was raised gradually with a screw device, until the box just started to slide down. The angle of the incline was read from a graduated scale. Angle of repose was determined by using the pipe method (Lawal 1989). The procedure was repeated twenty times. Hardness was determined by using a Rockwell Hardness tester, a direct reading instrument of high sensitivity. The rupture force was recorded in Newtons (N).

Results and Discussion

The results for all the parameters measured for pods and seeds as well as the frequency distributions of some of the physical properties are shown in Table 1 and Figs 1 and 2.

TABLE 1. SOME PROPERTIES OF LOCUST BEAN SEEDS

Physical property	Minimum value	Maximum value
Static coefficient of friction on		
(a) plywood parallel to grain	0.384(0.39±0.006)	0.414
(b) plywood perpendicular to grain	0.499(0.50±0.007)	0.543
(c) galvanized steel	0.456(0.47±0.008)	0.499
(d) glass	0.384(0.40±0.012)	0.477
Angle of repose (degrees)	30.7(37.6±3.19)	43.9
Hardness (Newton)	17.0(25.6±10.1)	70.03

Figures in parenthesis denote the mean values. Number of observations were 20 for all the tests, except for 100 observations in case of hardness.

Size : Major diameter of the pods ranged from 76 to 276 mm (Fig 1), although more than 60% were

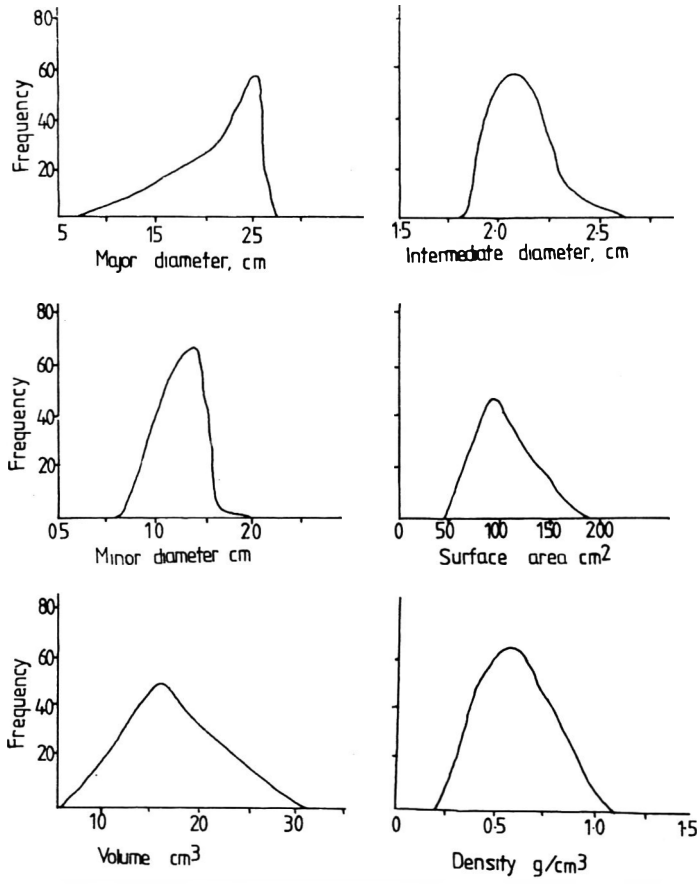

Fig. 1. Frequency distribution of some properties of locust bean pods.

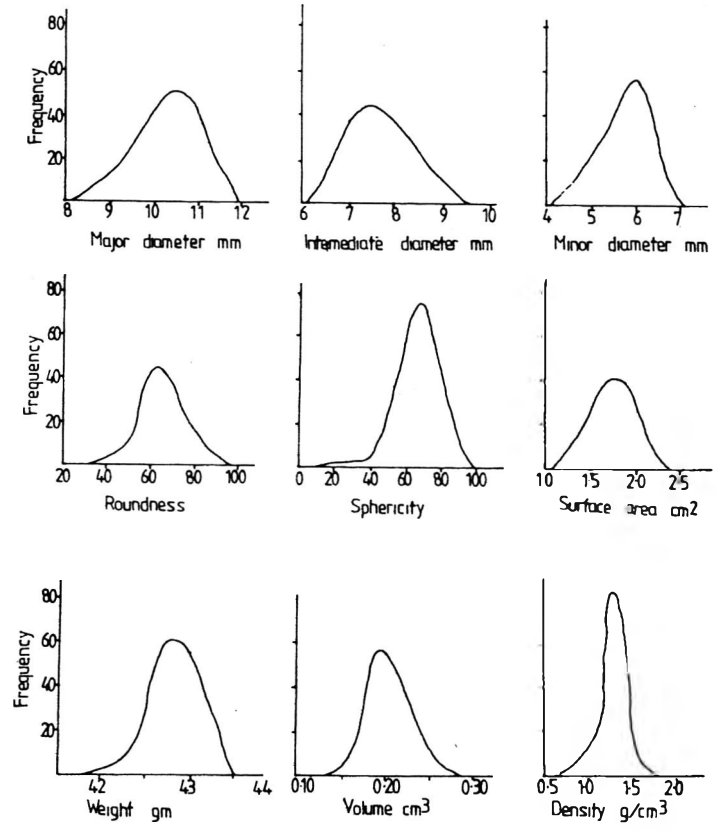

Fig.2. Frequency distribution of some properties of locust bean seeds.

between 200 and 270 mm. Nearly 50% of the pods had an intermediate diameter between 20 and 22 mm and minor diameters between 11 and 14 mm. Apart from the major diameter, whose frequency distribution is skewed to the right, all other distributions are quite close to the normal distribution. Over 70% of the seeds have major diameters between 9 and 11 mm, intermediate diameters between 7 and 9 mm and minor diameters between 4 and 7 mm. The frequency distributions of the three dimensions for seeds are shown in Fig. 2.

Sphericity and roundness : More than 75% of the seeds have roundness between 0.55 and 0.75. With these values, the seeds can roll with relative ease as well as slide on their flat surfaces. This property should help in the design of hoppers and dehulling equipment for the seed.

Weight, volume and density : The weight, volume and density have distributions that are close to those of normal distribution (Figs. 1 and 2). An interesting feature is that more than 85% of the seeds have densities between 1.12 and 1.35 g/cm^3. Also, all seeds have densities higher than 1.0 g/cm^3, which means that the seeds cannot float on water. About 96% of the pods have their densities below 0.95 g/cm^3. This characteristic can be used to separate the seeds from the pods during processing.

Surface area : More than 80% of the seeds have surface area ranging from 14 to 20 cm^2, although surface areas ranged from 1.1 to 2.3 cm^2. The pods have a very wide range of surface area between 43 and 172 cm^2. The distribution of the surface area of the pods is quite even. Any machine to be designed for processing the pods must take this wide range of surface area into consideration.

Static coefficient of friction : The static coefficient of friction for seeds was determined for four structural surfaces (Table 1). The values were highest for plywood with the grain perpendicular to the direction of motion and lowest for glass. The coefficient was higher than values for oilbean seeds (Oje and Ugbor 1991).

Angle of repose : The angle of repose of the seeds was found to be between 30.7 and 44°. This is considerably higher than those reported for oilbean seeds (Oje and Ugbor 1991). This is probably because the surface of oilbean seed is very smooth, making it easy for the seeds to slide on each other, while the surface of locust bean seed is relatively rough.

Hardness : This property is of special importance, as the ultimate objective is to develop a machine for dehulling the seed. Although the average hardness was 25.6 Newtons, the hardness ranged from 17 to 70 Newtons (Table 1). This should give an indication as to the amount of energy needed to crush the seed. This should normally be higher than the highest energy obtained here.

Investigations of various properties of oilbean seed revealed several interesting results, i.e., (a) the frequency distributions of most properties for pods and seeds approach the normal distribution, (b) the principal dimensions and surface area of locust bean pods vary widely. The seeds are more uniform than the pods, (c) locust bean seeds have values of sphericity and roundness that make it possible for them to roll with relative ease as well as to slide on their flat surfaces, (d) the seed is heavier, while the pod is lighter than water. This property can be used in separating the seeds from the pods, (e) hoppers and other unloading devices must be built fairly steeply, because of the high coefficient of friction of the seeds, and (f) the force required to break the seeds varies widely. The problems involved in the processing of pulses has been documented earlier (Kurien and Parpia 1968). This needs to be taken into consideration in the design of dehulling equipment.

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Quality Changes in Irradiated and Nonirradiated Boiled-Dried Anchovies After Inter-Country Transportation and Storage at 25°C

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Samples of nonirradiated and irradiated (5 kGy) dried anchovies (*Engraulis encrasicholus*) were transported from Korea to India for evaluation of microbiological, physico-chemical and organoleptic parameters during storage. The nonirradiated anchovies showed mould growth and an increase in total bacterial count by three log cycles over the initial load, after four months of storage at 25°C. However, 5 kGy irradiated samples packed with a laminated nylon/polyethylene (NY/PE) film exhibited 10^2 bacterial cells per g even after 6 months storage. Hunter's colour value, total volatile basic nitrogen, browning and lipid oxidation showed a good correlation with the organoleptic quality of stored anchovies. Partial changes in irradiated anchovies did not influence organoleptic acceptability. Differences in the levels of total volatile basic nitrogen and total volatile acid values in irradiated and nonirradiated samples may prove useful in distinguishing irradiated Korean anchovies from nonirradiated samples.

Keywords : Anchovies, Total volatile acids, Total volatile basic nitrogen, Total bacterial count, Hunter's colour value, Inter-country transportation, Irradiation preservation.

Annual production of fish and fishery products in Korea is about 4 million metric tonnes, while the demand for domestic consumption and export are on ever increase (Republic of Korea, 1991). Anchovies (*Engraulis encrasicholus*) account for about 150 thousand M.T. and are mainly processed as a boiled-dried product (moisture < 30%), before using in food processing and cooking. Due to the seasonal nature of the catch, long-term storage of dried anchovies is necessary for ensuring a continuous supply. At present, boiled-dried anchovies are preserved at below - 18° C in corrugated cardboard box packaging. A few studies have been done to evaluate the effect of chilling of fish (Balakrishnan Nair and Lahiry 1968), and also the effect of transportation in fibre board containers (Chattopadhyay and Bose 1978). Cost of packaging and storage as well as oxidative and microbial deteriorations during distribution are some of the problems encountered in effective utilization of Korean anchovies. Korean scientists have investigated different factors, such as water-activity (Kwon et al. 1990), non-enzymatic browning (Han et al. 1973), microbial contamination (Chang and Choe 1973), effect of anti-oxidants (Lee et al. 1965), efficacy of packaging methods (Lee et al. 1985), and control of temperature and relative humidities (Jo and Kim 1987), which determine the shelf-stability of dried anchovies. A few studies have been carried out on the preservation of fish by γ-irradiation in India (Banik et al. 1976). Nevertheless, no suitable preservation method has been developed to obtain a product having a shelf-life of six months at 25°C. The present work was carried out to examine the efficacy of irradiation preservation of dried anchovies, with respect to the quality aspects, during storage after transportation to India and back.

Materials and Methods

Samples : Boiled-dried anchovies, procured from a wholesale market in Seoul, were mid-sized (about 53 mm length and 660 mg body weight), and processed by using commercial procedure. Fresh anchovies were boiled on board ship in water containing 12% salt, and then brought to land for sun or hot air-drying. Approximately, 500 g of dried anchovies were packed in a laminated pouch (nylon 15μm/polyethylene 100 μm thickness) before irradiation at 5 kGy in a ^{60}Co-gamma irradiator (dose rate:0.2 kGy/h) at room temperature (26°C). The irradiated samples, together with the nonirradiated samples (total 3 kg each) were sent from Korea to Bombay, by air-mail for quality evaluation, during subsequent storage for 6 months at 25°C in the premises of Bhabha Atomic Research Centre.

Quality evaluation : For microbiological evaluation, a 10% homogenate of anchovies was prepared in 0.9% sterile saline and appropriate serial dilutions

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were placed on plate count agar (Difco) for total bacterial count (TBC). Potato dextrose agar (Difco) was used for mould count by pour plate method. The plates were incubated at 30°C for 48-72 h before counting the colonies as per standard method (APHA 1976). For determination of total volatile basic nitrogen (TVBN), a 10 ml of 10% fish homogenate was treated with an equal volume of 10% trichloroacetic acid, and the slurry was filtered through Whatman No. 1. filter paper, after 15 min contact time. One ml of TCA-filtrate was used to determine TVBN by Conway microdiffusion technique (Farber and Ferro 1956). Total volatile acids (TVA) were determined by treating 30 ml of fish homogenate with 5 ml each of 1 N sulphuric acid and 15% phospho-tungstic acid. After 15 min, the slurry was filtered through Whatman No. 1 filter paper, the filtrate (10 ml) was steam-distilled and 30 ml of the steam-distillate was titrated against 0.01 N sodium hydroxide, using 0.1% alcoholic phenolphthalein solution as indicator. TVA number was expressed as ml of 0.01 N NaOH required to neutralize volatile acids from 100 g fish (Venugopal et al. 1981). Thiobarbituric acid value (TBA) was determined by blending a homogeneous sample of fish (5 g) with 20 ml chilled aqueous 10% solution of TCA containing 0.5 M orthophosphoric acid for 1 min in a Sorval omnimixer. The homogenate was filtered through Whatman No. 1 filter paper and 5 ml of the extract was used to determine TBA value (Witte et al. 1970). Browning pigments were extracted into two fractions : chloroform - methanol (2:1, v/v) soluble fraction was attributed to lipid oxidation, while H_2O : methanol (1:1, v/v) fraction was considered as caused by Maillard type reaction (Chung and Tojomizu 1976). Colour and colour difference meter (ND-1001 DP, Nippon Denshokv, Kogyo, Japan) was used to measure the colour parameters of dried anchovies, e.g., Hunter's whiteness (L), redness (a) and yellowness (b).

Organoleptic evaluations : These were conducted by eight panel members using scoring difference test (Larmond 1970), with emphasis on the overall acceptability of the products in terms of colour, appearance and rancid odour. Sensory scores were : 6, excellent; 5, very good; 4, good; 3, fair; 2, poor; 1, very poor and score 2.5 was assumed to be the acceptable border line for a commodity. Sensory evaluation was also carried out at BARC using a 9-point Hedonic scale (Miyauchi et al. 1964).

Identification of irradiated semi-dried anchovies : The objective of this experiment was to develop a simple detection method to distinguish irradiated from nonirradiated anchovies for inspection purpose. For this, *Aeromonas hydrophila*, *Bacillus megaterium*, *Pseudomonas marinoglutinosa* and *Salmonella typhimurium* were grown in nutrient broth (Difco) overnight. The cultures were suitably diluted (10^5 cells/ml homogenate) and inoculated in 10% homogenates of nonirradiated and irradiated anchovies for overnight incubation at 30°C. Homogenate (1 ml), prepared from anchovies, served, as unirradiated natural microflora. After 18 h incubation, contents of TVA and TVBN were determined (Alur et al. 1991, 1992).

Results and Discussion

Proximate composition : Proximate composition of the sample was as follows : moisture 30.1%, protein 54.6%, fat 4.1% and ash 13.2%.

Microbiological evaluation : Initial microbial population of boiled-dried anchovies to be transported was 3.8×10^5 CFU/g of total bacteria, 2.3×10^5 CFU/g of halophiles, 1.0×10^2 CFU/g of moulds and 1.1×10^4 CFU/g of yeasts. The control samples transported to India showed a TBC of 1.2×10^7 CFU/g, which increased to 4.7×10^8 and 1.0×10^9 CFU/g after 4 and 6 months of storage, respectively. After 4 months of storage, the mould growth was observed in control samples, the count being 1.1×10^3 and 1.5×10^4 CFU/g at 0 h and 6 months storage at ambient temperature (25°C). However, TBC of the sample got reduced to 2.0×10^2 CFU/g in case of the sample with 5 kGy gamma-radiation. The dose, thus, is effective for preventing the microbiological deterioration of packed dried anchovies for 6 months, without any mould growth.

Physico-chemical qualities : TVBN and TBA values in both nonirradiated and irradiated samples were found to increase with storage time, thereby maintaining differences between control and irradiated samples. In contrast, the rancidity index and TBA were found to be higher in irradiated samples than in control during storage (Table 1). This tendency of dried fish has also been reported earlier (Kwon et al. 1990; IAEA 1989). Discolouration, a major factor contributing to quality loss of dried anchovies, is known to result mainly from lipid oxidative and non-enzymatic browning reactions (Han et al. 1973). Both lipophilic and hydrophilic browning were apparent in boiled-dried anchovies. These increased with storage time in nonirradiated and irradiated anchovies (Table 1). No noticeable difference was found in both the types of browning. Hunter's L value (whiteness) showed no significant changes, resulting from irradiation and subsequent

storage (Table 1). However, Hunter's a and b values (redness and yellowness) increased with storage time, and these changes can be correlated to lipid oxidation and browning pigment of the stored samples.

TABLE 1. CHANGES IN QUALITY ATTRIBUTES AND CORRELATION OF ORGANOLEPTIC QUALITIES WITH PHYSICO-CHEMICAL VARIABLES OF BOILED DRIED ANCHOVIES DURING STORAGE

Attribute	Storage period, months								Regression equation[a]	Correlation coefficient(r)
	0		2		4		6			
	A	B	A	B	A	B	A	B		
Total volatile basic nitrogen, mg/100 g	8.4	8.6	16.8	12.6	30.8	16.8	45.5	28.7	Y = 77.850x-12.384	-0.962
Thiobarbituric acid, mg/kg	1.24	1.36	1.85	2.16	2.44	2.67	4.35	6.67	Y = 6.681x-0.994	-0.935
Hydrophilic browning pigment	0.043	0.039	0.081	0.079	0.096	0.095	0.104	0.100	Y = 0.576x-0.058	-0.939
Lipophilic browning pigment	0.214	0.223	0.348	0.360	0.367	0.380	0.388	0.400		
Colour parameters										
Hunter's L	56.5	56.3	53.6	53.9	54.0	54.0	54.4	54.1	Y = 51.635x+ 0.705	-0.691
Hunter's a	0.5	1.0	3.0	2.9	3.1	3.0	3.3	3.3	Y = 6.439x-0.935	-0.895
Hunter's b	9.4	9.5	12.3	13.0	12.8	13.9	13.9	15.0	Y = 18.398x-1.480	-0.980

A = Control; B = Irradiated; Y = variables; x = Organoleptic quality. Samples were packed with laminated film stored at 25°C.

Organoleptic qualities : The sensory scores showed that both the control and irradiated samples were acceptable, even after 4 months of storage. Mould growth was observed in control samples after 6 months storage, thereby making the product unacceptable. However, irradiated samples were devoid of any mould growth, and were consequently organoleptically acceptable (Fig. 1). The data on sensory evaluation, carried out in India, concurred with the Korean studies. Further, no mould growth in irradiated anchovies on prolonged ambient temperature storage at 25°C was detected as against heavy mould infection observed in control samples. Overall organoleptic qualities showed good correlation with some quality indices of the sample, such as Hunter's colour b value (r=-0.980), TVBN (r=-0.962, browning (r=-0.939) and TBA (r=-0.935) (Table 1).

Method to identify irradiated dried anchovies : The TVA values in control samples varied from 230 to 296, depending upon the organisms inoculated, whereas, these values were low and in the range of 40 to 52 in irradiated samples (Table 2). Irradiated anchovies exhibited TVBN values of 100 mg%, irrespective of the spoilage organisms inoculated, whereas, the values ranged from 730 to 770 mg% in unirradiated samples, depending on the type of organisms inoculated. Thus, it is possible to distinguish nonirradiated and irradiated dried anchovies on the basis of differences in TVA and TVBN values. These results corroborate with the earlier reports on identification of irradiated fish and meat by the spoilage profiles of bacteria and the differences in the formation of volatile acids and bases (Alur et al. 1991, 1992).

As in the case of dried file fish (Yoo et al. 1985), the bacterial contamination (10^5-10^7 CFU/g) in dried Korean anchovies was high. This is in contrast to dried Indian fishes viz. Bombay duck (*Harpodon nehereus*), anchovies (*Anchoviella commersoni*), shrimp (*Penaeus indicus*) and Vietnam scad (*Alepes mate*), wherein comparatively low bacterial contamination (<10^4 CFU/g) was observed

TABLE 2. FORMATION OF VOLATILE ACIDS (TVA) AND VOLATILE BASES (TVBN) IN SEMI-DRIED IRRADIATED (5 kGy) AND NONIRRADIATED KOREAN ANCHOVIES STORED FOR 2 MONTHS AT AMBIENT TEMPERATURE (25°C).

Samples inoculated with	TVA number		TVBN (mg%)	
	Control	Irradiated	Control	Irradiated
None	258	52	770	100
A. *hydrophila*	232	48	730	100
B. *megaterium*	244	48	760	100
P. *marinoglutinosa*	250	50	730	100
S. *typhimurium*	296	42	730	100

The values indicated above are the average of three independent experiments.

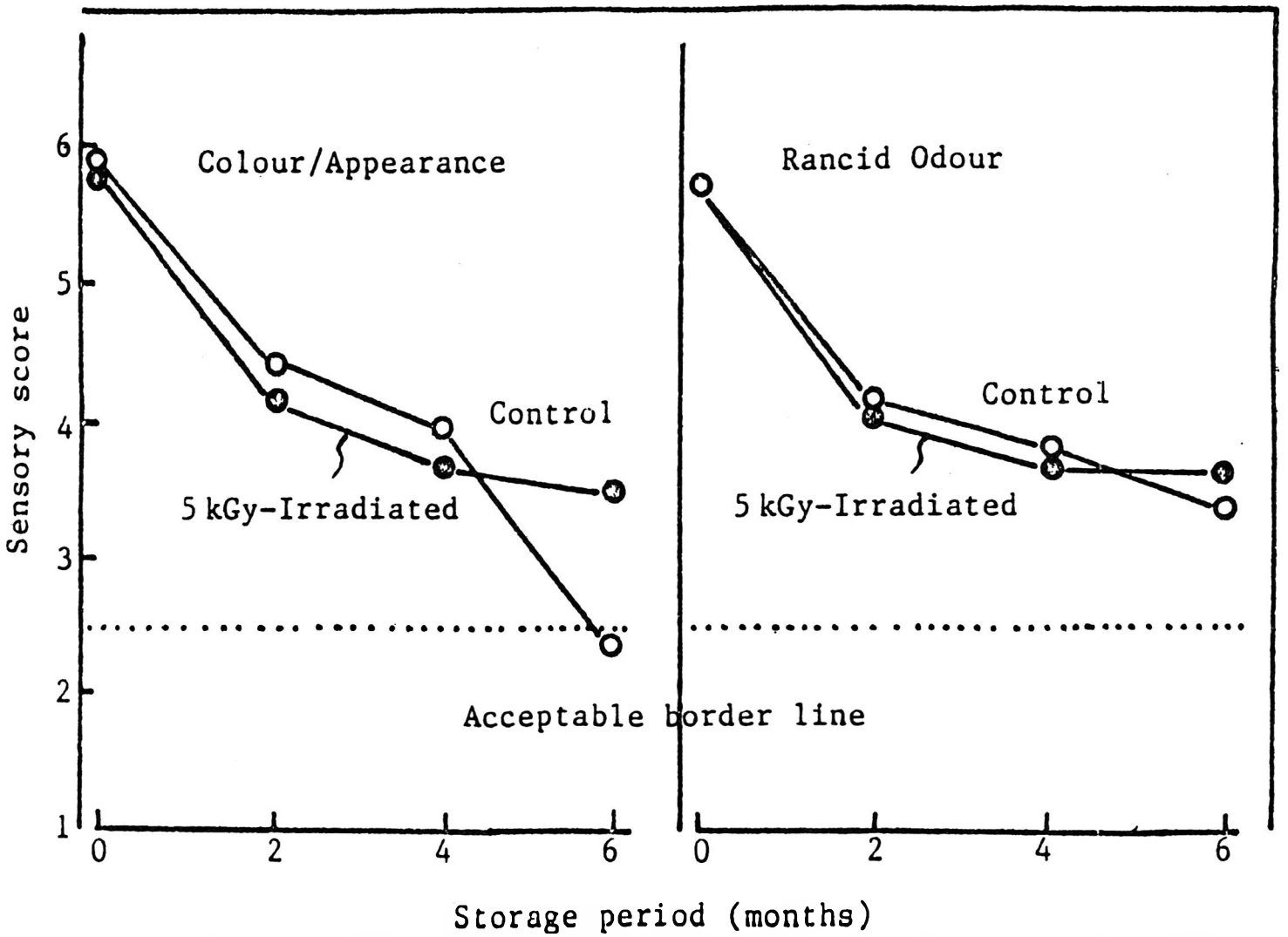

Fig.1. Changes in organoleptic properties on colour/appearance and rancid odour of boiled-dried anchovies during storage following gamma irradiation. Sensory scores were 6, excellent; 5, very good; 4, good; 3, fair; 2, poor; 1, very poor.

(Vinh et al. 1993). Further, Vietnam scad showed no sign of mould growth, even on prolonged storage for 4 months at ambient temperature (Vinh et al. 1993). The differences in bacterial contamination and growth in Indian, Vietnam and Korean dried fishes may be attributed to differences in moisture content. Moisture content of Korean semi-dried fish was 30%, as against that of 20% in Indian and Vietnam dried fish (Solanki and Sankar 1988). Since Korean semi-dried anchovies harboured high rate of bacterial contamination, probably due to higher moisture content and handling practices, it is imperative to employ gamma irradiation to reduce bacterial contamination substantially and achieve appreciable extension in shelf-life at room temperature (25°C). The efficacy of gamma irradiation in controlling mould growth in semi-dried anchovies is also evident. Thus, the irradiation would control the microbial growth of boiled-dried anchovies during storage, after transportation from Korea to India and back.

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Effect of Food Additives on Quality of Salted Pink Perch (*Nemipterus japonicus*)

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Wet salted pink perch, *Nemipterus japonicus* (Bloch), prepared using a curing mixture containing common salt, three preservatives (sodium benzoate, potassium sorbate and sodium dihydrogen phosphate), and an antioxidant (butylated hydroxy anisole), was found to have better keeping quality and longer shelf-life over those prepared using common salt alone. Thiobarbituric acid number (TBA number), free fatty acids (FFA), total volatile base nitrogen (TVBN), alpha-amino nitrogen (AAN) and total plate count (TPC) increased during storage, thereby resulting in decreased sensory scores for overall acceptability. Food additives were found to effectively enhance the quality, and extend the shelf-life of salted fish considerably. Further, the effect of the additives was observed to be slightly more pronounced at ambient temperature (26.8±3.3°C) than at cooler storage temperature (2.5±1°C).

Keywords : Pink perch, Wet salting, Curing mixture, Food additives, Storage, Shelf-life, Chemical changes, Total plate count, Sensory scores.

Besides drying, long-term preservation of fish by salting may be regarded as one of the practical and economic possibilities, for fishermen living in remote areas. Several studies have been conducted on the standardisation of salting procedure, in order to improve the quality and general acceptability as well as to minimise wastage and losses in production. However, in tropical countries like India, where sun-drying of salted fish is practised, the quality remains inferior due to absorption of moisture; attack by bacteria, mould, and insects; and development of rancidity and fibrous texture in the finished product. Some studies have also been carried out on the effect of storage on protein and related changes (Reddy and Srikar 1991). There are reports on dry salting of fish and its shelf-life, but very little or no information is available on the effect of wet salting of fish, in general, and for pink perch, in particular. Compared to dry-salting, the wet-salting affords better texture to the salted fish, as also typical flavour due to immersion of fish in brine during storage (Filsinger 1987). It is also known that Ca^{++} and Mg^{++} salts decrease the equilibrium moisture contents (Iyengar and Sen 1970). The present investigation was carried out to evaluate the effect of curing mixture (containing preservatives that are active against bacteria, mould, and also prevent oxidation of fatty acids) on wet-salted pink perch, which is one of the main shrimps-by-catch of Indian coastal waters, thereby contributing approximately to 3.5% of India's total marine fish landing (FAO 1988).

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Materials and Methods

Fresh pink perch, *Nemipterus japonicus* (Bloch), caught from Mangalore waters in May 1990 was transported in iced condition and kept in the anteroom of cold store (2.5°C±1°C), until further processing. Fishes were washed, eviscerated, dressed in butterfly style, cleaned thoroughly, and 4 lots of 8 kg each were stacked in 4 plastic troughs. Two lots were wet-salted by pouring saturated brine into the troughs, till it covered the stacks completely. For the remaining 2 lots, the saturated brine containing common salt and food additives was added. Curing mixture consisted of the following additives in proportion by weight in grams: common salt 100, sodium benzoate 0.25, sodium acid phosphate 1.50, potassium sorbate 0.50 and butylated hydroxy anisole 0.20 (Sen and Sripathy 1967). The troughs were covered tightly with polythene sheets to prevent exposure to atmosphere and insect attack. Weights were kept on the top to prevent floating of fish. One lot from each of the above treatments was stored at ambient temperature (26.8± 3.3°C), and the remaining two lots at cooler temperature (2.5±1°C).

Samples of fish drawn at definite intervals were analysed for moisture (AOAC 1975), salt content (AOAC 1975), water activity (a_w) (Doe et al. 1983), thiobarbituric acid number (TBA number) (Tarladgis et al. 1960), free fatty acids (FFA) (Takagi et al. 1984), total volatile base nitrogen (TVBN) (Beatty and Gibbons 1937), alpha-amino nitrogen (AAN) (Pope and Stevens 1939) and total plate count (TPC) (Speck 1976). For sensory evaluation, fish was immersed in tap water for 2 h to remove excess

salt, and cooked in boiling water (100°C) for 15 min. The products were presented separately and evaluated for overall acceptability by a panel of 8-10 experienced panelists, using a 5 point Hedonic scale (Prell 1976). Mean scores obtained were analysed for significant differences using analysis of variance technique (Snedecor and Cochran 1962). The shelf-life of the products, was assessed from the regression line of the mean overall acceptability scores on storage period (days), assuming the Hedonic score '2' to be the limit, below which the product was not acceptable.

Results and Discussion

In all the products, the moisture content of tissue decreased drastically after salting with simultaneous increase in salt content (Fig. 1a, b). It is evident that moisture of the fish decreased significantly during the first 7 days of immersion in brine with a significant increase in salt content during the same period. This is mainly due to osmosis followed by a collapse of the cell organisation as a result of protein denaturation and reabsorption of brine, which usually takes 3-4 days (Robert 1986). During subsequent storage, the decrease in

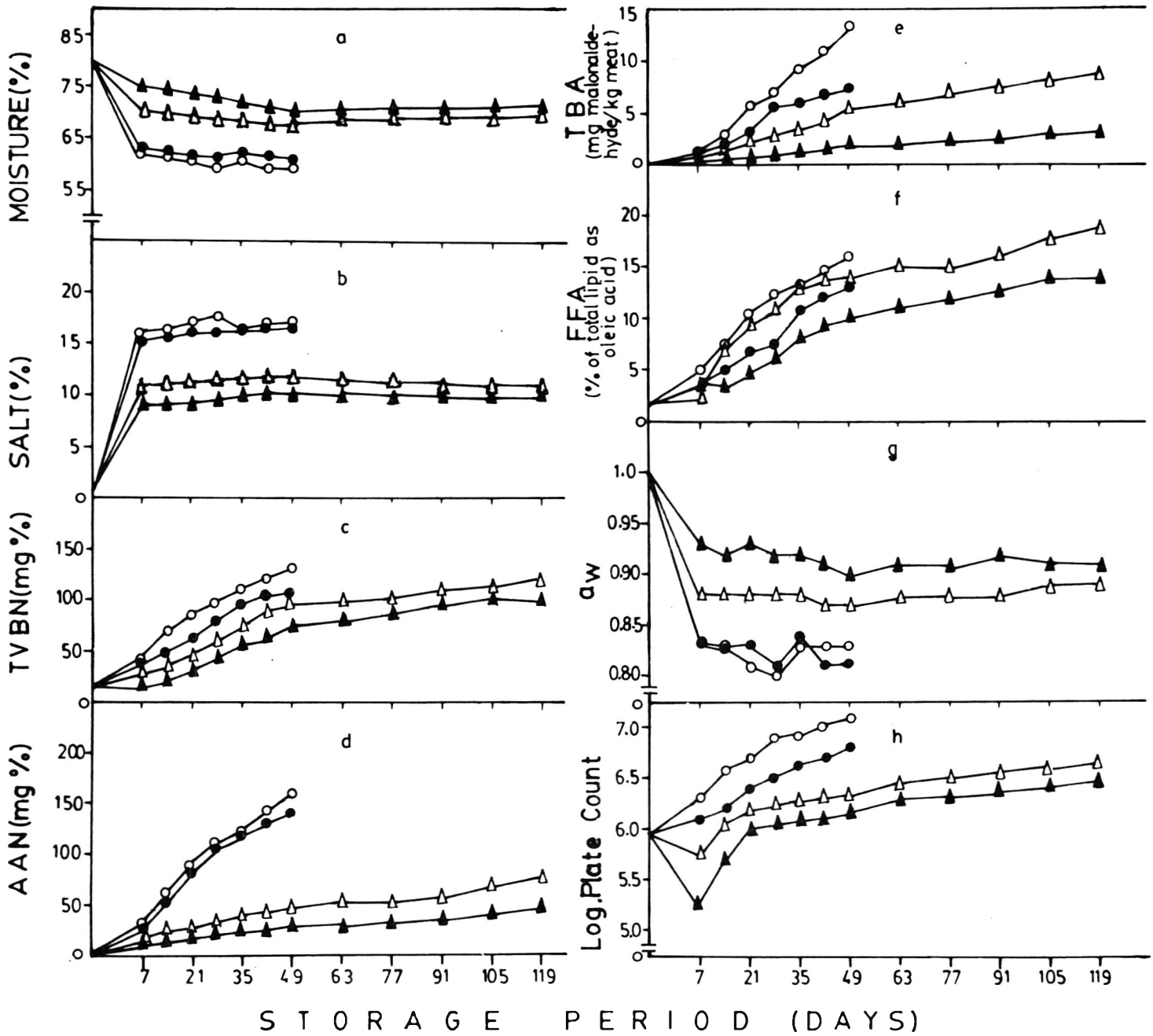

Fig. 1. Changes in moisture, salt content, water activity (a_w), thiobarbituric acid number (TBA), free fatty acids (FFA), total volatile base nitrogen (TVBN), alpha-amino nitrogen (AAN) and *Log* total plate counts (TPC) of salted pink perch.
●——● with additive at ambient temperature, ○——○ without additive at ambient temperature, ▲——▲ with additive at cooler temperature, Δ——Δ without additive at cooler temperature.

moisture was gradual, due to decreasing rate of mass transfer in both directions. Towards the end of storage at 2.5±1°C, a slight increase in moisture content was observed. Such increase in moisture content of salted fish during storage has been observed, and interpreted based on absorption of water by fish tissue (Wheaton and Lawson 1985), which takes place at the expense of complex compounds, formed by the salt and proteins (Akiba 1955). The higher moisture and lower salt contents, observed in products salted with additives, may be attributed to the insoluble BHA, which forms a complex coating on the fish surface and prevents mass transfer (Wheaton and Lawson 1985). Cooler temperature was found to retard the rate of mass transfer, which was reflected by the much higher moisture and lower salt contents in products stored at cooler temperature, as compared to those at ambient temperature. Such influence of temperature on salting rate corroborates with the findings on salting of jewfish (Narayanaswamy et al. 1980). Further, higher moisture contents in the products salted with additives can be attributed to the presence of phosphate, which absorbs the water by interacting with proteins.

Water activity (a_w) values decreased drastically in all the products, simultaneously with the influx of salt into the tissue and efflux of moisture (Fig. 1 g). The minimum a_w attained in the present study was 0.80. Theoretically, the minimum a_w attainable by salting alone is around 0.75, as sodium chloride molarity reaches saturation around 6.2 (Lupin et al. 1981). All the products of the present study come under the category of intermediate moisture foods, IMF (a_w=0.90-0.60), except the one salted with additives stored at cooler temperature, as it can be considered as a high moisture food, HMF (a_w=1.00-0.90). Results of the present study indicate that sodium benzoate and sodium acid phosphate (active against bacteria) and potassium sorbate (active against moulds) have been effective in enhancing the quality and extending the shelf-life of salted products despite their higher a_w.

In all the products, TBA number, FFA, TVBN, AAN and TPC increased during storage. The lower TBA number, observed in the products salted with curing mixture (Fig. 1 e), may be attributed to the anti-oxidant nature of BHA in the curing mixture. Lower FFA values, observed in the products prepared by employing the curing mixture, may be attributed to the effect of preservation in retarding the activity of microbes including the lipolytic bacteria (Fig. 1 f). TVBN, produced by microbial degradation of nitrogenous tissue components, was found to increase steadily in all the products (Fig. 1 c) and this corroborates with earlier findings on dry-, wet- and mixed-salted fish (Adebona 1978). However, the lower TVBN values in products with additives might have resulted from the effect of the preservatives in retarding microbial activity. Steady increases in AAN values were observed in all the products during storage (Fig. 1 d). Similar trend has been reported in salt preservation of lean and fatty herring (Bochkov and Safronova 1952) and ripening of barrel-salted anchovy (Durand 1981), thereby indicating the proteolysis of tissue proteins by tissue proteases and proteolytic bacteria. Retardation of bacterial activity by the preservatives used in the curing mixture might have resulted in the lower AAN, which was observed in the products salted with the curing mixture.

Throughout the period of storage, TPC showed an increasing trend (Fig. 1 h) in all the products, and this corroborates with earlier observations (Aschelong 1952). The lower TPC observed in products with additives is due to the action of the added preservatives on the microbes. Sodium benzoate and potassium sorbate have been reported to retard microbial activity by inhibiting various enzymes of the microbial cell, specifically those in the citric acid cycle (Lueck 1980).

The sensory scores for overall acceptability and the shelf-life of the products are shown in Fig 2.

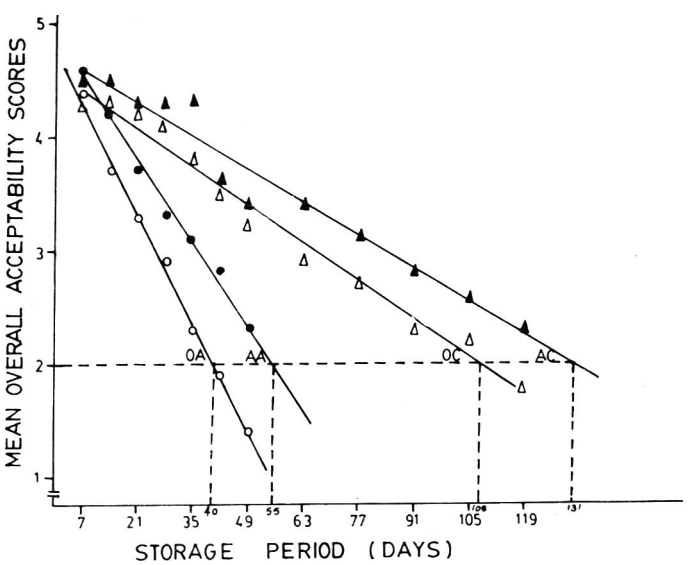

Fig. 2. Overall acceptability scores of salted pink perch during storage.

●——● with additive at ambient temperature, O——O without additive at ambient temperature, ▲——▲ with additive at cooler temperature, Δ——Δ without additive at cooler temperature

AA : Y = 4.90-0.0526X (r = - 0.9941), Shelf-life = 55 days
OA : Y = 4.79-0.0694X, (r = - 0.9976), Shelf-life = 40 days
AC : Y = 4.72-0.0208X, (r = - 0.9782), Shelf-life = 131 days
OC : Y = 4.57-0.0239X, (r = - 0.9895), Shelf-life = 108 days

Hedonic scale - Excellent : 5, Good : 4, Fair : 3. Acceptable : 2, Unacceptable : 1.

Analysis of variance of the mean overall acceptability scores indicated that there was a significant difference ($P<0.05$) due to the use of food additives. The better quality of salted pink perch with additives, than those salted without additives, was depicted by its higher scores, throughout the storage period. Apart from enhancing the quality, use of food additives was found to extend the shelf-life of products stored at ambient temperature by 1.4-fold and that of products stored at cooler temperature by 1.2-fold. This implies that the effect of food additives in extending shelf-life of salted pink perch is slightly more at ambient temperature than at cooler temperature.

The data clearly indicate that the addition of the preservatives to salt enhances the quality, and extends the shelf-life of pink perch.

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Effect of Xanthan Gum on the Quality of Bread

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Incorporation of different concentrations of the xanthan gum, an exocellular polysaccharide produced by obligately aerobic *Xanthomonas campestris* in wheat flour, has been studied to investigate its effect on the rheological and bread making quality of the wheat flour. Addition of 0.3% xanthan gum in wheat flour improved the water absorption from 59.8 to 61.4%, dough stability from 6.0 to 10.0 min, dough strength from 136.5 to 150.0 cm^2, and the overall bread making quality with special reference to crumb softness. In addition, the yield of bread increased by 3.5%. Though the xanthan gum has been approved by FDA for use in foods, its use in bread is not currently permitted in India. Considering its ability to significantly improve the bread quality and the yield, a need exists to clear it for use in bread manufacture.

Keywords : Xanthan gum, Incorporation in bread, Rheological effect on dough, Improvements in bread quality, Bread yield, Improved crumb softness.

Bread, a food product consumed by majority of population all over the world as part of daily diet, is becoming increasingly popular in India, due to various advantages, such as ready-to-eat convenience, cost competitiveness, better nutritive quality and enhanced shelf-life. Since the quality of bread depends on a number of factors, constant efforts are being made in the country to improve its quality. Incorporation of specific additives to the wheat flour has been found to have beneficial effects on improving the quality of the bread (Tsen and Hylnka 1967; Kulp 1981; Chamberlain 1981; Stauffer 1990; Knightly 1988; Schuster and Adams 1984). Besides, there are varieties of bread such as high protein bread (Sahni and Krishnamurthy 1975), soya fortified bread (Selvaraj and Shurpalekar, 1982) and the quality was improved by guar gum also (Venkateswara Rao et al. 1985). One such important additive is the gums from various sources (Anderson and Andon 1988; Klose and Glicksman 1972; Bayfield 1958). It is worth mentioning that the utilisation of gums, polysaccharides or hydrocolloids in food products has been traced to the dawn of history. The quality and quantity of the traditional gums are largely prone to various environmental factors (Lawson and Sutherland 1978). Moreover, the use of gums is highly specific, because of their predetermined functional characters (Margaritis and Pace 1985). Xanthan gum, a high molecular weight polysaccharide is produced by the plant pathogen *Xanthomonas campestris* (Pettitt 1982). The xanthan gum is composed of D-glucuronic acid, D-mannose and D-glucose units (Rocks 1971). Xanthan gum possesses an extraordinary combination of properties, which have resulted in its wide application to food systems, such as bakery fillings and icings (Glicksman 1982), cakes (Miller and Setser 1983), high protein breads (Christianson 1976) syrups, toppings, desserts and dairy products (Teague et al. 1982). Xanthan gum was used in cakes, cake mixes and pie fillings (Anderson and Andon 1988). In sponge mixes, xanthan gum showed good freeze/thaw stability, when eggs are replaced partially with xanthan gum (Hille 1982). Kulp et al (1974) reported the application of xanthan gum in conjunction with emulsifiers in the preparation of glutenless bread, meant specially for persons with celiac disease. Jongh (1961) reported that dough containing only starch and water forms a stable suspension in which repulsion forces exist between the starch granules, causing the suspension to demonstrate the rheological property of dilatency. Such a system lacked in structural coherency necessary to retain the gas, generated by yeast fermentation. Xanthan gum acted as binding agent, and helped in gas retention. The employment of xanthan gum is approved by FDA for use as a stabiliser, emulsifier, thickener, suspender, bodying agent or foam enhancer in foods (Glicksman 1969). However, xanthan gum is not permitted, at present, in India for use in bread making under the provisions of Prevention of Food Adulteration Act. Considering the widespread use of xanthan gum in foods in other countries and attractive beneficial uses, it is vitally important to move the matter for allowing its use in foods in India (Prasad et al. 1991). One of the best ways to achieve this aim

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is to demonstrate the advantages in Indian context. Exploratory work was, therefore, undertaken. This paper discusses the effect of incorporation of xanthan gum on the rheological characteristics and the bread making quality of wheat flour.

Materials and Methods

Materials : Commercial wheat flour (*Maida*) was obtained from the local market. *Xanthomonas campestris* is a natural isolate, grown on optimised medium containing carbohydrates, nitrogen and minerals for 72 h in a fermenter. The fermentation broth was processed suitably to obtain a cell-free product (Prasad et al. 1989). The food grade xanthan gum, a cream coloured powder, obtained as above, was used for the studies.

Chemical characteristics : Moisture, total ash, dry gluten, sedimentation value, falling number, diastatic activity and damaged starch content were determined according to AACC (1969) procedure.

Rheological characteristics : The rheological characteristics of the flour as influenced by the addition of xanthan gum at 0.1, 0.2, 0.3, 0.4 and 0.5% levels were studied using farinograph, amylograph and extensograph. according to the standard procedure (AACC 1969; Venkateswara Rao and Haridas Rao 1993).

Preparation and evaluation of bread : Prior to use, the xanthan gum was dispersed uniformly in water and kept aside for 60 min to accomplish hydration. The xanthan gum in the form of dispersion was added to the flour. Breads were prepared after adding 0.1, 0.2, 0.3, 0.4 and 0.5% xanthan gum, according to remix procedure with a reduced fermentation time of 120 min for the dough, instead of 165 min. Evaluation of breads was carried out for crust and crumb characteristics after 24 h of preparation by a panel of six judges (Irvine and McMullan 1960). Loaf volume was measured using rapeseed displacement method of Malloch and Cook (1930).

Results and Discussion

Chemical characteristics : Chemical characteristics of the flour indicated the medium strong characteristics. It contained 9.0% protein, 8.8% gluten and sedimentation value of 20 ml. The flour had low alpha-amylase activity, as shown by falling number (594) and diastatic activity (161.3 mg maltose/10 g flour).

Farinograph characteristics : The data showed a gradual increase in farinograph water absorption with the increase in the level of the xanthan gum (Table 1). Christianson (1976) studied the effect of

TABLE 1. EFFECT OF XANTHAN GUM ON THE DOUGH CHARACTERISTICS OF WHEAT FLOUR

Dough characteristics	Xanthan, %					
	0	0.1	0.2	0.3	0.4	0.5
Farinograph						
Water absorption, %	59.8	60.2	60.8	61.4	62.8	65.4
Dough development time, min	2.0	2.0	2.0	2.0	1.0	1.5
Dough stability, min	6.0	8.5	8.5	10.0	1.0	1.5
Departure time, min	7.5	10.0	11.0	9.5	2.0	2.5
Mixing tolerance index at 10 min, BU	80	60	70	80	100	140
Valorimeter value	46	50	50	56	48	42
Amylograph						
Gelatinisation temperature, °C	66.5	61.5	61.5	61.5	61.5	61.5
Pasting peak, °C	91.5	91.5	91.5	91.0	91.5	91.5
Peak viscosity, BU	1070	1120	1130	1150	1160	1160
Viscosity at 95°C, BU	760	800	840	920	860	780
Viscosity after 30 min cooking at 95°C, BU	610	680	710	700	730	740
Viscosity at 50°C, BU	1050	1130	1190	1170	1220	1220
Setback on cooling, BU	440	450	480	470	490	480
Extensograph						
Resistance to extension, R, BU	570	640	650	645	740	525
Extensibility, E, mm	162.5	161.5	149.5	150.0	146.0	158.5
R/E	3.51	3.93	4.34	4.29	5.11	3.30
Area, Cm^2	136.5	139.7	145.9	150.0	129.9	124.0

xanthan gum and gluten on the rheological properties of wheat flour dough and reported increase in water absorption and dough stability with addition of xanthan gum. The useful properties of gums are largely due to the physical effects, and primarily involves those dealing with their interaction with water. Gum interacts with protein molecules to provide suspension and solution stability, while it provides viscous and emulsification effects due to lipid molecules. By such interactions, gums perform their useful functions, which are mainly those relating to provision of the viscosity, solution stability and suspendability (Glicksman 1982). The dough development time remained unaffected up to 0.3% addition of xanthan gum and a decrease by 0.5 to 1.5 min was observed beyond this value. The stability of the dough showed an increase from 6.0 to 10.0 min with the use of 0.3% xanthan gum. However, a marked decrease to 1.0 and 1.5 min, respectively, was observed when the xanthan gum was used at 0.4 and 0.5% levels. Similarly, increase in mixing tolerance index was observed, when the xanthan gum level was increased beyond 0.3% in flour, thereby indicating adverse effect with respect to the weakening of the dough. There was a marginal increase in valorimeter value up to 0.3% level of the xanthan gum, which shows improvement in dough strength.

Amylograph characteristics : It is evident from Table 1 that there is no variation in the gelatinisation temperature with addition of different levels of the xanthan gum. The peak viscosity increased by 80-90 BU with 0.3-0.5% levels of the gum. Christianson et al. (1981) have also reported the increase in peak viscosity of wheat starch with the addition of the xanthan gum. Viscosity after 30 min cooking at 95°C showed an increase by 70 to 130 BU, as the xanthan gum level increased by 0.1 to 0.5%. Similarly, the viscosity at 50°C on cooling showed an increase by 80 to 170 BU, with the addition of 0.1 to 0.5% of xanthan gum. There was a marginal effect on set back value by 10-50 BU, which is related to retrogradation of starch.

Extensograph characteristics : It can be observed from Table 1 that the resistance to extension increased from 570 to 740 BU, with the use of 0.4% xanthan gum. In contrast, the resistance to extension decreased to 525 BU, with the use of 0.5% xanthan gum. Similarly, the extensibility decreased from 162.5 to 146 mm g with the incorporation of xanthan gum, in contrary to an increase of 158.5 mm, when xanthan gum was used at 0.5% level. The area increased from 136.5 to 150 cm^2 with 0.3%, thereby indicating increased strength, but it decreased with further increase in the concentration of xanthan gum.

Bread making characteristics : The results of bread making quality under different experimental conditions are presented in Table 2. The loaf volume of breads was not affected up to 0.4% addition of the xanthan gum. A gradual increase in the yield of the bread was noted. The increase in the yield of the bread ranged from 0.7-4.5% with use of 0.1-0.5% xanthan gum. The crust shape was normal, and the crust colour was golden brown for the breads emanating from all the experimental parameters. The crumb was creamish white with medium fine uniform grain in all the cases. The texture of the breads improved from soft to very soft with 0.3-0.5% addition of the xanthan gum.

TABLE 2. INFLUENCE OF XANTHAN GUM ON BREAD MAKING QUALITY* OF WHEAT FLOUR

Xanthan gum, %	Loaf				Crumb		Eating quality
	Weight, g	Volume ml	Specific volume, ml/g	Extra yield, %	Texture	Score**	
-	134.9	470	3.50^a	-	Soft	7	Normal
0.1	135.8	475	3.50^a	0.7^a	Soft	7	Normal
0.2	136.2	475	3.49^a	0.9^a	Soft	7	Normal
0.3	139.6	475	3.40^a	3.5^b	Very soft	7	Normal
0.4	140.5	465	3.31^b	4.2^c	Very soft	7	Slightly gummy
0.5	141.0	440	3.12^c	4.5^c	Very soft	7	Slightly gummy
SEM			±0.02	±0.029			

Means in the same column followed by different superscripts differ significantly ($p<0.05$)

* All the breads had normal crust shape, golden brown crust colour, creamish white crumb colour and uniform medium fine crumb grain

** Maximum score : 8

The stability of the xanthan gum to high temperatures appears to increase the retention of the moisture in the bread. The advantages of the increase in retained moisture coupled with the capability of the xanthan gum to complex with starch are of importance, and may inhibit retrogradation and consequently, lead to extension of the shelf-life of the baked goods (Pettitt 1982). The taste of the breads prepared by adding 0.1, 0.2 and 0.3% xanthan gum was normal. However, breads with xanthan gum at levels above 0.4% had slightly gummy mouthfeel.

The results highlight the increase in the yield of the bread and improvement in softness of crumb by the incorporation of xanthan gum.

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Studies on the Storage Characteristics of *Kodbale* - A Popular Indian Spicy Savoury

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Moisture sorption isotherm of *Kodbale*, a popular Indian spicy savoury was found to be sigmoidal in shape; typical of a starchy food. The equilibrium moisture content at 56% RH was found to be 10.01% (dry basis), which was critical from the point of view of crispness and acceptability. The Brunnauer-Emmett-Teller (BET) and Guggenheim-Anderson-de Boer (GAB) - monolayer moisture content was 4.60%. The product packed in polyamide, based coextruded film and metallized polyester-polyethylene laminate with ambient air and nitrogen gas flushing indicated shelf-life of 60 to 80 days, when stored at 38°C and 90% RH. At 27°C and 65% RH storage condition, the shelf-life was about 120 days. Gas flushing and polyamide based material gave added protection against deteriorative changes.

Keywords : Savoury *kodbale*; Moisture sorption; Gas packing; Storage behaviour.

Kodbale, a spicy savoury traditional food item, is popular throughout India, especially Southern States. It is a doughnut-shaped product made from rice flour, cumin, chilli, salt and asafoetida. Wheat flour (*atta*), in little quantity, is generally mixed with rice flour. The mixture is kneaded with water and deep-fat-fried. *Kodbale* is relished for its crisp texture and flavour. The product is generally prepared for home consumption, though commercial preparation by both home-scale and small-scale industries has become popular in recent years. Extension of the shelf-life by suitable packaging would help in the storage and distribution of the commercial product, over wider areas, as well as prevention of deteriorative changes. The results of such a study are reported in the present paper.

Deteriorative changes that occur in the product are loss of crispness, and development of hydrolytic rancidity, due to absorption of water and oxidative rancidity, due to interaction with oxygen, particularly when made using groundnut oil (Shanthi Narashimhan et al. 1986). It is also prone to tainting from external sources. It is well known that inert gas packaging and the use of water vapour and oxygen barrier packages would extend shelf-life of food products, such as chips (Satyavathi Krishnankutty et al. 1981). The studies were confined to the evaluation of the physico-chemical properties of two newly available composite packaging materials and their protective properties for storing the product under two storage conditions, to provide good keeping quality for 3 to 6 months.

Some studies on the storage aspects of other savoury foods, using various conventional packaging means, have been carried out by Bhat et al. (1982); Kumar and Sreenivasa Rao (1987); and Mahadeviah et al. (1979). They have indicated that suitable packaging means extend the shelf-life of the products. In the present study, two barrier materials were selected, (i) co-extruded film comprising outer high-density polyethylene, middle polyamide (poly caprolactam or nylon-6) and inner ethylene-acrylic acid copolymer (EAA) webs, and (ii) laminate of outer polyethylene terephthalate (PET or polyester) with polyethylene web. Inner EAA or PE would provide good water-vapour barrier property, sealability and impact strength, while the polyamide or polyester films would provide other requisite functions like oxygen and aroma barrier property (Kumar 1989).

Materials and Methods

Market type *Kodbale*, without any added chemical preservative or anti-oxidant, was obtained from the local market. The outer diameter of the samples varied between 2.5 and 3.0 cm and that of the central void between 1.5 and 2.0 cm. The weight of each piece ranged between 1.7 and 2.0 g.

Moisture sorption studies : Moisture sorption of *Kodbale* was studied at 27±1°C by exposing weighed quantities of the samples in petri dishes to relative humidities (RH) ranging between 11% and 90%, built in different desiccators, using saturated salt solutions (Rockland 1960). The samples were weighed periodically, till they attained constant weight (±0.001 g) or showed microbial growth. The samples, after equilibration, were analysed for sensory qualities.

Physico-chemical properties of packaging materials: The thickness, tensile strength and heat-seal

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strength were determined according to ASTM methods (1982). The water vapour transmission rate (WVTR) of the packaging materials and the oxygen transmission rates were determined, according ASTM procedure (1975, 1982).

Chemical analysis : The product was characterized, according to standard BIS procedures for moisture and fat. Free-fatty acid (FFA) content and peroxide value (PV) were determined, according to procedures of AACC (1976).

Storage studies : Unit pouches, filled with 100 g of sample were heat-sealed and exposed to two sets of storage conditions, (1) 90% RH and 38°C (accelerated condition) and (2) 65% RH and 27°C or normal or Bureau of Indian Standards (BIS) condition. The packaging materials used for storage study were (i) 100 micron coextruded yellow pigmented film of high-density polyethylene/bonding agent/polyamide/bonding agent/ethylene acrylic acid copolymer (Primacor, Dow chemicals) and (ii) laminate of metallized polyester/low-density polyethylene. Pouches were tested with ambient air and inert gas flushing with nitrogen (Nirovac-X-300 of Komet-Maschinen fabrik, Germany). The control samples packed in tin-plate containers were stored in refrigerated condition (4-6°C).

Sensory evaluation : Unit packages, stored under different conditions were periodically withdrawn from the two sets, and evaluated by a panel of judges (14 to 16) for colour, odour, taste, texture and overall acceptability. A five point sensory scale (5-like extremely, 4-like very good, 3-like moderately, 2-dislike slightly and 1-dislike extremely) was adopted with a score 3, as the limit for acceptance (Kumar 1987).

Statistical analysis : Of the three chemical parameters, moisture content, PV and FFA of the product, which change with time, were studied. For the moisture content, rate equation is well established by Paine (1955) and was fitted to Paine's equation. In the absence of any particular rate equations for PV and FFA (which are interdependent on many factors), they were fitted to polynomial regression equations upto third degree.

Results and Discussion

Sorption studies : Fig 1 shows the relative humidity moisture content relationship or moisture sorption (combination of adsorption and desorption) isotherm of *Kodbale* at 27°C. The Guggenheim-Anderson-de Boer (GAB) model, which generally fits upto a a_w of 0.92-0.94, when applied to the moisture sorption

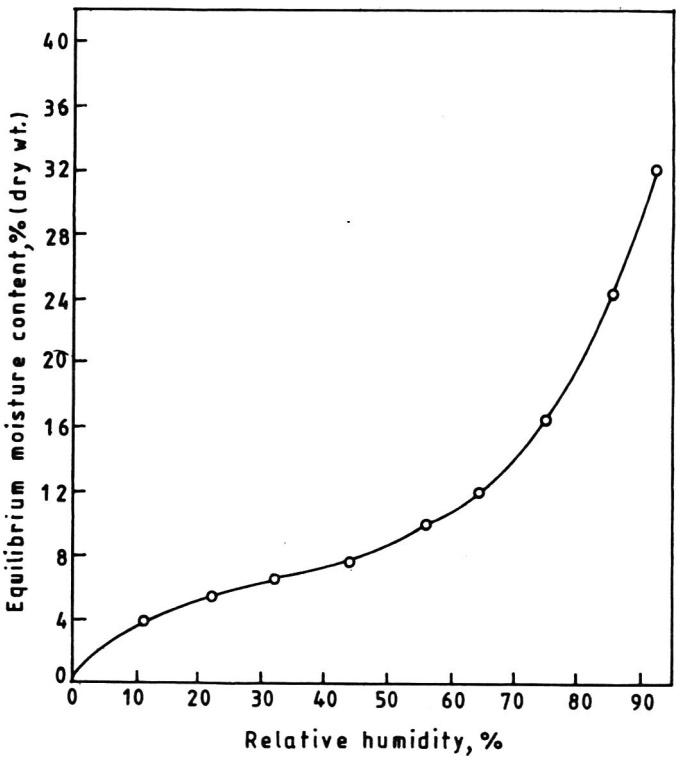

Fig. 1. Moisture sorption isotherm of *Kodbale* at 27°C.

data of the product, had a correlation co-efficient of 0.99 and a standard error of 0.0018.

The monomolecular moisture content as determined by the GAB-Plot (Schar and Ruegg 1985) was found to be 4.5%, which is almost the same as the Brunnauer-Emmett-Teller (BET) monolayer moisture content. With an initial moisture content of 5.8% (as is basis), corresponding to 6.2% on dry basis (db), the product was in equilibrium with RH of 30%. The ether-extractable (fat) content of the product was 9.0%.

The product, equilibrated to 11% RH, had an equilibrium moisture content (EMC) of 4.3% (db), and was found to be very hard and not relished. The products, equilibrated to RHs of 22, 32 and 44%, were crisp, and had retained desirable brown colour and good texture. However, the product at 56% RH with an EMC of 9.5% (wet basis) or 10.01% (db) was judged as just acceptable. *Kodbale*, equilibrated to 64 and 75% RH, had EMC values of 11.97 and 16.47% (db), respectively and had become soft and soggy, while at higher RH of 86 and 90%, the product developed microbial growth after 29 and 21 days of exposure, respectively. Thus, from the moisture sorption data obtained and sensory evaluation, it was inferred that moisture content between 5.02 and 9.50% (wet basis) or 5.33 and 10.01% (db) was acceptable. As such, it was considered that during storage, the product should retain moisture within these limits.

TABLE 1. PHYSICO-CHEMICAL PROPERTIES OF PACKAGING MATERIALS

Property	PNP co-extruded	MPP laminate
Thickness, μm	125	12/50
Tensile strength, KN/m,		
Machine direction	2.60	2.11
Transverse direction	1.89	1.72
Heat-seal strength, KN/m		
Machine direction	2.08	1.69
Transverse direction	1.50	1.38
Water vapour transmission rate, g/m². 24 hr.		
38°C/90% RH condition	3.02	4.13
27°C/65% RH condition	0.92	1.06
Oxygen transmission rate ml/m²/24 hr/atm at 27°C	45.4	55.4
Yield, m²/kg	8.2	17.8
Pouch dimension, cm.		
Length	14.8	17.8
Width	13.5	13.0

PNP : Polyethylene/Polyamide/Primacor Co-extruded film;
MPP : Metallized polyester/polyethylene laminate.

The BET-plot (not cited) as determined by the method of Iglesias and Chirife (1976) was 4.60% (db) at 27°C, below which the product would be susceptible to rapid oxidative deterioration.

Physico-chemical properties of packaging materials: From Table 1, it could be seen that the co-extruded film (PNP) with polyamide and laminate (MPP) with polyester webs had high tensile strength of 2.60 and 2.11 KN/m in the machine direction and 1.89 and 1.72 in the transverse direction. The WVTR values for PNP film were 3.02 and 0.92 g/m²/day at 38°C, 90% RH and 27°C, 65% RH conditions, respectively. The corresponding WVTR values for MPP laminate were 4.13 and 1.06 at the two conditions. These high values could be due to lesser metallization thickness, as the normal range for WVTR at 38°C/90% RH condition, as reported by Kumar (1989) was 0.5 to 1.5 g/m²/day. The oxygen transmission rates of the two materials were found to be 45.4 and 55.4 ml/m²/day/atm at 27°C and 65% RH. The high value for MPP laminate could be ascribed to the reason cited.

Storage studies

Accelerated condition : The changes in the MC, FFA and PV of the product are given in Table 2. It could be inferred that the co-extruded film PNP afforded better protection than the MPP laminate. The data analysis on moisture pickup of the product fits to the equation; log (Me-M) = Mt+log (Me-Mo) with a correlation coefficient of more than 0.99 and a low standard error estimate. The slope (M-value) was -2.2620×10^{-3} and -5.1328×10^{-3} for PNP pouches and MPP, respectively.

The time corresponding to log (Me-Mc) was found to be 59 and 80 days. The differences in the moisture contents between the air and gas flushed samples were not much (Table 2). In the case of MPP pouches, the initial moisture content of 5.90% increased gradually, reaching the critical value of 9.40% (as is basis) or 10.0% (db) at the end of 56 days, while in the PNP pouches, the moisture content was only 8.50% in the air-packed ones and almost similar values in the gas-flushed pouches also. The critical moisture content (CMC) was reached in PNP pouches at the end of 115 days (from graphs, not shown).

TABLE 2. CHANGES IN MC, FFA, PV AND OVERALL ACCEPTABILITY AT ACCELERATED CONDITION (38°C and 90% RH)

Storage period days	Package type	Moisture content, % Air and Gas	FFA, % Oleic acid		PV milli eqt. O_2 per kg fat		Overall acceptability	
			Air	Gas	Air	Gas	Air	Gas
0	-	5.90	0.94	0.94	39.2	39.2	-	-
20	PNP	6.81	1.19	1.16	126.4	64.1	4.0±0.4	4.5±0.4
	MPP	7.40	1.20	1.30	123.7	74.7	3.8±0.4	4.0±0.4
40	PNP	7.41	1.36	1.36	174.7	110.0	3.7±0.3	4.2±0.4
	MPP	8.60	1.46	1.57	184.7	122.6	3.5±0.4	3.8±0.4
60	PNP	8.07	1.43	1.48	191.3	156.8	3.2±0.3	4.0±0.3
	MPP	9.68	1.61	1.72	221.7	156.6	3.2±0.3	3.5±0.3
80	PNP	8.47	1.77	1.75	196.3	172.8	3.2±0.3	3.8±0.3
	MPP	10.45	1.93	1.92	207.1	190.2	3.0±0.2	3.2±0.3
90	PNP	8.72	1.84	1.83	188.0	176.5	3.0±0.2	3.6±0.4
	MPP	10.78	2.00	1.99	198.0	200.0	3.0±0.3	3.0±0.4

PNP & MPP : Designations same as in Table 1. All values are mean of three replicates.

The FFA value increased gradually in all types of pouches from the initial value of 0.94 to nearly 2.00%, at the end of 90 days storage. As could be seen from Table 2, the increase in FFA values were proportional to the increase in the moisture content of the stored samples. The changes in the FFA with time was fitted to polynomial models of different degrees, and their usage under the storage conditions was studied, with emphasis on storage condition in the two packing materials under both air and gas packed conditions. The data were found to fit best to the first degree equation and second and third degrees were compared to first degree. The values of coefficients of higher degrees were insignificantly low.

The oxidative change index as represented by PV of the product during storage also indicated an increase from the initial value of 39.2 m.eq. of oxygen/kg of fat, reaching a maximum of 207.1 at the end of 80 days storage and thereafter, decreased slightly in the product packed in air. Similar trend has been observed in case of shelled walnut (Jan et al. 1988) packed in different packages and stored at 40°C and 30% RH condition. The high initial value of PV may be due to the type of oil used for deep-fat-frying and/or the treatment given during the product preparation. The product in nitrogen gas-flushed packages indicated lower PVS than air-packed ones, while the difference between the two packaging materials was not very pronounced. This could be attributed to the same quantity of headspace and also similar oxygen gas transmission rates.

The data on PV with time were also fitted to polynomial models to third degree. In the air-packed pouches of PNP and MPP, where the rate of oxidation was fast, the equation fits well to third degree, and the second degree fit was also comparable. However, the first degree fit was very poor with low correlation coefficient (0.86) and higher standard error estimates. In the gas-flushed pouches, where the rates of reactions were lower, eventhough the third degree was best, first and second degrees were also comparable with a correlation coefficient of more than 0.98. However, standard error estimate was higher, probably due to non-uniformity in gas-flushing.

The results of the sensory rating tests (only the overall quality ratings are indicated) have shown that at the end of 20 days, MPP pouch-packed samples were rated inferior to those packed in PNP pouches. Further detailed analyses regarding odour, taste and texture revealed that air-packed product in MPP had very slight rancid odour, and was good in texture only. At the end of 40 days storage, the product in MPP pouches had developed perceptible rancid odour and gas-flushed PNP film packed sample was rated higher. Similar trend was observed at the end of 60 and 80 days. The final analyses at the end of 90 days indicated loss of crispness, development of bitter taste and perceptible off-odour, especially in the air filled MPP laminate pouches. However, *Kodbale* samples in PNP pouches were rated acceptable.

It could be concluded that the shelf-life with respect to acceptability was 80-90 days in MPP laminate pouches with ambient air and gas-flush treatments. The product packed in PNP remained in acceptable condition, even at the end of 90 days.

Normal condition : The changes in the chemical parameters and sensory quality are given in Table 3.

TABLE 3. CHANGES IN MC, FFA, PV AND OVERALL ACCEPTABILITY AT ACCELERATED CONDITION (27°C and 65% RH)

Storage period days	Package type	Moisture content, % Air and gas	FFA, % Oleic acid		PV milli eqt. O_2 per kg fat		Overall acceptability	
			Air	Gas	Air	Gas	Air	Gas
0	-	5.90	0.94	0.94	39.2	39.2-	-	
30	PNP	5.99	1.26	1.20	61.3	53.8	4.0±0.4	4.2±0.4
	MPP	5.96	1.26	1.24	65.1	62.8	4.0±0.5	4.2±0.4
60	PNP	6.08	1.54	1.52	96.3	80.5	3.6±0.3	4.0±0.4
	MPP	6.07	1.54	1.54	95.0	93.0	3.8±0.3	4.0±0.3
90	PNP	6.22	1.82	1.74	121.4	109.5	3.4±0.5	3.8±0.4
	MPP	6.19	1.94	1.94	121.4	112.6	3.7±0.5	3.9±0.3
120	PNP	6.38	2.23	1.94	154.5	138.5	3.2±0.4	3.6±0.4
	MPP	6.36	2.30	2.33	154.5	140.2	3.4±0.3	3.5±0.4

PNP & MPP : Designations same as in Table 1. All values are mean of three replicates.

As is evident, the rate of increase in MC, FFA and PV were less pronounced than in the accelerated condition. The moisture content pickup under this condition fits the Paine's equation with a correlation coefficient of 0.98 and with a very low standard error estimate. The slope (M-value) was 4.1899 x 10^{-3} and 4.0104 x 10^{-3} for PNP and MPP pouches, respectively. At the end of 120 days, it was observed that the moisture contents in the stored product were 6.35 and 6.40% in air and gas-packed PNP film pouches. Similarly, the moisture contents in the MPP laminate pouches were 6.34 and 6.38%, indicating little difference between the two types of pouches.

The FFA values steadily increased in all types of pouches with packed product. In general, the rates followed the pattern of increase in the moisture contents. Moreover, the rates were in consonance with the WVTR of the packaging materials. The changes in FFA values fit best to the 2nd degree equation. However, the first degree fits also yielded comparable results. The changes in the PV of the stored samples at standard condition, as shown in Table 3, indicate that changes were less pronounced than the other condition of storage. Also, it was observed that the PV did not decrease, after reaching a maximum value.

The sensory ratings of the various quality parameters of the stored product under normal condition indicated less drastic reduction in the overall acceptability scores. Further, the total rating scores were also not very different between the two types of packaging materials. This could be due to the equal barrier properties of the two types of MPP laminate at the condition of 65% RH and 27°C. But, the gas-flushed samples were rated superior to those packed with ambient air. The experimental results have shown that gas-flushing and use of barrier material packages, which include polyamide or polyester, improve the keeping quality of the product.

In conclusion, it can be said that the % moisture content changes fit to the established Paine's equation with a correlation coefficient of more than 0.98. In general, PV and FFA changes

TABLE 4. REGRESSION EQUATIONS FOR MC, PV AND FFA CHANGES IN *KODBALE*

Parameter	Storage condition	Packaging material and form	Regression equation	r^2	Standard error
% MC	90% RH, 38°C	PNP Air and N_2	$\log (13.4-m_0) = -0.0023t+0.8711$	0.995	0.0064
		MPP Air and N_2	$\log (13.4-m_0) = -0.0051t+0.8793$	0.995	0.0047
	65% RH, 27°C	PNP Air and N_2	$\log (10.37-m_0) = -0.00042t+0.6544$	0.984	0.0042
		MPP Air and N_2	$\log (10.37-m_0) = -0.00039t+0.6540$	0.983	0.0040
PV	90% RH, 38°C	PNP Air	$PV = -3.32\times10^{-2}t^2+4.58t+42.5$	0.995	5.6
		PNP N_2	$PV = -7.14\times10^{-3}t^2+2.30t+32.52$	0.989	11.0
		MPP Air	$PV = -3.92\times10^{-2}t^2+5.32t+36.9$	0.988	5.5
		MPP N_2	$PV = -4.93\times10^{-3}t^2+2.28t+36.6$	0.997	4.3
	65% RH, 27°C	PNP Air	$PV = 9.60\times10^{-4}t^2+0.85t+38.1$	0.997	3.6
		PNP N_2	$PV = 2.47\times10^{-3}t^2+0.55t+37.9$	0.998	2.8
		MPP Air	$PV = 8.65\times10^{-2}t^2+0.85t+39.2$	1.000	1.6
		MPP N_2	$PV = -2.86\times10^{-4}t^2+0.87t+38.8$	0.988	3.3
FFA	90% RH, 38°C	PNP Air	$FFA = 1.49\times10^{-5}t^2+8.32\times10^{-3}t+0.97$	0.988	0.06
		PNP N_2	$FFA = 3.80\times10^{-6}t^2+9.40\times10^{-3}t+0.95$	0.996	0.03
		MPP Air	$FFA = -5.17\times10^{-6}t^2+1.22\times10^{-2}t+0.95$	0.997	0.04
		MPP N_2	$FFA = -6.92\times10^{-5}t^2+1.76\times10^{-2}t+0.95$	0.988	0.03
	65% RH, 27°C	PNP Air	$FFA = 1.43\times10^{-5}t^2+8.75\times10^{-3}t+0.96$	0.997	0.04
		PNP N_2	$FFA = -1.75\times10^{-5}t^2+1.06\times10^{-2}t+0.93$	0.999	0.02
		MPP Air	$FFA = 1.59\times10^{-5}t^2+9.43\times10^{-3}t+0.94$	0.999	0.03
		MPP N_2	$FFA = 6.35\times10^{-5}t^2+1.02\times10^{-2}t+0.93$	0.999	0.04

The notations are as in Table 1.

during storage can be explained by quadratic regression model of the type $Y = A+Bt+Ct^2$ with a correlation coefficient of more than 0.90, where Y is PV or FFA, t = storage period in days and A, B and C are constants of regression model. Table 4 gives the quadratic regression equations for PV and FFA. However, in some cases, linear relationship of the type Y = A+B holds good to explain the changes with a correlation coefficient of more than 0.99 and can be seen in Table 4 by insignificant values of constants of the second degree coefficient. These relationships are applicable upto the point of interest (upto the shelf-life of the product), as the polynomial relationships cannot be extended very much beyond the last data point.

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Gamma Radiation in the Control of Important Storage Pests of Three Grain Legumes

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A dose of 1 kGy of gamma radiation completely killed adult pulse beetle (*Callosobruchus chinensis* Linn) within a week, but the dose of 0.5 kGy required two weeks to achieve the same level of mortality. For six month storage, 1.0 kGy dose was effective for control of natural infestation of *mung* beans and gram by *C. chinensis* alone. The dose was also sufficient for the management of weevilling in gram and lentil, which were additionally infested with *C. chinensis*, rust red flour beetle (*Tribolium castaneum* Herbst), and lesser grain borer (*Rhizopertha dominica* Fabricius). For short (one and half months) storage of *mung* bean and lentil, a dose of 0.25 kGy was adequate. The test of significance for doses of radiation, storage periods and their interactions, however, indicated that 1.0 kGy is the preferred dose for the control of weevilling during storage upto 6 months.

Keywords : Storage pests, Grain legumes, Weevilling, Control, Gamma radiation

Insects have been reported to cause 29.6 and 2.2% damages during storage in grams and lentil, respectively, thereby reducing their nutritive value (Mukherjee et al. 1970; Shehnaz and Theophilus 1975). This could be prevented by fumigation, but the presence of residue, unsatisfactory penetration, need for repeated application and impossibility of gassing the products in pouches are some of the limitations of fumigation method. Irradiation, another method for preventing the storage losses, is known to be highly efficient in killing of all stages of storage insects (Anon 1985; Bongirwar et al. 1981). Moreover, a single treatment with irradiation is good enough for insect control. The joint FAO/IAEA/WHO expert committee (Anon 1981) has declared that foods treated with a dose upto 10 kGy are wholesome, and do not pose any health hazard. The irradiation dose required for disinfestation varies with developmental stages and age of insects as well as the time of the storage (Kloft 1984). Study with *C. chinensis* employing 0.05-3.0 kGy of radiation, showed the dependence of mortality on the dose, while complete control of adults in 24 h could be achieved by 3 kGy dose (Khattak and Hamed 1989). Studies have also shown that eggs, larvae and pupae require lesser doses for mortality than those by the adults (Dawes et al. 1985; Brzostak and Ignalowiez 1989; Khattak and Hamed 1989). It has been stressed that older the insect, greater is the radiation resistance (Hekel and El-Kady 1987). However, negligible work has been done in India to control storage pests of pulses by irradiation, though considerable amount of work has been done for the control of storage pests of other crops, such as wheat (Rao et al. 1976). Hence, an attempt has been made in this study to control the storage pests of pulses by gamma radiation.

Materials and Methods

To study the mortality of *Callosobruchus chinensis* on irradiation, 25 g healthy *mung* beans (variety 'ML-370') were sterilised at 60°C for 1 h, transferred to plastic vials (6x4 cm size) with perforated lids, infested with 25 freshly emerged adults (12 males and 13 females) of the insect and irradiated immediately thereafter. Dead insects were counted at weekly intervals for two weeks, but the progress of weevilling in this, and all the other experiments described below, were recorded at 45 days interval by counting weevilled grains in samples (triplicate) drawn randomly from each of the 3 replicates per treatment.

Naturally weevilled whole grains of Bengalgram (*Cicer arietinum* L. variety - 'Pusa 408') and *mung* beans (*Vigna radiata* L. varieties - 'ML 370' and 'PDM-84-186') were employed to control the insect causing weevilling. The insect responsible for these natural infestations was identified as *C. chinensis* and reared on sterilized *mung* beans. Gram variety 'Pusa 209' and lentil (*Lena esculents* L., variety '4076') - were deliberately infested with 4 insects of *C. chinensis*, 2 each of *Tribolium castaneum* and *Rhizopertha dominica*. Freshly emerged males and females in the ratio of 1:1 were used in this experiment. These insects were allowed to grow and cause further weevilling in the grains for 4 weeks before irradiation (^{60}Co) at a dose rate of 0.07 kGy/min. Grains(250 g lots, triplicates per treatment) of all the varieties mentioned above, were taken in

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polythene pouches (400 gauge), and irradiated with the required dose. Percentages of weevilling in both the naturally and additionally infested grains were noted before irradiation. All these experiments were carried out at ambient conditions (temperature 30±1°C, relative humidity 50-70%). The data in all the Tables, except those in Table 3, were analysed statistically after angular transformation of the original valves. (Snedecor and Cochran 1968).

Results and Discussion

The data on mortality of *C. chinensis* (Table 1) showed that the extent of insect death was dose dependent. The dose of 1.0 kGy was required to complete kill within a week, whereas the same effect could be produced by 0.5 kGy after 2 weeks. The analysis of variance of data on weevillings in beans showed significant differences among storage periods, treatments and their interactions. The data also showed that 0.25 kGy was as effective as higher doses studied upto 3 months for control of weevilling, but subsequently, other two doses (0.5 to 1.0 kGy), were more effective (Table 1). Both the doses (0.5 to 1.0 kGy), were, however, equally effective after 6 months of storage for reducing the weevilling caused under the experimental condition.

The data in Table 2 revealed that the *mung* beans, naturally infested with *C. chinensis* alone, could be stored for longer than 6 months with irradiation dose of 1.0 kGy. The efficiency was significantly lower with 0.25 kGy and 0.5 kGy doses. Dawes et al (1985) also found that the adults of sweet potato weevil died within 7-11 days on irradiation with 1.0 kGy, depending on temperature of rearing. Likewise, a dose of 1.0 kGy has been reported to cause 100% mortality of bean weevils in 9 days, though complete kill was obtained after 3 days within 2.25 kGy dose (Brzostek and Ignalowicz 1989).

The data on lentil indicated that 1.0 kGy was the best dose for 3-6 months storage (Table 3). Anova for average weevilling on irradiation in this case showed significant differences among treatments, storage periods and their interactions. Significant interaction between treatment and storage periods was indicated by significant increase in average weevilling from 3 to 6 months storage periods in 0.25 kGy dose, but in other two doses, this increase was not significant.

TABLE 1. MORTALITY OF *CALLOSOBRUCHUS CHINENSIS* AND PROGRESS OF WEEVILLING IN *MUNG* BEANS 'VARIETY ML-370; IRRADIATED IMMEDIATELY AFTER DELIBERATE INFESTATION WITH 25 INSECTS.

Dose (T),	Av. mortality at storage weeks, %		Av. weevilling at storage months (P), %			
kGy	1	2	1.5	3	6	Mean
0.00	18.9 (10.5)	35.0 (32.9)	27.4	27.8	35.4	30.2
0.25	17.8 (9.3)	43.9 (48.0)	4.9	5.1	6.5	5.5
0.50	64.5 (81.3)	90.0 (100)	4.2	4.6	5.0	4.6
1.00	90.0 (100)	-	3.9	3.9	4.0	3.9
Mean			10.1	10.4	12.7	-
CD at 5%	4.1	3.1	C.D at 5%	T:1.1;	P:0.9;	PxT:1.83

Figures in parenthesis are percentages of mortality. T = Treatment, P = Period and P x T = Period x Treatment interactions.

TABLE 2. WEEVILLING OF IRRADIATION OF NATURALLY WEEVILLED *MUNG* BEANS.

Dose (T), in kGy	Average weevilling of grains (over periods) for *mung* varieties (V), %			Average weevilling of grain (over two varieties) for storage months (P), %			
	'ML 370'	'PDM-84-146'	Mean	1.5	3	6	P(Mean)
0.00	16.48	9.03	12.76	10.32	11.70	16.24	12.76
0.25	4.94	4.84	4.89	4.53	4.91	5.24	4.89
0.50	4.80	4.12	4.46	4.31	4.46	4.62	4.46
1.00	4.50	3.70	4.10	4.09	4.11	4.11	4.10
Mean	7.67	5.43	-	5.81	6.30	7.55	-
CD at 5%	V=0.28	VxT=0.57	-	t:0.40	P:0.35	PxT 0.70	-

Initial weevilling in 'ML-370' and 'PDM 84-146' was 0.6 and 0.4%, respectively.

TABLE 3. WEEVILLING ON IRRADIATION OF LENTIL ADDITIONALLY INFESTED WITH THREE SPECIES OF INSECTS.

Dose (T), in kGy	Average weevilling of grains at storage after months (P), %			
	1.5	3	6	Mean
0.00	35.1	37.3	46.4	39.9
0.25	21.3	22.3	29.9	24.5
0.50	16.5	16.7	17.2	16.8
1.00	13.6	13.6	13.5	13.6
Mean	21.8	22.5	26.2	-
CD at 5%	T: 0.68	P: 0.58	TxP : 1.19	-

Initial infestation was 0.5%

The grams (variety 'Pusa-408'), naturally infested with *C. chinensis*. and those of the variety 'Pusa-209' (with an initial weevilling of 3.0% at the time of additional infestation with the 3 spp. of insects used), had same (7.0) percentage of weevilling as 'Pusa 408' at the time of irradiation. After 6 months of storage, the infestation in unirradiated 'Pusa 209' and 'Pusa 408' increased to 32.5 and 26.9%, respectively. The increase in weevilling was found to be significant even after 6 months in the grains treated with 1.0 kGy (Table 4). This dose, thus, constitutes the best treatment in the control of *C. chinensis* alone or multiple infestation by 3 spp. of insects, namely, *C. chinensis*, *T. castaneum* and *R. dominica* for the period of storage studied for 1.5, 3 and 6 months.

The validity of conclusion is borne by the significant effects of varieties, treatments, period of storage and their interactions. For example, interaction of storage period and treatment was indicated by significant increase in weevilling from 3 months to 6 months with the use of 0.25 kGy dose. On the other hand, the weevilling did not increase from 3-6 months, in case of 0.3 to 1.0 kGy doses (Table 2). Similarly, the significant reduction in weevilling occurred in 'Pusa 408', but the reduction in weevilling was non-significant, in case of 'Pusa 209', thereby indicating the presence of variety and treatment interactions (Table 4). Studies in Bangladesh (Bhuiya et al. 1987) confirmed 100% kill of pests of pulse at 1.0 kGy. Several workers (El-Kady 1983; Huda and Rezaur 1982) have suggested the dose in the range of 0.5-3.0 kGy for disinfestation of pulse pests. Studies in Egypt indicated the effective doses in the range of 0.4-0.8 kGy for disinfestation of cowpea and broad bean (Khattack and Hamed 1989). The minimum dose of disinfestation depends on insect species as well as commodity, and a dose of 0.5-1.0 kGy has been suggested for pulses meant for export purposes (Kloft 1984). Pulses require storage for about 6-12 months i.e., till the next harvest season. The tests of significance for various radiation doses, storage periods and their interactions confirmed that 1.0 kGy is efficient for such a storage for controlling storage pests of pulses.

TABLE 4. WEEVILLING ON IRRADIATION OF NATURALLY INFESTED GRAM, VAR 'PUSA 408' AND ADDITIONALLY INFESTED GRAM VAR. 'PUSA-209' WITH 3 SPECIES OF INSECTS.

Dose (T), in kGy	% Average weevilling of grains (over periods) for *gram* varieties			% Average weevilling of grain at storage months			
	'Pusa 409'	'Pusa 209'	Mean	1.5	3	6	Mean
0.00	22.55	28.39	25.47	22.23	24.40	29.78	25.47
0.25	11.07	8.35	9.71	9.09	9.49	10.55	9.71
0.50	8.95	7.57	8.26	7.78	8.30	8.71	8.26
1.00	7.19	7.14	7.17	7.11	7.12	7.28	7.17
Mean	12.44	12.86	-	11.55	12.35	14.08	-
CD at 5%	V=0.36	V x T =0.52	-	T: 0.37	P: 0.32	T x P: 0.64	

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Storage Changes in Fortified Mango Bars

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Changes in chemical, textural and sensory characteristics of three types of mango bars (plain mango, mango-desiccated coconut powder and mango-soy protein concentrate bars) during 90 days storage at -18°C, 27±3°C (65% RH) and 38±1°C (92% RH) were studied. Moisture, acidity and reducing sugars of the mango bars increased significantly during storage in all the cases. Reduction in total and free SO_2, total carotenoids and beta carotone, and an increase in non-enzymatic browning (NES) were observed. Losses of carotenoids and non-enzymatic browning were found to be more in unsulphited bars than in sulphited bars. Storage decreased the overall acceptability and textural characteristics. The deteriorative changes were minimum in mango stored at -18°C.

Keywords : Coconut, Fruit bars, Intermediate moisture foods, Mango drying, Soy protein concentrate, Texture.

Mango bar is prepared by drying the pulp of ripe fruits. Addition of ingredients like sugar, citric acid, pectin and potassium metabisulphite to pulp facilitates drying, and improves the product quality (Heikal et al. 1972; Jayaraman 1988; Mathur et al. 1972; Nanjundaswamy et al. 1976; Rao and Roy 1980a). The storage characteristics of mango bars obtained by drying pulp in air cabinet drier have been studied (Rao and Roy 1980b). The resulting bars were found to be hygroscopic in nature, and had a shelf-life of about one year under ambient conditions, when wrapped in butter paper and canned (Nanjundaswamy et al. 1976). Storage conditions, activity and residual SO_2 content of the product are known to affect the quality of mango bar (Rao and Roy 1980b). Khedkar and Roy (1983) studied the absorption and retention of sulphur dioxide in raw mango slices during drying and dehydration. These parameters influenced the extent of changes in non-enzymatic browning (NEB), reducing sugars, acidity, carotenoids and SO_2 during storage. Fortified mango bar with desiccated coconut powder (DCP) and soy protein concentrate (SPC) has been prepared by Mir (1990). The results of studies on the changes in physico-chemical and sensory characteristics of fortified mango bars during storage are reported in this paper.

Materials and Methods

Materials : Pulp of ripe mangoes ('Langra' variety) was heated to 91-93°C for 2 min to inactivate enzymes (Nath and Ranganna 1980), cane sugar powder was added to raise total soluble solids (TSS) of the puree to 30° Brix, acidified with 0.6% citric acid (CA) and sulphited with 1734 ppm potassium metabisulphite (KMS) (1000 ppm SO_2). A part of puree was stored unsulphited at -18°C. This puree was dried as such (plain mango bar), or after adding (i) 2% desiccated coconut powder (DCP) (mango-DCP bar) or (ii) 4.5% soy protein concentrate (SPC) (mango-SPC bar). DCP was procured from the local market and SPC from M/s Tata Oil Mills Co. Ltd., Bombay. The blend was dried for 14-15 h in a cross-flow cabinet drier (63±2°C, tray load 9.8 kg/m^2). Drying reduced the thickness of mango sheets from 1.0 cm to 0.5 cm. Dried products (mango bars) were cut into 3x10 cm size and packed individually in polyethylene (92 micron) pouches.

Storage : Dried and packed samples of mango bars were stored for 90 days under three conditions : (i) in deep freezer (about -18°C), (ii) under ambient temperature (27±3°C), 65% RH, and (iii) at 38±1°C, 92% RH. In case of the storage conditions (ii) and (iii), the samples were stored in desiccators equilibrated for 1 week with saturated aqueous solutions of sodium nitrate (65% RH) or ammonium phosphate (92% RH), respectively. The storage experiment was designed on 3 factor CRD pattern (3x4x3) representing numbers of formulations, storage intervals and storage conditions (Federor, 1955).

Analysis : Samples of mango bars were analyzed for moisture by vacuum oven method, reducing sugars by Lane and Eynon method, free-and total-SO_2 by modified Ripper's titration method and beta-carotene by AOAC method (Ranganna 1986). The method of Arya (1981) was used to estimate total carotenoids. The extent of non-enzymatic browning (NEB) was measured as optical density of 60% aqueous ethanolic extract of samples at 420 nm (Ranganna 1986).

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Textural characteristics of the stored samples were determined on Instron-6021, using cylindrical samples of 12.5 mm diameter and 5 mm thickness in 5 KN load cell (Bourne 1968). Two cycles of compression were conducted on samples to give first and second bite at room temperature. Textural profile curves were prepared with time on X-axis and force on Y-axis. Hardness, cohesiveness, springiness, gumminess and chewiness values were calculated from these curves. Sensory evaluation was carried out by Numerical Scoring Method and data were statistically analysed using 3 factor CRD technique for any significant difference at $p \leq 0.05$ (Ranganna 1986).

Results and Discussion

Chemical characteristics : Moisture content of the samples increased with storage period, the increase being greater at higher temperature (Fig.1). The

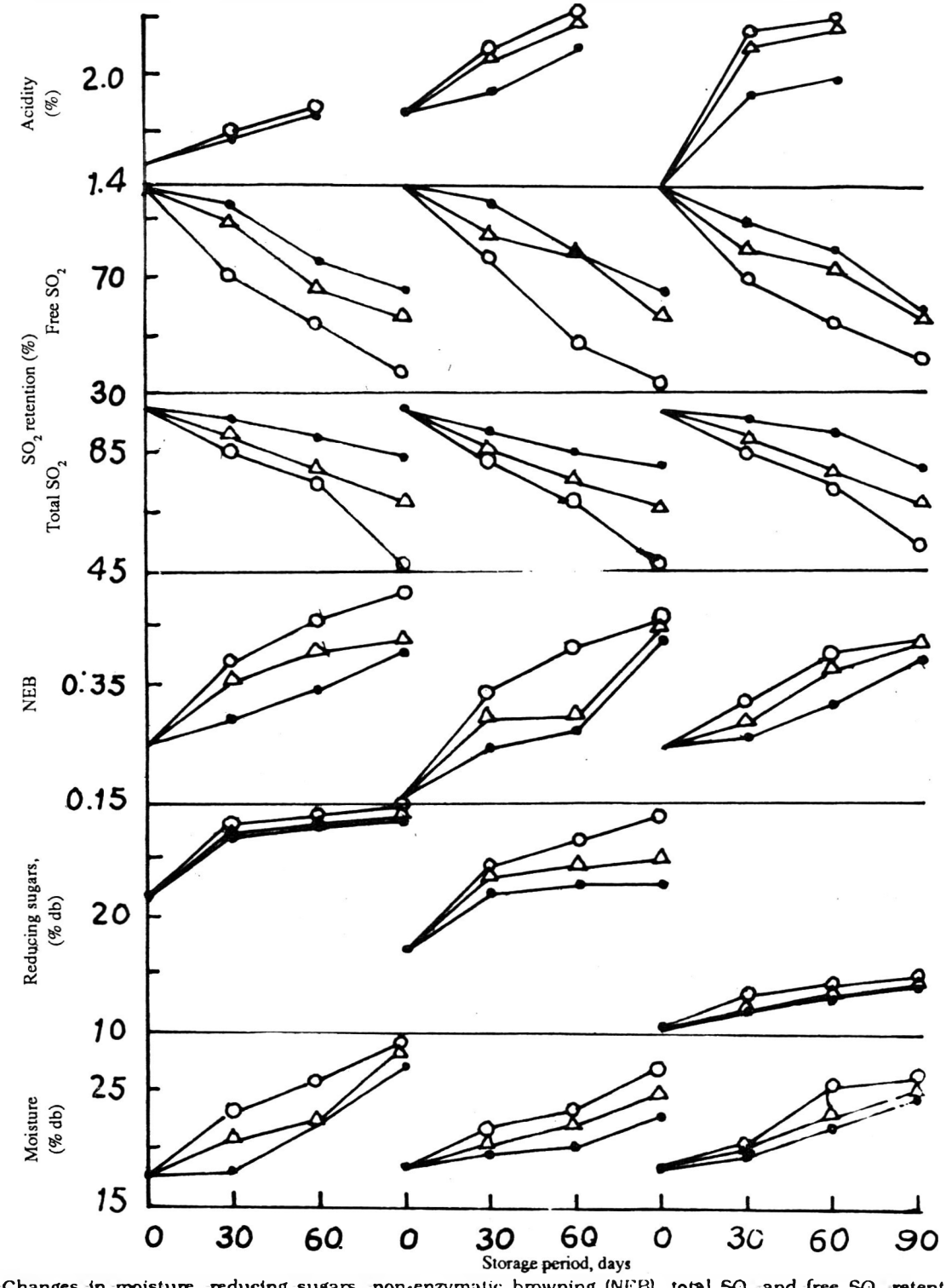

Fig. 1. Changes in moisture, reducing sugars, non-enzymatic browning (NEB), total SO_2 and free SO_2 retention, and acidity during 90 days storage of mango bars at -18°C (● ●), 27°C & 65% RH (Δ——Δ), and 38°C & 92% RH (○——○).

product may have absorbed moisture from the external environment or the change may be due to chemical changes such as browning reactions (Karel 1975). Plain mango bar exhibited greater increase in moisture content and browning, during 90 days storage. Similarly, the reducing sugars (RS) and acidity increased during storage (Fig. 1). Rao and Roy (1980b) and Ammu et al (1977) had also reported an increase in RS during storage of mango leather and freeze-dried mango powder, respectively. The increase in RS was probably due to acid hydrolysis of sucrose (Labuza et al. 1970). Increase in acidity of certain other fruit products has been ascribed to the formation of sulphurous acid from slightly higher in sulphited bars than in unsulphited bars. They decreased during storage and the losses were greater at higher temperature (Table 1). The losses could be due to non-oxidative changes (Cis-trans isomerization, epoxide formation or thermal degradation) or oxidative changes. Such changes altered the product colour from red-orange to pale-yellow orange, and lowered the flavour and nutritive value of the product (Eskin 1979; Land 1962). Changes in carotenoids have been reported in other mango products (Goday and Rodriguez-Amaya 1987; Ranganna and Siddappa 1961). Greater retention of beta-carotenes and total carotenoids observed in sulphited samples was due to the protective action

TABLE 1. CHANGES IN CAROTENOIDS OF MANGO BARS DURING 90 DAYS STORAGE

	Total carotenoids (mg % db)				Beta-carotene (mg % db)			
		After 90 days				After 90 days		
Sample	0 day	I	II	III	0 day	I	II	III
Plain mango bar								
Unsulphited	9.2	7.0	-	6.5	4.9	4.0	-	2.4
Sulphited	9.5	8.6	8.4	7.1	5.4	4.5	4.3	2.5
Mango DCP bar								
Unsulphited	8.7	7.1	-	5.9	4.7	3.4	-	2.3
Sulphited	8.8	7.5	6.7	6.3	5.3	4.2	3.8	3.4
Mango-SPC bar								
Unsulphited	8.2	6.8	-	5.7	4.2	3.2	-	2.1
Sulphited	8.5	7.6	6.9	6.2	5.0	3.9	3.8	3.3

I :18°C; II : 27°C, 65% RH; III:38°C, 92% RH

SO_2, ascorbic acid degradation or hydrolysis of pectin (Cruess 1958; Seth 1985). Since the reactions for the above changes were temperature-dependent, the rates of changes were more at higher temperature (Fig. 1).

Colour of mango puree darkened during drying. But, the changes were of lower magnitude in sulphited samples. Consequently, the NEB in the samples was lower (0.17-0.25) than in unsulphited samples (0.21-0.29). Sulphited samples darkened during storage, probably due to increase in NEB to 0.40-0.51 (Fig. 1), but their colour was still lighter than that of unsulphited samples (NEB 0.45-0.64). Changes were of greater magnitude at higher temperatures, as expected from the Arrhenius equation. Increase in NEB was concomitant with loss of total and free SO_2 (Fig 1). This loss could be due to reaction of SO_2 with food constituents (sugars, pectins, proteins, lipids), oxidation or due to volatilisation (Bolin and Boyle 1972; Echkoff and Okos 1986).

Total carotenoids and beta-carotene content (mg% db), at the beginning of storage period, were of SO_2 (Bolin and Stafford 1974; Foda et al. 1972; Rao and Roy 1980b).

Textural characteristics : Textural characteristics of mango bars changed during 90 days storage (Table 2). Calculated values of hardness, springiness, gumminess and chewiness of mango-SPC bars were higher than those for other two bars. However, cohesiveness of plain mango bar was higher, probably due to higher protein content of the former. Presence of DCP prevented compact structure in the mango -DCP bar and therefore, it had lower values for textural characteristics. All the values decreased significantly ($P \geq 0.05$) during storage and the changes were greater at higher temperature. A significant interaction was observed between formulation and storage time as well as the storage time and temperature.

Sensory attributes : Initial sensory scores for colour, texture, aroma and taste for mango-DCP bar were higher than those for other samples. But, the pattern changed after 90 days storage and plain mango had the higher rating. The scores for overall acceptability are given in Table 2. Storage at higher

TABLE 2. CHANGES IN TEXTURAL CHARACTERISTICS AND SENSORY QUALITY OF MANGO BARS DURING 90 DAYS STORAGE

	Plain mango bar				Mango-DCP bar				Mango-SPC bar			
	0 day	90 days			0 day	90 days			0 day	90 days		
		I	II	III		I	II	III		I	II	III
Hardness (Newtons)	1840	997	757	307	1037	560	557	273	2167	1620	1447	593
Cohesiveness (A_2/A_1)	0.20	0.13	0.17	0.07	0.12	0.13	0.08	0.08	0.18	0.18	0.16	0.09
Springiness (cm)	0.33	0.37	0.27	0.20	0.37	0.30	0.23	0.13	0.40	0.37	0.30	0.23
Gumminess (Newtons)	361	126	82	21	126	70	45	15	392	292	234	54
Chewiness (Newton-cm)	119	47	22	4	47	21	10	2	157	108	70	12
Score[1]	4.2	3.7	3.3	2.5	4.3	3.3	2.8	2.4	3.3	2.6	2.3	2.3

[1]Maximum overall acceptability score 5; I, II, III as in Table 1.

temperatures caused greater loss in sensory scores. These changes were similar to those observed for chemical and textural attributes in this study.

Statistical analysis of the data revealed that the changes in chemical, textural and sensory characteristics were significant ($P \leq 0.05$) for the type of bar, storage period, and storage conditions. The changes were minimum in sample stored at -18°C.

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Effect of Sprouting and Kilning Temperatures and Germination Time on Cyanide Content in Sprouted Sorghum : A Statistical Approach

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Data on sorghum sprouts showed significant effect of kilning temperature (50-65°C), temperature of sprouting (20-32°C) and period of sprouting (1 - 7 days) on cyanide levels. The effect of sprouting period was parabolic in contrast to the linear effects of germination and kilning temperatures.

Keywords : Sprouting, Kilning, Germination, Cyanide content, Sorghum.

Need to conserve foreign exchange and dwindling economic situations has compelled developing countries including Nigeria, to search for newer raw materials. Local production of barley, used mainly in brewing, is not sufficient to meet the demands in Nigeria. Therefore, some breweries started looking for alternative sources, such as sorghum, maize, rice and millet. Their use, however, involves the possibility of forming hydrogen cyanide, under certain chemical conditions. Studies on suitability of sorghum (Aniche 1989; Aniche and Okafor 1987), showed that lager beer had the clear sparkling straw-colour, when sprouting and, kilning were carried out at 20 and 65°C, respectively. The work on germination and drying conditions revealed that the highest hydrogen cyanide was formed in sprouts on third day at 30°C (Aniche 1989, 1990). The effects of sprouting and kilning temperatures and germination time on cyanide content are reported in the present communication.

Seven samples of the 'Local Red' variety ('LRV') of sorghum grain (each 1.4 kg) were given the same

TABLE 1. ANOVA TABLE FOR MODEL USED AND THEORETICAL AS WELL AS EXPERIMENTAL VALUES

	THEORITICAL VALUES			
Source of variation	Degrees of freedom	Sums of squares	Mean square	Expected mean square
A_i	P-1(3)	SSA(5261.0)	MSA(1753.7)*	$\sigma_E^2+\sigma_G^2+\frac{28\Sigma A^2 i}{3}$
B_j	q-1(6)	SSB(17446.9)	MSB(2907.8)*	$\sigma_E^2+\frac{7\Sigma\Sigma C^2 ij}{18}+\frac{28\Sigma B^2_j}{6}$
C_{ij}	[P-1][q-1](18)	SSC(315.7)	MSC(17.4)	$\sigma_E^2+\frac{7\Sigma\Sigma C^2 ij}{18}$
D_{ikl}	q[n-1](42)	SSD(14948.7)	MSD(355.9)*	$\sigma_G^2+\sigma_G^2+4\sigma_\ell^2$
G_{ikl}	q[n-1][p-1](126)	SSG(1072.3)	MSG(8.51)	$\sigma_E^2+\sigma_C^2$
Total	pqn-1(195)	SST(39044.6)		

* Significant at P<0.05. Figures in round bracket give experimental values. F values calculated for experimental results were 2606.1, 165.8 and 41.8 for kilning temperature, sprouting temperature and period of sprouting, respectively. These were significant at P<0.05.

sterlisation, washing and steeping treatments in nylon bags for avoiding contamination by moulds and bacteria (Aniche 1989). These were placed in seven shallow stainless steel trays (with fine mesh bottom), covered with wet cloth and allowed to germinate in refrigerator for seven days at temperatures between 20 and 32°C (at interval of 2°C). At 24 h intervals, 10 g sample was removed for determination of cyanide content (AOAC 1980). Another 100 sample was kilned at 50 - 65°C (at 5°C interval) before determining the cyanide content. A linear statistical model was used for the analysis of the data (Scheffe 1956) :

$$Y_{ijk} = U + A_i + B_j + C_{ij} + D_{k(j)} + G_{ik(j)} + E_{ijk}$$

$$i = (1,4);\ j = (1,7);\ K = (1,7).$$

Where Y_{ijk} is the cyanide content (µg) from the ith kilning temperature, jth sprouting temperature and kth sprouting period; U = the grand mean; A_i = the effect of the ith level of the kilning temperature; B_j = the effect of the jth level of the sprouting temperature; C_{ij} = the effect of interaction between the ith level of the kilning temperature and the jth level of the sprouting temperature; $D_{k(j)}$ = the effect of the kth level of sprouting period, nested under the jth level of the sprouting temperature; $G_{ik(j)}$ = the interaction effect between the ith level of the kilning temperature and the kth level of sprouting period; and E_{ijk} = random error.

$$\sum_{i=1}^{4} A_i = 0; \quad \sum_{j=1}^{7} B_j = 0; \quad \sum_{i=1}^{4} C_{ij} = 0; \quad \sum_{j=1}^{7} C_{ij} = 0.$$

$$D_{k(j)} \sim NID(0, 6^2_E);\ G_{ik(j)} \sim NID(0, 6^2_G);\ E_{ik(j)} \sim NID(0, 6^2_E)$$

In order to determine the appropriate test statistics for the effects of the proposed mixed model, the expected mean squares, according to the procedure of Gaylor and Hartwell (1969), are shown in Table 1. The expected mean squares for G is a pooled value for $6^2E + 6^2G$ and for C is also a pooled value for

$$6^2E + \frac{n}{(p-1)(q-1)} \sum_{i=1}^{4} \sum_{j-1}^{7} C^2_{ij}$$

because E_{ijk} is a dummy variable (Siner 1962). The hypotheses to be tested are :

(a) $H_0 : A_i = 0$ for all; $H_1 : Ai \neq 0$ for at least one i

F_0 = MSA/MSG

(b) $H_0 : B_j = 0$ for all j; $H_1 : B_j \neq 0$ for at least one j

F_0 = MSB/MSC

(c) $H_0 : 6^2_D = 0$; $H_1 : 6^2_D > 0$;

F_0 = MSD/MSG

Analysis of variance has been used to confirm that all the three factors under study are significant. Duncan's multiple range Test (Montgomery 1976), was used to test the significance of the range of the differences in mean cyanide contents.

Table 1 shows that the effects of the three factors under investigation are significant ($p<0.05$) for cyanide content. Table 2 shows the mean cyanide content of sorghum sprouts at each level of the factors in ascending order of magnitude. For

TABLE 2. MEAN CYANIDE CONTENT (µg) FOR THE LEVELS OF THE FACTORS

Kilning temperature °C	Germinating temperature °C	Period of germination (days)
$\bar{Y}_{4..} = 41.2^a$	$\bar{Y}_{.1.} = 38.4^l$	$\bar{Y}_{..1} = 33.3^u$
$\bar{Y}_{3..} = 46.0^b$	$\bar{Y}_{.2.} = 41.1^m$	$\bar{Y}_{..7} = 44.7^v$
$\bar{Y}_{2..} = 52.2^c$	$\bar{Y}_{.4.} = 41.4^m$	$\bar{Y}_{..2} = 44.7^v$
$\bar{Y}_{1..} = 54.4^d$	$\bar{Y}_{.3.} = 44.0^n$	$\bar{Y}_{..6} = 49.2^w$
	$\bar{Y}_{.5.} = 52.0^o$	$\bar{Y}_{..3} = 52.9^x$
	$\bar{Y}_{.6.} = 55.4^p$	$\bar{Y}_{..5} = 54.8^y$
	$\bar{Y}_{.7.} = 66.8^q$	$\bar{Y}_{..4} = 59.5^z$

(i) Any two mean values in the same column containing different superscripts differ significantly ($p < 0.05$).

(ii) $$S_{\bar{Y}i..} = \sqrt{\frac{MSE}{n}} = \sqrt{\frac{8.51}{49}} = 0.4$$

$$S_{\bar{Y}j.} = \sqrt{\frac{8.51}{28}} = 0.6$$

$$S_{\bar{Y}..k} = \sqrt{\frac{8.51}{28}} = 0.6$$

the kilning temperature, the highest value of 53.4 µg was obtained at 65°C. These results agree with earlier finding by Aniche (1990). The mean cyanide contents of sorghum sprouts for varying temperatures of sprouting (Table 2) show the highest value of 66.6 µg at 32°C and the lowest value of 38.4 µg at 20°C. This outcome may be explained by the fact that the cotyledons have not properly developed for the production of hydrogen cyanide at low temperatures. The cyanide content

rises slowly from 33.3 µg on the first day of sprouting to 59.5 µg on the fourth day. The maximum value of cyanide content at this period is due to full development of cotyledons. Subsequently, a steady decline was observed with further increase in the sprouting period, the mean cyanide on the seventh day being 44.7 µg. The sprouts had developed leaves on sixth and seventh days, thereby allowing the loss of hydrogen cyanide through transpiration. The withering of the leaves further reduced hydrogen cyanide.

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Alteration in Carbohydrate Components of Glycoproteins of Rat Liver by Feeding Dietary Fibre from Unripe Banana

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Feeding isolated dietary fibre from unripe banana to rats has been found to have significant effect on the carbohydrate components of glycoproteins in the liver. Many of the carbohydrate components of glycoproteins showed increases in rats fed fibres due to resultant decrease in the activity of glycohydrolases. *In vitro* inhibition of glycohydrolase activity by bile acids is known in most cases and thus, explains the *in vivo* effect observed in the present studies.

Keywords : Neutral detergent fibre; Carbohydrate components; Glycoproteins; Glycohydrolases; Bile acids; Rats, Dietary fibre, Unripe banana.

Most of the earlier work on dietary fibre has been on its effect on metabolism of cholesterol and bile acids (Reddy et al. 1980; Trowell 1975; Cummings 1973; Jayakumari and Kurup 1979; Menon and Kurup 1976). The components of dietary fibres are capable of binding bile acids and carcinogens, depending upon the composition of fibre and other factors (Agte and Joshi 1991). The effect of detergent fibre from blackgram, barley, ragi and rice and its hypocholesterolemic action in rats has also been reported (Molly Thomas et al. 1990). It has been observed in this laboratory that the feeding of dietary fibre (Neutral detergent residue - NDR), isolated from unripe banana *(Musa paradisiaca)* increased glycogenesis, and the activity of many glycolytic enzymes, while suppressing gluconeogenesis in addition to its well established hypocholesterolemic effect in the serum and tissues (Usha et al. 1989). This effect was attributed to the increase in the concentration of hepatic bile acids produced by the dietary fibre. Bile acids, both cholic and chenodeoxy cholic acids, have also been found to affect the *in vitro* activity of many of the glycolytic and gluconeogenic enzymes (Usha et al. 1989). In addition, the metabolism of the carbohydrate components of glycoproteins in the liver was altered by feeding this dietary fibre. The results obtained in this respect are discussed from the point of view of the changes in the concentration of hepatic bile acids caused by the dietary fibre and the *in vitro* effect of the bile acids on the activity of glycohydrolases.

Dietary fibre, as neutral detergent fibre (NDF) from unripe banana, was prepared as described by Goering and Vansoest (1970). Male albino rats ('Sprague-Dawley' strain average weight 80 g) were divided into two groups of 12 rats each. Group I - rats were fed fibre-free diet and served as control, while group II - rats were fed the diet containing NDF from unripe banana. The rats were housed individually in polypropylene cages with wire mesh floors. The diet had the following composition (g/100 g) :

		Diet	
Portion	Constituents	Fibre-free	NDF
A	Dextrin	67	42
	Casein (Vitamin and fat-free)	20	20
	Fibre	-	25
	Groundnut oil	8	8
B	Salt mixture	4	4
	Vitamin mixture	1	1

Portion A (13.1g) and NDF diet supply the same calories (42 cal), as 10 g of portion A of fibre-free diet. The caloric intake of the two groups was kept the same by adjusting the intake of portion A. The intake of salt mixture and vitamin mixture was also kept the same in the two groups by giving the same amount of portion B (0.75 g/rat). Portions A and B were mixed in the required weight for feeding purposes. NDF was taken to contribute very little towards caloric value. Water was provided *ad libitum*. The rats were maintained on the respective diet for 75 days. At the end of this period, they were deprived of food overnight, stunned by a blow at the back of the neck and killed by decapitation. The liver was removed and stored in ice-cold containers for use in various estimations. The liver tissue was worked up for the estimation of carbohydrate components of glycoproteins, while total hexose, fucose and sialic acid were

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TABLE 1. EFFECT OF DIETARY RAW BANANA FIBRE ON HEPATIC HEXOSE, FUCOSE, SIALIC ACID AND GLYCOHYDROLASES IN ALBINO RATS

Hepatic Constituent	Group I	Group II
Total hexose*	92.30±1.94	153.38±4.45[a]
Fucose*	16.60±0.35	32.93±0.95[a]
Sialic acid*	14.05±0.30	6.50±0.19[a]
ß-Glucosidase**	59.80±1.44	52.70±1.20[a]
ß-Fucosidase**	14.30±0.30	11.56±0.33[a]
ß-Galactosidase**	86.30±1.81	64.75±1.88[a]
ß-N acetyl hexosaminidase**	72.60±2.11	66.10±1.93

* mg/g **mg p-nitrophenol liberated/h/g protein
Average of the values from 6 rats ± SEM, a = p < 0.01

estimated, as described elsewhere (Latha et al. 1991). The activities of ß-N acetyl hexosaminidase, ß-glucosidase, ß-galactosidase and ß-fucosidase were estimated, as described before (Latha et al. 1991). Extraction of bile acids from the liver was carried out, as described by Okishio et al. (1967), and estimated enzymatically, as per the method of Palmer (1969). Protein was estimated in the enzyme extracts, after TCA precipitation by the method of Lowry et al. (1951). The enzyme activity in each case was assayed in the presence of varying concentrations of cholic and chenodeoxy cholic acids with appropriate controls. Sodium salt of the bile acids was used. Statistical analysis was carried out by Student's 't' test (Bennet and Franklin 1967). The NDF from unripe banana contained (%): hemicellulose 86.8±2.69, cellulose 4.91±0.1, cutin 2.5±0.05, lignin 4.1±0.1 and silica 0.19±0.006 (Usha et al. 1984). Rats fed NDF from unripe banana showed significant increase in the concentration of total hexose and fucose in the liver, when compared to control rats fed fibre-free diet (Table 1). On the other hand, the concentration of sialic acid in the liver showed significant decrease in the rats fed NDF. The activity of ß-glucosidase also showed decrease in the liver of the rats fed NDF, as compared with rats fed fibre-free diet. The activity of ß-fucosidase and ß-galactosidase in the liver also decreased significantly, while the activity of ß-N-acetyl hexosaminidase was not significantly altered.

There was a significant increase ($p < 0.01$) in the concentration of total bile acids in the liver of the rats fed NDF (37.90±0.99 mg/100 g wet tissue), when compared to those fed fibre-free diet (27.98±0.68 mg/100 g). *In vitro* activities of ß-glucosidase, ß-fucosidase and ß-galactosidase in the liver were significantly inhibited by both cholic and chenodeoxy cholic acids (Table 2). However, the activity of ß-N-acetyl hexosaminidase was not affected.

The results indicate that feeding NDF from unripe banana has significant effect on the concentration of the carbohydrate components of glycoproteins in the liver. The increase in the concentration of many of the carbohydrate components (total hexose and fucose) observed in the liver may be due to the decrease in the activity of the glycohydrolases in the tissue. These glycohydrolases are involved in the degradation of the carbohydrate components of the oligosaccharide chain in the glycoproteins. The changes produced by feeding NDF from banana on the carbohydrate components of glycoproteins are, however, similar to those observed in the atheromatous rat liver (Satakopan and Kurup 1977), even though unripe banana fibre has been reported to be hypocholesterolemic (Usha et al. 1984).

TABLE 2. *IN VITRO* EFFECT OF BILE ACIDS ON HEPATIC GLYCOHYDROLASES

Attribute	ß-Glucosidase	ß-Fucosidase	ß-Galactosidase	ß-N Acetyl hexosaminidase
	(mg p - nitrophenol liberated/h/g protein)			
Enzyme activity, (no bile acid) control	59.90±2.06	12.79±0.50	82.30±1.66	70.99±1.65
Enzyme activity in presence of cholic acid				
0.01 mg/ml reaction mixture	52.50±1.45[a]	8.49±0.41[a]	72.40±2.11[a]	70.79±1.94
0.05 mg/ml reaction mixture	46.60±1.08[a]	8.20±0.37[a]	64.20±1.37[a]	70.69±.172
0.10 mg/ml reaction mixture	40.30±1.09[a]	7.69±0.39[a]	57.37±1.48[a]	70.03±2.13
Enzyme activity in presence of chenodeoxy cholic acid				
0.01 mg/ml reaction mixture	56.30±1.75	10.80±0.44[b]	64.54±1.94[a]	73.99±1.61
0.05 mg/ml reaction mixture	55.20±1.63	10.40±0.45[a]	56.23±1.82[a]	72.81±1.88
0.10 mg/ml reaction mixture	48.40±1.37[a]	10.60±0.47[a]	41.89±2.04[a]	72.41±1.52

Average of the values from 6 rats ± SEM. Experimental groups have been compared with control group
a = p < 0.01, b = p between 0.01 and 0.05

The results of *in vitro* studies indicate that the activity of many of glycohydrolases is inhibited by cholic or chenodeoxy cholic acid, either alone or in combination. As the concentration of bile acids increased significantly in the liver of rats fed NDF, the decreased activity of glycohydrolases in this tissue may possibly be due to this increase.

Thus, it is possible that the observed alteration in the concentration of the carbohydrate components of glycoproteins, brought about by dietary fibre, may be mediated via its effect on hepatic bile acids.

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Moisture Sorption Behaviour Of Weaning Foods

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Water activity - equilibrium moisture content data were obtained for commercial weaning foods A and B at 20, 30, 40 and 50±1°C using dynamic method. Commonly used models such as Brunauer Emmette Teller (BET); Caurie's; Henderson's; Chung and Pfost; and Guggenheim Anderson deBoer (GAB) were fitted to the observed values of moisture sorption data to find their suitability to predict the moisture sorption behaviour of these foods. Guggenheim Anderson deBoer model described the moisture sorption of the two weaning foods, better than other equations considered. Henderson's; Chung and Pfost; and Caurie's equation could predict equilibrium moisture content values more correctly with two pairs of their constants.

Keywords : Water activity, Weaning foods, Moisture sorption isotherm, Equilibrium moisture content, Models.

Water, one of the constituents of foods, is bound partially to specific sites of food e.g., carboxyl group of polysaccharides, amino group of proteins, etc. The monolayer moisture is equivalent to this bound water, and this value is of interest to processors, as food is highly stable at this moisture content. In this communication, a study has been made on commercially available weaning foods A and B, and a few selected models were tested for their suitability to predict the moisture sorption characteristics of these foods.

Water activity - equilibrium moisture content data were obtained for weaning foods A and B at constant temperatures of 20, 30, 40 and 50±1°C, for both absorption and desorption processes. Dynamic method was, used to obtain these data. An apparatus for dynamic method was developed, which worked on the principle described by Smith (1965). Composition of weaning foods A and B is as given in Table 1.

TABLE 1. COMPOSITION OF WEANING FOODS.

Ingredients	g/100 g weaning foods	
	A	B
Fat	9.0	1.0
Proteins	15.5	6.0
Carbohydrates	65.7	86.0
Moisture	2.5	4.0
Ash	3.2	0.7
Dietary fibre	4.1	2.1

Commonly used models such as Henderson's 1952); Brunauer Emmette and Teller (1938); Caurie's (1981); Chung and Pfost (1967); and Guggenheim Anderson deBoer model (Sawhney 1989) were considered for studying the moisture sorption behaviour of these weaning foods activity and is given by

$$1 - Aw = e^{-KT(Xe)^n} \quad \ldots (1)$$

The BET model is represented as

$$\frac{Aw}{m(1-Aw)} = \frac{1}{Cb \cdot Mb} + \frac{(Cb-1)Aw}{Cb \cdot Mb} \quad \ldots (2)$$

The value of Mb is known as monolayer moisture content.

Caurie's model is an improvement over the BET model and it has been used widely for the moisture absorption isotherm, fractionation of bound water and storage stability of coriander powder (Selot et al. 1991). According to Caurie's model, the moisture-adsorption takes place in multilayers:

$$\frac{1}{m} = \frac{1}{Cc \cdot Mc}\left[\frac{1-aw}{aw}\right]^{(2\,Cc/\,100 \cdot Mc)} \quad \ldots (3)$$

The moisture content, at which a food material becomes stable, is the value of Mc. The number, N of the so-called monolayers is given by

$$N = 100\ Mc/\ Cc \quad \ldots (4)$$

Chung and Pfost (1967) proposed the following model:

$$\ln[\ln(1/Aw)] = \ln(A/RT) - B \cdot Me \quad \ldots (5)$$

The GAB model is applicable over a wider range of water activity.

$$\frac{Aw}{M} = \frac{K(1-C)}{Mm \cdot C}Aw^2 + \frac{C-2}{Mm \cdot C}Aw + \frac{1}{Mm \cdot C \cdot K} \quad \ldots (6)$$

where, Mm = Moisture content equivalent to monolayer value, g water/ g dry solid; C, K = Constants

Equilibrium moisture data obtained at constant temperatures for weaning foods A and B are presented in Table 2. The isotherms are found to have smooth sigmoidal shape of BET type II curves.

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TABLE 2. EMC - WATER ACTIVITY DATA AT DIFFERENT TEMPERATURES

Temp (±1°C)	WATER ACTIVITY													
	0.05		0.10		0.25		0.40		0.55		0.75		0.95	
	Ad	De	Ad	De	Ad	De	Ad	De	Ad	De	Ad	De	Ad	De
Weaning Food A														
20	0.016	0.022	0.025	0.033	0.038	0.052	0.067	0.066	0.080	0.082	0.111	0.125	0.237	0.253
30	0.014	0.021	0.026	0.031	0.036	0.043	0.058	0.055	0.068	0.072	0.110	0.125	0.221	0.225
40	0.011	0.016	0.022	0.025	0.030	0.036	0.053	0.048	0.065	0.069	0.101	0.113	0.193	0.196
50	0.010	0.012	0.018	0.021	0.026	0.031	0.050	0.045	0.059	0.061	0.092	0.100	0.181	0.186
Weaning Food B														
20	0.024	0.029	0.055	0.060	0.091	0.104	0.124	0.127	0.139	0.142	0.183	0.193	0.244	0.246
30	0.018	0.024	0.052	0.057	0.084	0.096	0.115	0.118	0.138	0.141	0.169	0.176	0.223	0.226
40	0.017	0.019	0.035	0.040	0.057	0.068	0.083	0.090	0.109	0.112	0.141	0.148	0.200	0.200
50	0.015	0.017	0.028	0.031	0.046	0.056	0.071	0.072	0.080	0.091	0.128	0.136	0.193	0.196

Ad = Adsorption, De = Desorption

Adsorption and desorption isotherms are found distinctly separate at all temperatures for both the foods. A discontinuity in adsorption isotherm prevails in the water activity region of 0.42 to 0.57. The reason for this occurrence of discontinuity as suggested by Varshney and Ojha (1977) and Saltmarch et al. (1980), is crystallization of lactose from amorphous form. On the basis of high total water soluble sugar content of the products (which is present in amorphous form), it is inferred that the discontinuity is due to crystallization of water soluble sugars. Sugar in the amorphous form, sorbs moisture, until it is diluted to an extent that molecules attain sufficient mobility to become

TABLE 3. COEFFICIENTS OF VARIOUS MODELS AT DIFFERENT TEMPERATURES

Temperature °C	Process	Henderson's model Two st.lines		Chung & Pfost model Two st.lines		Cauric's model Two st.lines		GAB model		
		K	n	A × 10^{-7}	B	Mc	Cc	Cg	Mg	Kg
Weaning Food A										
20	Ad	0.1816	1.671	1.000	24	0.0539	1.427	-4.423	0.0834	-1.1
		0.0577	1.178	0.012	0	0.0604	1.200			
	De	0.4792	2.080	1.300	27			-13.255	0.0600	-0.97
		0.0517	1.164	0.370	14					
30	Ad	0.1995	1.665	1.100	29	0.0515	1.345	-5.493	0.0666	-1.1
		0.0551	1.125	0.390	15	0.0560	1.163			
	De	2.2408	2.475	1.700	37			-16.705	0.0478	-0.98
		0.0541	1.152	0.500	16					
40	Ad	0.2107	1.608	1.00	30	0.0471	1.279	-3.121	0.0781	-1.2
		0.0672	1.173	0.480	19	0.056	1.088			
	De	1.1973	2.167	1.500	39			-9.014	0.0509	-1.0
		0.0720	1.241	0.600	20					
50	Ad	0.1893	1.522	0.990	32	0.0435	1.257	-1.759	0.0959	-1.4
		0.0636	1.106	0.420	20	0.0519	1.039			
	De	0.3637	1.741	1.200	38			-4.871	0.0564	-1.1
		0.0738	1.211	0.510	20					
Weaning Food B										
20	Ad	0.0044	0.863	1.0	12	0.774	2.706	-3.566	0.2481	-0.75
		0.2866	2.384	4.4	24	0.1153	1.181			
	De	0.0059	0.990	1.1	12	0.1013	4.925	-5.975	0.204	-0.68
		0.4041	2.633	5.1	24	0.1135	1.211			
30	Ad	0.0030	0.715	1.1	14	0.0742	2.905	-1.350	0.1975	-0.74
		0.3508	2.419	16.0	32	0.1158	1.114			
	De	0.5206	2.684	1.1	14	0.1104	6.419	-4.198	0.2450	-0.64
		0.5206	2.684	3.4	35	0.1109	1.158			
40	Ad	0.0110	1.019	1.0	18	0.0597	1.774	-1.239	0.2916	-1.10
		0.1702	1.831	4.5	29	0.0985	1.071			
	De	0.0079	0.962	1.1	18	0.0657	2.222	-2.598	0.2049	-0.91
		0.2659	2.097	6.9	31	0.0933	1.108			
50	Ad	0.0214	1.140	1.1	22	0.0538	1.595	-1.091	0.2346	-1.30
		0.1247	1.591	2.2	27	0.0745	1.097			
	De	0.0190	1.143	1.2	23	0.0583	1.856	-2.812	0.1373	-1.10

Ad = Adsorption, De = Desorption, St.lines = Straight lines

TABLE 4. COMPARISON OF MODELS FOR SORPTION DATA ON WEANING FOODS

Model	Errors in prediction (%)							
	20°C		30°C		40°C		50°C	
	Ad	De	Ad	De	Ad	De	Ad	De
Weaning Food A								
Cauries								
two lines	3.94	-	6.7	-	7.8	-	7.6	-
Henderson's								
one line	25.1	23.0	27.9	24.7	25.6	22.6	29.2	27.2
two lines	6.5	2.4	5.3	3.0	5.9	3.4	6.5	3.6
Chung & Pfost								
one line	7.1	3.3	8.8	5.8	11.7	7.3	11.2	7.4
two lines	3.7	1.1	4.7	2.7	7.2	3.1	10.0	2.7
G.A.B.	6.4	2.3	5.7	3.6	5.7	5.2	6.3	3.4
Weaning Food B								
Cauries								
Two St. lines	7.32	2.93	9.4	3.6	5.4	7.0	6.0	6.2
Henderson's								
Two St. lines	1.8	1.8	1.9	0.7	2.72	0.81	2.1	2.5
One St. line	17.2	17.0	22.1	20.1	10.6	12.8	4.06	4.71
Chung & Pfost								
Two St. lines	5.40	6.70	8.30	8.30	2.30	4.20	3.40	2.70
One St. line	23.4	21.2	28.9	25.1	19.5	20.8	16.0	16.1
G.A.B.	6.4	5.6	7.7	6.6	3.6	3.8	3.0	3.3

Ad = Adsorption, De = Desorption, St. line = Straight line

oriented in stable non-hygroscopic crystals. Due to rapid rate of moisture gain, the product gains an extra moisture, which is desorbed after the completion of crystallization. This causes discontinuity in adsorption isotherm.

Caking and slight swelling of the product are observed, when products are equilibrated in environment of 75% RH at 20°C. At higher temperatures, caking and swelling are initiated at low relative humidities. Equilibrium moisture values are higher in weaning food B than those in A, under similar surrounding conditions. Reason for this could be the higher sorptive capacity of starch, than gluten. Weaning food B, being rice-based food, has higher amounts of starch and is free from gluten which, otherwise, is available in food A.

Testing of MSI models: Moisture sorption isotherm equations, as mentioned above, were fitted to moisture sorption data of these products. Values of the constants involved in these models were computed through a computer program, and are presented in Table 3. The correlation coefficients between dependent and independent variables of these equations were computed. The absolute values of correlation coefficient is found to be higher than 0.95 at all temperatures for all models, except BET and GAB models. For BET equation, the value of correlation coefficient is always higher than 0.8, but less than 0.98. For GAB model, correlation coefficient was much low.

The percentage errors in predicting EMC values for different models are presented in Table 4. It is inferred that moisture-sorption characteristics of weaning food B can be predicted with accuracy, by two straight line Henderson's equation, while that of weaning food A by two straight line Chung and Pfost equation. Further, monolayer moisture values, obtained from Caurie's equation, were found to be higher than BET monolayer values for both products, but in the vicinity of safe moisture content range obtained from 'local' and 'stability' isotherms of these products. The monolayer values of 5.45% (db) and 7.79% (db) were estimated for weaning foods A and B, respectively, at 20°C. The values decreased with an increase in moisture content.

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Studies on the Safety of Water Stored in High Density Polyethylene Water Bottles

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Studies were carried out to find out any possible safety hazard that may arise upon consumption of drinking water stored in high density polyethylene water bottles due to leaching of some toxic compounds in the stored water during storage for 3 days. The albino rats were fed with such water upto a period of 6 months spread over two generations. No significant difference in body weight, food consumption, feed efficiency ratio and organ to body weight ratio was observed in both the generations, as compared to the control group. The data indicate safety of such stored water, and the results are of specific importance to the personnel from Defence Services, as they have to depend on such water under the operational conditions, because of the lack of facilities to get fresh water at operation locations.

Keywords : Stored water, High density polyethylene bottles, Safety, Organoleptic quality, Leaching of toxic substances, Animal feeding.

Metallic water bottles covered with felt are authorised for use by Defence troops to carry and store drinking water. High density polyethylene (HDPE) water bottles offer several advantages such as light weight, convenience of handling and ease in cleaning over the conventional containers, made of glass or metal. Besides, glass bottles and pouches are widely used for milk and ghee (Kadan and Bhanumurthy 1984). However, HDPE materials may pose problems, due to their temperature sensitivity and susceptibility to cracking. Since plastic polymers are made of monomers with addition of certain additives, the possibility of leaching of some of these additives into the stored water on continued contact over a long time may occur and thus, pose a health hazard. This calls for a detailed examination of the safety aspects of such plastic materials used for drinking water. There are legislation limits for the additives that could be used in such plastic materials for use in packing food in many countries (US-GSA 1983). In contrast, there is no such comprehensive legislation in India on the additives used in packaging materials, and the data on the possible toxic hazards arising due to leaching of these additives are scarce. Studies on these aspects, therefore, have assumed critical importance with respect to safety of foods, food products and drinking water stored/packed in plastic materials.

A study was, therefore, undertaken in this laboratory to investigate the safety of drinking water stored at ambient temperature in HDPE water bottles for possible hazards from leached materials, development of odour and microbiological contamination. The toxicity has been determined by feeding the water to albino rats.

High density polyethylene (HDPE) water bottles of one litre capacity were procured from local market for use in the experiments. The tests were conducted by using the methods recommended by the Expert Commttee on Food Additives (FAO/WHO 1958). The most accepted method for packaging of foods and beverages, is to feed the leaching materials to, at least two species of animals, one rodent and another non-rodent, and observe them for symptoms for abnormalities over a sufficiently long periods. Moreover, maximum life span of the experimental animal is to be observed in the feeding trial. For this purpose, a period of six weeks to 90 days is considered adequate in case of albino rats (FAO/WHO 1958), being the experimental animals used in the present study.

Twenty albino rats of the 'Wistar' strain, maintained at this laboratory, were selected for testing. The weaning rats selected were of 21-28 days old and in the weight range of 35 to 45 g. These were grouped in randomised block design, based on the initial weights, as per the procedure of Snedecor (1950). They were housed in individual cages with wire screen bottoms. The rats were divided into two groups consisting of ten rats each, and each group consisted of 5 males and 5 females. First group of rats were fed with the water stored in HDPE bottles (at ambient temperature : 19-26°C) for a period of 3 days. The second group of rats, which served as control, were fed with the water stored in Pyrex glass water bottles over the same period. The components of the standard diets were prepared as per the methods of ISI (1974). The diet contained (%) : protein derived from skim milk powder 10, fat from refined groundnut oil 9, fat

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soluble vitamins from shark liver oil 1, other vitamins 1, salt mix 4 and corn starch 75. The food and water under evaluation were given *ad libitum*.

At the end of 4, 8 and 13 weeks, the gain in body weight, growth rate, food consumption and feed efficiency ratio of each rat were recorded. Organs to body weight ratio was determined for 3 males from 1st and 2nd group, to evaluate gross abnormalities in heart, liver, spleen, kidney, adrenals and gonads. These studies were also carried out in case of first and second generations and compared with the control. At the end of 13 weeks, the rats of both the groups were regrouped in breeding cages, each with 2 females and one male, and the respective diet and water were continued during mating and reproduction stages. After 21st day of littering, the weaning rats were used for second generation study (Satyanarayana Rao et al. 1974). To study the development of any odour, taste etc. in the water samples during storage at ambient temperature, a trained panel of judges was given water, stored in HDPE and glass container, for comparing organoleptic quality against the fresh tap water. The panelists were asked to give comparative evaluation with respect to colour, taste, odour, appearance and overall quaity. Studies were also carried out simultaneously on the various microbiological aspects, to examine the safety with respect to total viable bacteria, coliforms and *Escherichia coli* using standard methods (Harrigan and McCance 1966).

During the course of this study, diet and water were given *ad libitum*. No death or abnormalities in behaviour as well as the appearance of the animals occurred during the study involving two generations. There was no significant difference between the control and experimental groups with respect to the rate of weight gain, food or water consumption in both the generations (Table 1). The water consumption was of the order of 35 ml/day/rat in both experimental and control groups, and it remained same in both the generations. This clearly shows that the rats maintained their normal intake of water, food and growth rate pattern. The relative organ : body weight ratio of six important organs such as liver, heart, spleen, kidney, adrenals and gonads at the end of 13 weeks, showed that the ratios were nearly the same in all cases, except for liver weight (3.83) (Table 2). In the second generation, the relative ratio of liver was only 2.90 in control group, but the difference is not significant. Statistical analysis of the data could not be done due to small sample size. Further examination of the livers for fat, moisture and protein contents, showed that there was no abnormal difference. It is evident from the data that the values for all organs studied, are more or less equal. Similar trend was also found among the data collected on animals maintained on stock diet for the purpose of comparison. The triangular test of experimental, control and fresh water (without storage) showed that there was slight development of plastic smell, which was not significant and did not affect the overall acceptability of water.

The microbiological examination of water stored in the HDPE containers and also in glass bottles showed that there was no development of unusual growth of organisms during storage. However, the viable bacterial count was in the range of 106 to 54 × 100/ml in experimental sample and 48/ml in the control sample. This is, within, the acceptable

TABLE 1. WEEKLY GAIN IN WEIGHT, FOOD CONSUMPTION AND FOOD EFFICIENCY RATIO OF EXPERIMENTAL AND CONTROL GROUPS OF RATS IN 1st AND 2nd GENERATIONS

	Initial weight (g)	Gain in weight (g) (wks)			Food consumption (g) (wks)			Food efficiency ratio (g) (wks)		
		4	8	13	4	8	13	4	8	13
I Generation										
(a) Experimental	39.6	71	132	197	62	69	80	1.15	1.9	2.46
(b) Control	40.3	78	137	207	75	85	86	1.05	1.6	2.43
II Generation										
(a) Experimental	48.4	65	112	168	60	77	97	1.08	1.5	1.73
(b) Control	48.2	52	105	176	61	76	98	0.85	1.4	1.80

TABLE 2. PERCENTAGE ORGANS BODY WEIGHT RATIOS OF EXPERIMENTAL AND CONTROL GROUPS OF 1st GENERATION AND 2nd GENERATIONS*

Attribute	Organ weight (g)						
	Body weight, g	Liver	Heart	Spleen	Kidney	Adrenals	Gonads
(a) Experimental							
1st Generation	280	3.14	0.29	0.15	0.63	0.01	0.94
2nd Generation	241	3.83	0.29	0.16	0.56	0.01	0.13
(b) Control	200	2.90	0.31	0.24	0.58	0.01	1.20
		(3.05)	(0.28)	(0.17)	(0.67)	(0.01)	(0.75)

* Average of data from 3 rats in each group at the end of 13 weeks of feeding. Figures in parenthesis indicate the literature values of the relative organs.

limits prescribed by public health authorities for water meant for drinking purpose (ICMR 1975: HMSO 1969).

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Sorghum Grain Moisture : Its Effect on Popping Quality

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Ten sorghum genotypes, viz. 'SPV-462', 'Pant Chari', 'SPV-475', 'SPV-881', 'CSH-9', 'CSH-6', 'CSH-11', 'SPH-504', 'CSH-10' and 'SPH-509', were tempered to 12-20% grain moisture level and evaluated for popping %, popping volume, expansion volume and flake size. The popping % of the genotypes differed significantly at different levels of grain moisture. SPV-881 had the highest popping volume of 23.22 ml at 12% grain moisture, while genotypes 'SPV-462', 'SPV-881' and 'CSH-6' exhibited highest expansion volume at same grain moisture. 'SPV-881', 'SPH-504' and 'CSH-10' had the highest flake size at the same level of grain moisture.

Keywords : Sorghum, Popping quality, Grain moisture.

Sorghum (*Sorghum bicolor* L. Moench) can be popped, extruded, shredded and flaked to produce delightful snacks. The popping quality of several varieties of sorghum has been studied by various workers (Murthy et al. 1982; Savithri and Meera Rao 1985; Thorat et al. 1988). Popped sorghum is tender, has less hull, does not clog in the space between teeth and causes less noise, when eaten as compared to popcorn. Also, its flavour and nutritive value compare well with popcorn (Subramanian 1956). Varietal differences observed in popping quality of cereal grains have been ascribed to kernel structure, amount and distribution of protein, starch composition, tightness of glumes enveloping the grains and differences in processing conditions (Srinivas and Desikachar 1973). Popping of sorghum at about 17% grain moisture level and at a grain moisture content of 9-10% have been reported (Chandrashekar and Desikachar 1984; Prasad Rao and Murthy 1981). The present investigation has been undertaken to evaluate the effect of grain moisture on popping quality of sorghum.

Ten sorghum genotypes viz. 'SPV-462', 'Pant Chari', 'SPV-475', 'SPV-881', 'CSH-9', 'CSH-6', 'CSH-11', 'SPH-504', 'CSH-10', and 'SPH-509', were obtained from the Department of Plant Breeding, College of Agriculture of the University. After estimation of their original moisture content (AACC 1962), they were tempered to a moisture level of 12%, 14%, 16%, 18% and 20%. The samples were analysed in triplicate for popping %, popping volume, expansion volume and flake size (Porderimo et al. 1990). Sample (2 g) was placed in an iron pan, containing 100 g of salt maintained at a temperature of 160°C, covered with a lid and stirred briskly for 40 to 60 sec. Immediately, the mixture containing popped grains and salt was passed through 40 mesh sieve to remove salt from popped grains. The other parameters were computed as follows :

$$\text{Popping \%} = \frac{\text{Number of popped kernels}}{\text{Number of total kernels}} \times 100$$

Popping volume was estimated by rapeseed displacement method.

$$\text{Expansion volume} = \frac{\text{Total popped volume, ml}}{\text{Original weight of raw kernels, g}}$$

$$\text{Flake size} = \frac{\text{Total popped volume, ml}}{\text{Number of popped kernels}}$$

The data on popping %, popping volume, expansion volume and flake size are presented in Table 1. The results on popping % reveal that genotypes exhibited significant differences in popping % at different moisture levels. However, the grain moisture level, at which highest popping % was obtained, differed from one genotype to another. Of all the genotypes, SPV-881 had the highest popping volume at 12% grain moisture level. The genotypes 'CSH-6', 'SPH-504', 'CSH-10', and 'SPH-509' exhibited the highest popping volume at 12% grain moisture level, whereas 'SPV-462', and 'SPV-475', at 16% grain moisture level. The expansion volume of genotypes 'Pant Chari', 'SPV-475', 'CSH-9', 'CSH-11', 'SPH-504', 'CSH-10', and 'SPH-509' did not differ significantly at all the grain moisture levels. The genotypes 'SPV-462', 'SPV-881', and 'CSH-6', exhibited the highest expansion volume at a grain moisture level of 12%. With regard to flake size, the highest values were obtained for genotypes 'SPV-881', 'SPH-504', and 'CSH-10' at a grain moisture level of 12%, whereas 'Pant Chari' had

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TABLE 1. POPPING (%), POPPING VOLUME (ml), EXPANSION VOLUME (ml/g) AND FLAKE SIZE (ml) OF SORGHUM GENOTYPES AT VARYING MOISTURE LEVELS

Grain moisture level*	Genotypes									
	'SPV-462'	'Pant chari'	'SPV-475'	'SPV-881'	'CSH-9'	'CSH-6'	'CSH-11'	'SPH-504'	'CSH-10'	'SPH-509'
					POPPING (%)					
1	86.15	4.55	83.00	89.95	63.05	87.69	84.10	85.30	78.40	88.40
2	82.37	8.40	81.50	86.87	62.28	85.38	84.60	83.84	77.69	87.60
3	86.10	6.17	84.60	87.60	63.80	86.10	83.50	83.07	76.92	86.87
4	83.80	7.60	84.60	86.87	61.50	83.84	82.84	83.07	78.40	84.60
5	83.80	6.20	83.00	86.87	62.20	85.30	83.00	83.80	76.90	83.70
		CD_1 = 1.21; CD_2 = 0.94								
					POPPING VOLUME (ml)					
1	19.51	1.50	19.01	23.22	12.40	20.30	19.40	19.41	13.01	23.05
2	19.40	1.75	18.99	20.26	12.40	19.40	19.20	19.26	12.75	22.91
3	20.21	1.50	19.54	21.02	12.40	20.20	19.21	19.21	12.75	22.40
4	19.21	1.75	19.44	20.26	12.40	19.21	19.20	19.01	12.15	21.77
5	19.21	1.62	19.01	20.26	12.40	19.40	19.01	19.21	12.07	22.91
		CD_1 = 0.36; CD_2 = 0.28								
					EXPANSION VOLUME (ml/g)					
1	14.35	0.75	9.50	11.60	6.22	10.15	9.72	9.70	6.50	11.50
2	9.70	0.87	9.40	10.13	6.21	9.73	9.60	9.63	6.27	11.40
3	10.10	0.75	9.70	10.51	6.20	10.12	9.60	9.60	6.27	11.18
4	10.00	0.87	9.70	10.13	6.20	9.60	9.60	9.50	6.07	10.88
5	9.60	0.75	9.50	10.15	6.21	9.73	9.50	9.60	6.03	4.40
		CD_1 = 1.05; CD_2 = 0.82								
					FLAKE SIZE (ml)					
1	0.34	0.33	0.36	0.39	0.19	0.35	0.29	0.25	0.41	0.32
2	0.34	0.32	0.35	0.36	0.19	0.34	0.29	0.24	0.38	0.31
3	0.35	0.37	0.35	0.36	0.19	0.35	0.29	0.24	0.39	0.32
4	0.35	0.35	0.35	0.35	0.19	0.35	0.29	0.23	0.39	0.31
5	0.35	0.36	0.35	0.35	0.19	0.34	0.29	0.23	0.40	0.32
		CD_1 = 0.11; CD_2 = 0.83								

*1 = 12%, 2 = 14%, 3 = 16%, 4 = 18%, 5 = 20%
CD_1 = at 5%, between two genotypes at the same level of moisture
CD_2 = at 5%, between two moisture levels in the same genotype

the highest flake size at grain moisture level of 16%. Rest of the genotypes did not show significant differences in flake size at various grain moisture levels.

It is concluded that suitable grain moisture for popping of sorghum varied from genotype to genotype. In general, 12% level of grain moisture was observed to be suitable for popping for most of the sorghum genotypes.

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Palatability of Spray-Dried, Foam-Mat-Dried and Freeze-Dried Whole Egg Powders Packed in Different Packaging Materials

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Freeze-dried and foam-mat-dried whole hen's egg powders, prepared from egg melange of uniform composition, and commercial spray-dried egg powders were packed in cans and in flexible pouches with and without air. Drying conditions and packaging materials did not significantly influence the acceptability of egg powders during storage at 4°, 19-27°, 37° and 42°C in all three types of egg powders upto a period of one year.

Keywords : Dried egg powders, Scrambled egg, Palatability, Packaging materials, Storage flavour, Acceptability.

The scoring of organoleptic quality of foodstuffs is one of the prime quality evaluation methods used mostly in research and in industrial quality control (Ninabarylko and Metelski 1964). It is based on evaluation of quality factors such as colour, odour, juiciness, tenderness, palatability, according to adopted scale. Using these scores (and corresponding descriptive terms) for individual quality factors, overall acceptability can be expressed as a combined score. Such a score is necessary in many cases, especially in quality control, because it is more comprehensible and easier to interpret than tabulation of the series of several individual quality factors. The combined score should illustrate the overall quality of the product in the best possible way. A systematic study was undertaken to evaluate the flavour acceptability of the samples of the egg powders by preparing scrambled egg after storage at different temperatures. The effect of packaging materials on this attribute was also evaluated.

Fresh eggs of "White Leg Horn" birds were procured from local market, washed with water and then, with Tween 80 solution (2%) to remove the adhering material. The cleaned eggs were soaked in 2% bleaching powder solution for 30 min. Finally, they were washed with water and surface-dried at room temperature to remove surface moisture. The eggs were, then, broken manually and inspected visually for any spoilage. The egg white and yolk were homogenised with a mechanical stirrer. The melange was filtered through muslin cloth. Desugaring of egg melange was done as described in an earlier study (Satyanarayana Rao and Murali 1985) using 600 mg Baker's yeast (wet) for 100 g melange, followed by pasteurisation at 64°C for 3 min (Satyanarayana Rao et al. 1987) in water bath and immediately cooled to 4°C. Spray-dried egg powder, manufactured as per the method of Iyengar et al. (1969), was procured from M/s Foods and Inns Ltd., Bombay. The freeze-dried and foam-mat-dried whole egg powders were prepared according to the methods described earlier by Satyanarayana Rao et al. (1987).

All the three types of egg powders (150 g) were packed in (a) paper-aluminium foil-polyethylene laminate consisting of 60 g brown casing (BC) paper, 0.02 mm aluminium foil-polyethylene, 150 gauge pouch (PFP), (b) butter size cans (401 × 300) with and without air and (c) a high density polyethylene (HDPE), 300 gauge pouches. The packages were stored 4°, 19-27°, 37° and 42°C for different periods and analyzed for palatability score.

Egg is mainly consumed as scrambled egg or fried egg. The greatest advantage in selecting the scrambled egg for evaluation is that it is easy to prepare. It is the only preparation, where flavour of egg can be detected without any interference and difficulty. Scrambled egg was prepared by taking 30 g egg powder, 100 ml water and 0.31 g salt. These were mixed in round bottom flask and shaken for 30 min. The reconstituted sample was cooked in a stainless steel vessel over a water bath (80-90°C) for 5-6 min by constant stirring, till coagulation. The samples were kept over a water bath at 50-58°C, till served. The organoleptic evaluation of the reconstituted samples was done by a trained panel of judges (8-10). The same judges were continued throughout the study, and were asked to grade only for degree of storage flavour and to ignore other parameters. The flavour score ratings are graded as per the method of Wilson and Slosberg (1973).

The effect of storage temperatures, packaging materials and nitrogen atmosphere on the palatability of three types of egg powders are given in Table 1. As can be seen from the data, the spray-dried egg powder had an initial flavour score of 8.0, whereas foam-mat-dried and freeze-dried egg powders had scores of 7.5 and 7.8, respectively. During storage at 42°C for the first 180 days, the score of spray-dried sample was between 6.0 and 7.3, whereas the scores of the foam-mat-dried and freeze-dried samples were between 6.5 and 7.3, in all the packaging materials. Thus, the data indicate an increase in development of storage flavour with the increase in the storage period. Among the packaging materials, the development of flavour was slightly higher in PFP and HDPE packages, than in the cans, probably because the former two packaging materials did not provide good barrier to oxygen and moisture. This probably causes lipid oxidation which, in turn, led to development of lipid-amine-aldehyde compounds (Satyanarayana Rao and Murali 1989). These compounds are shown by Kline et al. (1951) as a source of off-flavour in stored egg powders. A score of 6 (Fryd and Hanson 1945) was considered as an acceptable score, and it was taken as a guideline for overall acceptability of the products. Any score less than 6 was considered to be indicative of development of off-flavour.

TABLE 1. CHANGES IN PALATABILITY OF EGG POWDERS PACKED IN DIFFERENT PACKING MATERIALS AND STORED AT DIFFERENT TEMPERATURES

Egg Powder type	Storage period, days	Cans		PFP laminate		HDPE
		N_2 pack	Air pack	N_2 pack	Air pack	
			4°C			
Spray-dried	180	7.8	7.8	7.8	7.8	7.5
	365	7.8	7.8	7.6	7.6	7.5
Foam-mat-dried	180	7.5	7.2	7.5	7.5	7.4
	365	7.3	7.2	7.5	7.5	7.0
Freeze-dried	180	7.5	7.3	7.4	7.3	7.3
	365	7.5	7.5	7.6	7.0	7.0
			19-27°C			
Spray-dried	180	7.5	7.5	7.4	7.4	7.3
	365	7.4	7.2	7.0	7.0	7.0
Foam-mat-dried	180	7.4	7.5	7.2	7.2	7.0
	365	7.0	7.0	7.0	7.0	7.0
Freeze-dried	180	7.5	7.3	7.7	7.3	7.0
	365	7.0	7.0	7.0	7.0	6.8
			37°C			
Spray-dried	180	7.5	7.4	7.0	7.0	7.3
	365	7.0	7.0	7.0	7.0	7.0
Foam-mat-dried	180	7.2	7.2	7.0	6.5	6.5
	365	7.0	6.0	7.0	6.0	6.0
Freeze-dried	180	7.6	7.0	7.6	7.0	7.3
	365	7.0	7.0	7.0	6.0	6.3
			42°C			
Spray-dried	120	7.3	7.1	7.6	7.0	7.6
	240	7.3	6.7	7.0	6.0	6.0
	365	7.0	6.6	7.0	6.0	6.0
Foam-mat-dried	120	7.0	7.0	7.0	6.8	7.4
	240	7.1	6.3	7.0	6.0	6.3
	365	7.0	6.0	7.0	6.0	5.8
Freeze-dried	120	7.0	7.0	7.2	7.0	7.0
	240	7.2	7.0	7.0	6.6	6.6
	365	7.0	7.0	7.0	6.4	6.0

The initial values were : spray-dried 8.0, foam-mat-dried 7.5 and freeze-dried 7.8.

There was a slight decrease in flavour in all the three types of egg powders, and the judges were unable to differentiate any change in colour, flavour and texture as well as general appearance in the product, stored at 37°C upto a period of 365 days. A similar observation was made on the palatability of egg powders in compressed form by Srivastava et al (1973). As expected, the rates of changes in flavour scores at room temperature (19-27°C) were less than those at 42° and 37°C. Boggs and Fevold (1946) have reported a maximum life of acceptability of spray-dried egg powders at 20°C for 8 months, while only one month at 36.5°C, when compared to the products stored in air. They also reported that the storage under nitrogen atmosphere had little beneficial effect. It was observed that the flavour score remained unchanged in the control sample (4°C), as compared to the samples stored at other temperatures studied.

It is worth mentioning that the palatability is a necessary judgement of desirability of foodstuffs, and correlation with palatability is regarded as an important factor in the choice of methods. Thistle et al. (1943) have found that moisture, pH, water values and potassium chloride values are all interrelated to one another. Among all these methods, they found that the measurement of potassium chloride values and fluorescence were most sensitive methods, which associated closely with the palatability ratings of dried whole egg powder. Fryd and Hanson (1944) tried to find out the relations between flavour and chemical characteristics of egg powder, as the flavour of reconstituted dried egg is of considerable importance and its determination by taste panels on a large number of samples, presents obvious difficulties. Boggs and Fevold (1946) have studied various factors affecting the palatability of stored egg powders. The results indicated the effect of decreasing moisture content, gas packing and

acidification on the shelf-life of dehydrated egg powders during storage at 36.5°C. Egg powders of low moisture value, retained their palatability better than those containing high moisture. Acidification, plus CO_2 and N_2 packing, brings about the retention of palatability during storage, the shelf-life being 5-6 times that of non-acidified air packed powders. Kline and Sanoda (1951) are of the view that although glucose removal from pulp prior to drying prevents Maillard reaction, it does not permit evaluation of the role of glucose in the development of off-flavour in the dried product. Recent evidence (Western Regional Research Laboratory, California) points out the role played by glucose in such reactions in egg powders (Satyanarayana Rao 1979). Kline et al. (1951) have compared the characteristics of glucose-free egg powder with those from which glucose had not been removed. It was observed that the palatability retention at 37.5°C is much greater for glucose-free than the glucose containing dried egg. This is true both in lyophilised and spray-dried egg powders with 2 to 5% moisture and also for nitrogen and air-packed samples. The present studies have indicated that the drying conditions, packaging materials and storage conditions have no significant effect on the palatability and acceptability of the product.

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Studies on Chilled and Frozen Stored Ready-to-Eat Buffalo Beef Sausages Prepared by Incorporating Skeletal and Offal Meats with 20% Pork Fat

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Emulsion-type buffalo beef (buef- a new word coined to denote buffalo beef) sausages, prepared by incorporating 70 and 30 parts of skeletal and offal meats, respectively with 20% pork fat, were stored at chilled and frozen temperatures. Sausages stored well at 4°C - 5°C for 7 days and at -10°C for 8 weeks. Sliminess did not develop, till 7 days in chilled storage. However, a significant ($P < 0.01$) increase in aerobic plate count from 3.75 to 3.89 log/g was observed. In frozen storage sausages, a significant ($P < 0.01$) reduction in aerobic plate count from 3.75 to 3.53 log/g was observed after 7 days. The changes in plate count were marginal at 8 weeks of storage. The initial thiobarbituric acid value of 0.49 mg of malonaldehyde/kg showed a decreasing trend and it was 0.14 mg at 8th week.

Keywords : Buffalo-beef, Offal meats, Skeletal meats, Sausages, Shelf-life, Aerobic plate count, Thiobarbituric acid.

In India, buffalo beef is produced as a byproduct of dairying, and only old buffaloes are slaughtered for meat production. Several studies have been carried out on the meat quality (Kondaiah et al 1986), its organoleptic properties (Prabhakar and Narayana Rao 1986) and its amino acid content (Madhavi et al 1982). The resulting meat is usually tough and with less flavour as well as taste. Such meat can be processed into a novel, tasteful, convenient and ready-to-eat emulsion-type buef sausages. A meat product, to become popular, shall have good keeping quality. Chilling and freezing are convenient methods to increase shelf-life without altering the appearance. Therefore, the shelf-life of ready-to-eat emulsion-type buef with pork fat, was studied, and the results are presented in this communication.

Ready-to-eat emulsion-type sausages, incorporating 70 parts of skeletal meat (SM) : 30 parts of offal meats (OM) with 20% pork fat, were prepared and processed as per Krishnan and Sharma (1990). Sausage samples weighing about 75 g were packed in polyethylene bags and sealed. Six samples were stored in refrigerator maintained at 4° - 5°C. Ten samples were stored in deep-freezer maintained at -10°C. Samples were withdrawn and studied for various attributes at 48 h intervals upto 7 days in refrigerated samples and at weekly intervals upto 8 weeks in frozen samples. The frozen samples were thawed for 18 h at 4° - 5°C, prior to testing.

The odour, colour and surface slime were estimated using a 5- point scale by a panel of 5 trained persons. For pH measurement, about 10 g sausage sample, in duplicate, was homogenized with 50 ml distilled water for 10 - 15 sec in a Waring blender. The pH was recorded using a digital pH meter (Naina NIG - 333). The moisture content was estimated as per AOAC (1980) method, while the method of Tarladgis et al. (1960) was followed for the determination of thiobarbituric acid (TBA) value. For aerobic plate count (APC), a serial 10-fold dilutions were made from 10 g sample in 0.1% sterile peptone water. From appropriate dilutions, 1.0 ml was plated in plate count agar (HiMedia) by pour plate technique. The plates were incubated at 35°C for 48 h. After incubation, plates containing 30 - 300 colonies were counted and expressed in log number/g.

The data were subjected to statistical analysis as per the standard procedures of Snedecor and Cochran (1967).

Refrigeration storage: No significant changes were observed in pH, % moisture (Table 1), and the score for colour (4.0), odour (5.0) as well as surface changes (5.0) throughout the storage period. This is probably due to the reduction in the number of bacteria and destruction of enzymes as a result of heat treatment. Heat treatment also imparts the desired colour, flavour and consistency (Stiebing 1985). Negligible loss of moisture during storage was probably due to the property of polyethylene, which has a very low moisture vapour transmission rate (Gokalp et al. 1978).

A significant ($P < 0.01$), but gradual increase in APC was observed with the increase in storage period. The observed APC was less than 4 log/g at the end of 7-day storage period, even when offal meat

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TABLE 1. MEAN AND SE QUALITY CHARACTERISTICS OF READY-TO-EAT EMULSION-TYPE BUEF SAUSAGES CONTAINING 20% PORK FAT STORED AT CHILLED (4° - 5°C) AND FROZEN (-10°C) TEMPERATURES.

	Chilled storage period, days			
	0	3	5	7
pH	6.3 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
Moisture, %	65.5 ± 0.8	65.7 ± 0.6	65.0 ± 1.6	64.9 ± 0.4
Aerobic plate count, log/g	3.75^{b}	3.79^{b}	3.84^{ab}	3.89^{a}

	Frozen storage period, weeks							
	0	1	2	3	4	5	6	7	8
Moisture, %	66.7 ± 0.2	65.8 ± 1.1	65.3 ± 1.4	65.5 ± 1.4	65.3 ± 0.9	65.6 ± 1.1	64.7 ± 0.8	64.9 ± 0.9	64.7 ± 0.8
Aerobic plate count, log/g	3.75^{a}	3.53^{b}	3.56^{b}	3.60^{b}	3.55^{b}	3.55^{b}	3.56^{b}	3.64^{ab}	3.57^{b}
TBA value, mg malonaldehyde/kg	0.49^{a}	0.51^{a}	0.40^{ac}	0.32^{bc}	0.14^{de}	0.18^{be}	0.19^{be}	0.15^{de}	0.14d^{e}

pH was 6.3 ± 0.1 throughout. Means bearing different superscripts in rows differ significantly ($P < 0.01$).

was used in the preparation (Table 1). A similar finding was reported by Stamenkovic (1984), who observed aerobic mesophilic counts in the range of 2600-3000 organisms/g (about 3.42 - 3.48 log/g) in cooked sausages, stored at 4° - 10°C for 17 days. APC of less than 4 log/g, recorded during a 7-day period of refrigeration storage, might be due to the combined effects of the use of polyphosphate, in addition to curing and seasoning agents. Libelt (1984) reported that sausages stored at 4°C were fit for consumption upon ≤ 120 h, and no change occurred in sausages at 10°C in ≤ 96 h.

Frozen storage: No significant changes were observed in moisture content, and pH was 6.3 ± 0.1 throughout 8 weeks storage period (Table 1). The scores for colour (4.0), odour (5.0) and surface changes (5.0) remained the same during frozen storage also. However, a significant ($P < 0.01$) reduction in the APC was observed, especially at the end of first week of frozen storage. Neither off-flavour nor surface changes were observed probably due to low bacterial counts (less than 4 log/g). The TBA values of frozen stored sausages were very low indicating that no spoilage occurred due to rancidity of fat. As the frozen storage period increased, the TBA values decreased. The lower TBA values might be due to the effect of tetrasodium pyrophosphate, which decreases fat oxidation and moderates flavours (Keeton 1983; Matlock et al. 1984). The spices too would have prevented the fat oxidation. Further, the presence of nitrates, nitrites and carbohydrates might have influenced and brought down the TBA value (Koniecko 1979) in subsequent period of storage.

It is concluded that ready-to-eat emulsion-type buef sausages, properly packed in polyethylene bags, can safely be kept for a minimum period of 7 days at 4° - 5°C and 8 weeks at -10°C. They may even keep well beyond these periods, but extended periods of storage studies need to be carried out to confirm such possibility.

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A Simple Method for the Separation of Stones from Coriander Seeds Based on the Use of Fluidization Technique

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Coriander seeds available from the market invariably contain about 5% stone particles. These, in turn, increase the ash content of the coriander powder and can damage the grinder, if not removed. A simple method has been developed for the removal of stones from coriander seeds. It works on the principle of minimum fluidization velocity differences of stones and coriander seeds, when the mixture is subjected to fluidization.

Keywords : Coriander seeds, Stone particles separation, Simple method, Fluidization technique, Minimum fluidization velocities, Material balance.

Coriander (*Coriandrum sativum*) seed is an important spice used in many common food preparations in the powder form. A number of studies have already been carried out on aflatoxin (Misra 1987) and insecticide residues (Jain et al. 1987) and also its microbiology (Krishnaswamy et al. 1975) to find its suitability for culinary use. A number of industries manufacture coriander powder. Contaminants like stones, sand and clay particles lead to the increase in the ash content of the powder, and may even damage the grinding mills. A survey conducted by us have shown that all the industries are using either manual method or the wet water washing method for the removal of stones and other contaminants. Manual method is used generally by tiny industries and the water washing method by small scale industries. Both the methods are highly labour intensive, while the latter needs energy for the drying of the resulting moist seeds.

A dry process is developed in our laboratory for the separation of stones and sand particles from coriander seeds, using the principles of fluidization with air as the medium (Thomas et al. 1991). The minimum fluidization velocity of material depends upon particle size and density (Kunii and Levenspiel 1977; Peeler and Huang 1989; Chyang et al. 1989). When a mixture of coriander seeds and stone is fluidized at minimum fluidizing conditions, the particles, with minimum fluidization velocity higher than those of coriander seeds, do not fluidize and settle at the bottom of the fluidization column. In contrast, those particles (fines) with minimum fluidization velocity less than or equal to those of coriander seeds get mixed up with the coriander seeds. These fine particles are smaller than those of coriander seeds, and can be removed by sieving.

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The minimum fluidization velocities and the separation efficiency of coriander seeds and stones were determined experimentally in a laboratory set up (Fig. 1). A stainless steel fine wire mesh distributor plate was fixed at the bottom of the glass column using the flanges. Material present in the portion below the hole is termed as bottom fraction, while that above the hole as top fraction. Material balance diagram is depicted in Fig.2.

Materials used in the study : Coriander seeds (moisture content 6%) were purchased from local market and cleaned to remove all contaminants. The particle size of coriander seeds varied from 2 to 5 mm. River sand (stones) was collected and segregated into three groups based on the sizes viz., (i) 2 to 3 mm (ii) 3.5 to 4.5 mm and (iii) 4.5 to 5.0 mm. Fine sand (beach sand) was segregated into two groups with sizes ranging from 426 microns to 1 mm and 426 to 300 microns, respectively.

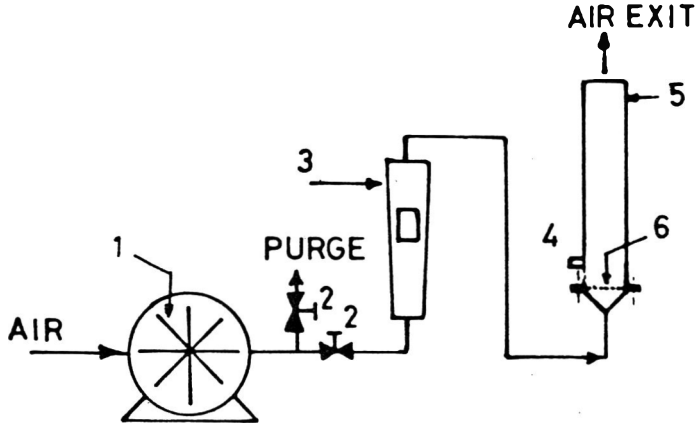

Fig. 1 Experimental set-up 1: Blower, 2: Control valves, 3: Rotameter, 4: Rubber Cork, hole, 5: Glass fluidization column, 6: Distributor plate.

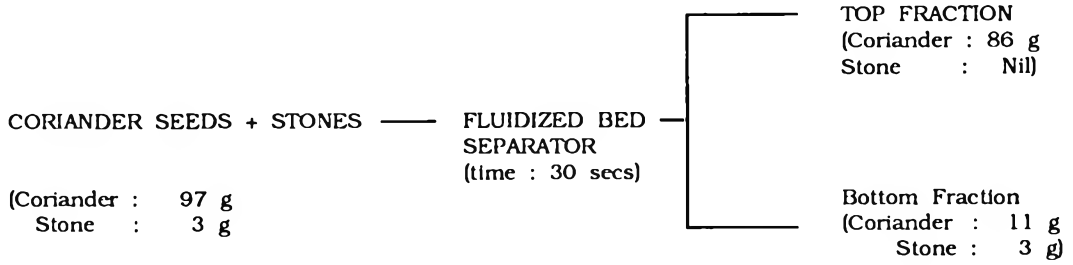

Fig. 2 Material balance diagram.

Determination of minimum fluidization velocity: One hundred g of material was poured into the glass column, and air was passed through the material by gradually increasing the flow rate, and the pressure drop was noted corresponding to the flow rates. The readings were taken till the pressure drop remained constant. The results are presented in Table 1.

TABLE 1. MINIMUM FLUIDIZATION VELOCITIES OF MATERIALS (100 g) BATCH SIZE

Material	Particle size range, mm	Minimum fluidization velocity, metres/sec
Coriander seeds	2-5	0.80
Stone	4.5-5.0	1.50
"	3.5-4.5	1.20
"	2.0-3.0	1.05
Beach sand	0.43-1.0	0.35
"	0.30-0.43	0.30

Separation of stones from coriander seeds : Weighed quantities of cleaned coriander seeds were thoroughly mixed with the stones of the specific size range and was fed into the column. Air was admitted into the column, and flow was regulated in such a way that the air velocity was slightly above the minimum fluidization velocity of coriander seeds. The fluidization was continued for 5 sec in the first experiment. After each experiment, the fluidization column was removed and the top and bottom fractions were separately collected through the side holes, and percentage (by weight) of stone in each fraction was determined. The results are presented in Table 2.

The data on minimum fluidization velocity show that stones above 2 mm size have minimum fluidization velocity higher than those of the coriander seeds. For sand particles of less than 1 mm size, the minimum fluidization velocity is less than that of the coriander seeds. As particle size increases, the minimum fluidization velocity also increases for material having the same density. It was observed that the coriander seeds fluidized and the stone particles settled at the bottom of the fluidization column, when the mixture of stone particles and coriander seeds was subjected to minimum fluidization. But, the sand and dust particles, with minimum fluidizing velocity less than those of the coriander seeds, did not settle down; but got mixed up with the coriander seeds. A simple sieving, with 2 mm sieve, removed all the sand particles. It could also be seen from Table 2 that the time of fluidization also increased for complete separation, as the quantity of material fluidized increased. For higher proportion of stones in coriander seeds (to the extent of 10%), the effect on the time of separation was only marginal.

The experiments were also extended to a bigger fluidization column of 250 mm diameter and 500

TABLE 2. EFFECT OF FLUIDIZATION TIME AND STONE CONTENT ON THE SEPARATION OF STONES FROM CORIANDER SEEDS

Quantity of coriander-stone mixed fluidized,	Stone in coriander, %	Volumetric air flow rate, l/min	Time of fluidization* sec			Quantity of stones* (>2mm) in top fraction, g		
			a	b	c	a	b	c
50	3	85	5	10	20	0.026	0.014	Nil
50	6	85	5	10	20	0.4	0.25	Nil
50	10	80	-	10	20	-	.036	Nil
100	3	92.5	10	20	30	1.3	0.3	Nil
100	6	92.5	10	20	30	2.7	0.4	0.02
100	10	92.5	10	20	30	1.9	0.1	0.031
150	3	100	-	20	30	-	1.3	0.4
150	6	100	-	20	30	-	4.8	0.1
150	10	97.5	20	30	50	9.1	0.7	Nil

*a, b, c in the two columns correspond to each other

mm height, which could fluidize a batch size of 5 kg. Coriander seeds from different sources were subjected to minimum fluidization in batches of 5 kg. In all the cases, it was found that the fluidization time of 5 min followed by the sieving of top fraction, using 2 mm size sieve was sufficient for complete separation of stones and sand particles from the coriander seeds.

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Colour Changes During Drying of Apricot

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Effect of pretreatments viz, heat application, chemical dipping and sulphur fumigation, during drying of apricots to prevent discolouration was investigated. Maximum yield was recorded in control followed by steam-blanched fruits. Lye-peeled apricots retained lower moisture than steam-blanched fruits. Maximum and minimum brownings were observed in untreated samples and the fruits subjected to sulphur fumigation, respectively. Non-enzymatic browning showed statistically significant differences among varieties and treatments. 'Chuli' - a wild variety had the maximum browning, while 'Suffaida' the least. Among various pretreatments, sulphur fumigation and lye-peeling were more effective than the rest, while 'Kaisha' variety was rated as the best.

Keywords : Apricot, Pretreatment, Sun-drying, Dehydration, Residual SO_2, Discolouration.

Apricot is extensively grown in North and North-Eastern India. A few good varieties are also grown in Himachal Pradesh. The annual production in India is 19000 tonnes (FAO 1988). Because of short harvesting season and sensitivity to storage at low temperature, apricots are preserved in different forms (Woodroof and Luh 1985). The physical and chemical changes that take place have been studied (Sharma et al. 1992) and the biochemical changes that take place on storage have also been studied (Deshpande and Salunke 1969). Drying is one of the commonly used methods (Bolin et al. 1983). About 19% of total production of apricot is dried, of which 15% is mechanically dehydrated, in cross-flow dryer, while remaining are sun-dried either as whole or as halves. Changes in colour and water-holding capacity occur during dehydration, and are responsible in causing deterioration of the quality of dehydrated fruits. Browning reaction is a major reason for colour change in fruit and possible impairment of flavour and nutritive value (Stadman 1948). Effects of dehydration processes on flavour compounds and texture characteristics of apricots have been reported by Lee et al (1966). This communication presents data on the changes in colour of dried apricots under a range of conditions, following sun-drying and dehydration.

Three Himachal grown cultivars of apricot viz., 'Charmaghz', 'Kaisha' and 'Suffaida' and one wild variety 'Chuli' were purchased from the orchards of Kinnaur District. Firm, mature and medium-sized fruits were selected. Apricots were washed thoroughly in running water and divided into 2 lots. One lot of fruits was peeled in 1% boiling lye for 30 sec, followed by washing under running water. Another lot of apricots was steam-blanched for 3 min, followed by cooling in water.

Peeled and blanched fruits were further divided into 2 lots each. One lot each was sulphured in sulphur chamber by burning sulphur at a rate of 5 g/kg of prepared fruit for 1 h, and second lot was dipped in 0.4% potassium metabisulphite solution for 15 min. Fruits, prior to sulphuring and sulphiting, were dipped in water, containing a thin layer (2-3 mm) of wild apricot oil to prevent sticking during drying. Pretreated fruits were loaded in perforated trays at the rate of 8 kg/m^2, and were divided into 2 lots. One lot was dehydrated in a cross-flow dryer at 50±2°C, and the second lot was sun-dried (day temperature 30-32°C with 14 h day light). In both the cases, the dehydration of fruits was carried out to a recommended moisture level of 20-24%. Fruits were packed in 300 gauge polyethylene bags, soon after drying, and sealed. Dehydrated fruits were drawn periodically for studies on colour changes, during 180 days of storage at ambient conditions (20-40°C and 85-90% R.H.), at an interval of 45 days. One lot of apricot, which was not given any pretreatment was used as control.

Moisture in the dried fruit samples was determined by drying 10 g of shredded dried samples to a constant weight in hot air oven. Sulphur dioxide (SO_2) was determined by Monier Williams method (AOAC 1985). The dried samples were evaluated for non-enzymatic browning by the method given by Ranganna (1986). One g each of

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the dried fruit was treated with 15 ml of 70% alcohol for 12 h at room temperature and filtered. Intensity of the filtrate was measured at 440 nm in Spectronic 20 (spectrophotometer, Bousch and Lomb) against alcohol as blank.

The results are expressed on moisture-free basis, and were analysed statistically following the procedure of Snedecor and Cochran (1967). Results reported are the means of duplicate samples.

The drying time in cross-flow dehydrator was about 36 h, as against that of 96-108 h, required for sun-drying. Table 1 shows yield of dried apricot, which was maximum in control, followed by steam-blanched apricots. The yield, in general, were low in 'Suffaida'.

There were appreciable differences in moisture levels in dried fruit (Table 1). 'Charmaghz' had relatively low moisture content, while 'Chuli', the highest among the varieties. Average moisture content was not affected much, due to methods of drying. It was noted that lye-peeled and sulphite-treated apricots differed significantly from all other treatments with respect to moisture level under similar drying conditions. Lye-peeled apricots,

TABLE 1. EFFECT OF VARIETY, TREATMENT AND DRYING METHOD ON MOISTURE CONTENT, RESIDUAL SULPHUR DIOXIDE AND NON-ENZYMATIC BROWNING OF DRIED APRICOTS AT ROOM TEMPERATURE.

Variety	Treatment										CD (0.05)	
	Lye peeling and sulphuring		Lye peeling and sulphiting		Blanching and sulphuring		Blanching and sulphiting		Control			
	S	D	S	D	S	D	S	D	S	D		
					Yield %							
'Charmaghz'	21.3	20.8	21.7	23.5	25.2	29.1	25.6	28.4	32.2	30.5		
'Chuli'	21.8	22.7	23.5	21.4	25.8	23.4	26.8	24.5	30.5	31.3		
'Kaisha'	22.1	20.1	23.2	22.3	29.4	23.4	26.5	21.6	33.5	27.0		
'Suffaida'	16.3	18.0	17.0	18.8	19.3	19.0	17.9	20.0	32.5	23.9		
					Moisture %							
'Charmaghz'	21.5	19.8	18.6	21.2	21.3	22.7	20.4	22.1	21.9	17.7	V	0.035
'Chuli'	24.4	21.0	23.1	20.0	21.3	22.6	23.4	22.6	19.6	21.9	T	NS
'Kaisha'	21.8	22.0	20.9	21.1	22.2	19.7	24.1	23.1	21.2	24.8	M	0.25
'Suffaida'	23.1	22.6	18.3	20.6	22.4	20.4	20.5	18.9	21.2	23.7	V × T	0.79
											T × M	0.79
											V × M	0.50
					Residual SO_2 % moisture free basis[a]							
'Charmaghz'	89.1	91.2	91.4	91.6	90.0	90.5	89.4	89.4	-	-	V	1.40
	(2310)	(2425)	(2150)	(2225)	(2050)	(2210)	(1800)	(2150)	-	-	T	0.99
'Chuli'	82.2	86.2	83.6	85.7	80.0	83.7	80.6	83.2	-	-	M	0.99
	(2120)	(2300)	(2200)	(2150)	(2115)	(2150)	(1880)	(2000)	-	-	V × T	NS
'Kaisha'	88.2	90.2	88.6	89.4	87.6	88.0	88.5	87.2	-	-	V × T	NS
	(2350)	(2560)	(2010)	2350)	(2075)	(2570)	(1750)	(2150)	-	-	V × M	1.95
'Suffaida'	85.9	89.0	83.4	89.7	82.5	89.6	83.3	88.8	-	-		
	(2230)	(2350)	(1975)	(2150)	(2150)	(2350)	(1800)	(1900)	-	-		
					Non-enzymatic browning, moisture free basis[b]							
'Charmaghz'	0.29	0.22	0.34	0.25	0.36	0.23	0.39	0.30	0.58	0.51	V	0.02
	(0.2)	(0.18)	(0.31)	(0.23)	(0.31)	(0.20)	(0.35)	(0.3)	(0.54)	(0.48)	T	0.01
'Chuli'	0.36	0.29	0.36	0.39	0.39	0.39	0.44	0.34	0.69	0.69	M	0.01
	(0.32)	(0.27)	(0.35)	(0.29)	(0.36)	(0.33)	(0.4)	(0.3)	(0.6)	(0.6)	V × T	0.04
'Kaisha'	0.25	0.16	0.29	0.19	0.37	0.19	0.35	0.22	0.62	0.45	T × M	NS
	(0.18)	(0.09)	(0.24)	(0.15)	(0.28)	(0.18)	(0.31)	(0.2)	(0.45)	(0.41)	V × M	0.01
'Suffaida'	0.25	0.19	0.26	0.19	0.24	0.29	0.27	0.34	0.46	0.48		
	(0.18)	(0.18)	(0.17)	(0.13)	(0.18)	(0.28)	(0.25)	(0.3)	(0.43)	(0.48)		

a = values within parenthesis denote intial SO_2 content in ppm b = values within parenthesis denote initial OD at 440 nm
S = Sun-dried, D = Dehydrated; V = Variety; T = Treatment; M = Drying Method

however, retained low moisture than steam-blanched ones.

The results of residual sulphur dioxide (SO_2) in dried fruits (Table 1) indicated that varieties behaved differently in retention of SO_2. Drying methods had significant effects on SO_2 retention. Cross-flow dehydrated samples seemed to have retained more of SO_2, than the sun-dried samples, and the former retained their characteristic colour during prolonged storage. This is similar to the findings of Mc Bean et al. (1964), who reported that 5% SO_2 might be retained under slow drying conditions and uptc 40%, if water removal was very rapid. Sulphur-fumigated fruits showed higher retention of SO_2 and were found to prevent the loss of ascorbic acid, and inhibit browning during drying and storage, than sulphite-treated samples. Lye-peeling and sulphur-fumigation treatment had been found to retain the maximum percentage of SO_2. Most of the sun-dried samples, containing low SO_2 initially, showed low residual SO_2 values on storage. On the other hand, dehydrated samples treated with SO_2 showed retention of reasonably high quantity in proportion to the initial concentration of SO_2, even upon storage. During storage for 180 days, continuous reduction in the total SO_2 was observed.

Optical density (OD), which represents the index of browning of dried fruits was affected by all treatments and methods of drying. The alcoholic extracts of fruits, dehydrated in hot air, gave the lowest OD, while those of sun-dried samples, the highest. Maximum browning were recorded in control samples and minimum in samples pre-treated with SO_2. This could be attributed to the fact that pretreated dried fruits had more residual SO_2. Browning was found to decrease with sulphur fumigation. Of varieties, 'Chuli' had the maximum browning and 'Suffaida' the least. Browning increased in the dried fruits with storage. Samples with relatively high residual SO_2 were found to have little increases in OD upon storage of dried fruits. The experiment supports the hypothesis that the browning index is inversely proportional to the residual SO_2 in the stored product (Mc Weeny et al. 1969)

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Physical Chemistry of Foods : Edited by Henry G Schwartzberg and Richard W. Hartel. Published by Marcel Dekker Inc. New York, pp: 747, price US $ 69.75, 1992

To meet the demands of teaching and reference materials dealing with physical chemistry of foods, an important symposium on the different aspects of the subjects was organised by International Union of Food Science and Technology (IUFST) in 1991. Major topics covered in this symposium are published in this book, and the areas of physical chemistry covered are phase equilibria, reaction kinetics, physical chemistry of gels and disperse systems and physico-chemical effects occurring during consumption of food and their use.

In chapters 1 and 2, thermodynamic basis of vapour liquid equilibria, solid-liquid equilibria and crystallization phenomena have been discussed. Statistical-mechanical theory based on lattice models and UNIQUAC equations for interpreting excess Gibbs thermodynamic functions have been examined, with rigour to treat experimental data on solutions of different types of carbohydrates used in foods. Keeping carbohydrate-water mixture in view, phase diagrams, crystal formation, solubility, supersaturation, metastability and nucleation phenomena for different food systems have also been critically examined on the basis of laws of thermodynamics. In chapter 3, glass transitions in different food polymer systems have been extensively discussed from the physico-chemical treatment of synthetic polymer chemistry, earlier developed by Flory and others. The importance of glass transition temperature for storage stability of foods, kinetics of heat/moisture processes for foods, "glass dynamics" and "water dynamics" in food science have been critically examined from the standpoint of physical chemistry. In chapters 4-7, physico-chemical aspects of surface thermodynamics based on contact measurements equilibrium and kinetic aspects of protein adsorption, production of gels and theories of gelation, mechanical properties of gels, theories on emulsion stabilities, steric stabilization of emulsions, coalescence and flocculation of emulsions, solid and protein stabilized emulsions have been well presented and their applications to the development of food products have been extensively referred. In chapters 7-9, engineered structures of gels, physico-chemical behaviour of muscle tissue and bread dough have been described in detail under variety of conditions, which may be of considerable technological importance. Physico-chemical behaviour of variety of food nutrients, roles of olfaction and taste in flavour detection and disintegration and segregation kinetics of dry food particulates have been elaborated in chapters 8 to 12. Based on our knowledge on physical chemistry of polymeric materials, mechanistic basis of rheological behaviour of foods has been critically examined in chapter 13. Two chapters (chapters 14 and 15), on the kinetics of non-enzymatic browning and kinetics of lipid oxidations, are of interest to the R&D scientists in food science and food engineering. Chapter 16 dealing basically with thermal denaturation of proteins, is quite interesting to biological chemists in particular and food scientists, in general. The role of protein denaturation by heat is a very important factor in the formulation and production of many protein-rich foods.

There is no book available so far, which deals in detail and rigour the application of principles of physical chemistry to different types of model or actual food systems. The publication of the present book in the light of this observation is most welcome. Different authors in this book have written different aspects of physical chemistry, related to food with enormous number of current and relevant references so that the book will be useful to scientists carrying out research in a particular branch of Food Science. The food technologists may also find guidance from this book for model or actual designing of food products. Elegance of physical chemistry lies in clarity of fundamental concepts of different simple and complex systems under consideration and also in rigorous mathematical approach of the derivation of equations used for explaining physico-chemical phenomena. The balance between these two aspects in dealing with 16 different chapters is, however, not well-maintained, but every chapter is self-complete with relevant and concise discussions and critical comments at the end. In our opinion, additional chapters on electrochemistry and spectroscopy in relation to Food Science could have been introduced in this book for further understanding. The book, in its present form, may be recommended as an advanced text for a new course on "Food Physics" or "Food Physical Chemistry" in M. Tech or M.Sc. levels in Food Technology or Food Science in Indian Universities with a perquisite of one semester course on general physical chemistry in undergraduate level.

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Food poisoning handbook of natural toxins (Vol. 7): Edited by Anthony, T.Tu. Marcel Dekker Inc, New York, 1992, pp. 656. Price : US $185

This book is the 7th volume of a comprehensive Handbook on Natural Toxins. The previous volumes had covered several natural toxins from fungal, bacterial, animal and marine sources. The present volume deals mainly with toxins present in human food, which are principally derived from plant sources. Some of the plant and fungal toxins have also been described in volume 1 of the series.

The present volume deals mostly with toxins present naturally in foods as well as those arising from contamination by bacteria and fungus, and from the environmental sources. The foods discussed cover not only plant foods, but also foods of animal origin like fish, meat, milk and eggs, which carry toxins derived from feeds. The food toxins discussed in the present volume are covered in 10 parts and 20 chapters written by 34 experts in the field. Each part deals with toxins with common characteristics, while chapters discuss individual food or toxin.

Food poisoning due to toxins has been discussed as arising from three main sources. Pertaining to the first category are the toxins elaborated by bacteria or fungi contaminating the foods, due to unhygienic and improper handling of the foods. These toxins from the microbial sources are more of a public health or post-harvest food storage problem. These problems are discussed in parts II and III and the public health aspects are discussed in part I. The second category of toxins is the wide range of chemical compounds naturally present in foods, like alkaloids, lectins, cyanogenic compounds, phenolic compounds and other chemically identified toxins. Some of the chemicals with beneficial function, like nutrients, which can become toxic, when ingested in excess are also discussed. The third category covers contamination of foods from the external sources, accidentally entering the food chain or wilfully added, viz., foreign seeds, pesticides, antibiotics, nitrates, heavy metals etc. Some of these toxic compounds are present in the food in the form of precursors or glycosides, which are converted into their active form in the body.

Each class of toxins has been described in great detail with regard to their chemistry, mode of action and manifestations of their toxicity like enterotoxicity, teratogenicity, allergy, neurotoxicity, goitrogenicity and hepatic, pulmonary and renal toxicity. The metabolism, tolerance, method of their detection and estimation and methods of prevention of their toxicity are discussed in detail. The effects of heat, cooking and processing on inactivation of the toxins or in reducing their levels are also described. Emphasis on these aspects in the discussion of a toxin varies from one toxin to the other, perhaps depending upon the interest of the author. The foods which are discussed in the present volume are the habitually consumed ones by man, and the toxic manifestations are not routinely seen. This is because toxicity of any compound depends upon its level in the food, its toxicity potential and its metabolism and detoxification in the body. At low levels normally present in many foods, they may not exhibit toxicity in normal individuals, but they may do so in susceptible individuals. Such susceptibility is often of genetic origin, which is particularly seen in the case of allergic manifestations.

The problems of food toxicity and food poisoning described in this book relate mostly to the conditions prevailing in the developed countries of the world. Since public awareness and public health and environmental health standards have attained high levels in these countries, incidence of food poisoning may not be really very high, and may not pose much of risk to the population. The present book hardly covers the situation with regard to food toxicity and food poisoning prevailing in the developed countries. Due to widespread poverty and low education levels, poor environmental and personal hygiene, diseases, particularly the enteric ones due to food and water contaminants, are much more widespread in these countries, and contribute significantly to the morbidity and mortality among their population. Widespread under-nutrition may further aggravate the manifestation of toxicity. Considerable information on these aspects based on researches carried out in these countries do exist both on food - borne diseases and the endemic diseases due to toxins present in staple foods. Some aspects of these food toxicity problems prevailing in the developing countries should have been covered. Such information may provide more insight into the human aspect of the food poisoning problem.

Notwithstanding this limitation, the present book provides a lot of useful information on various types of food toxins and poisons, their chemistry metabolism and toxic manifestations. The book wil be most useful to a wide range of readers, especiall

food toxicologists, nutritionists, dieticians, and public health workers. This is a most welcome addition to the currently available book on food toxicity and food poisoning.

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Fenitrothion: Environmental Health Criteria-133. Published under the joint sponsorship of the UNEP, ILO and WHO. Drafted by Dr J. Sekizawa (Japan), Dr M. Eto (Japan), Dr. J. Miyamoto and Dr. M. Matsuo (Japan) under the International Programme on Chemical Safety (IPCS) 1992, pp 184; Price SW Fr. 22 (15.4 for developing countries).

This report is an outcome of the meeting of the WHO task group on Environmental Health Criteria for fenitrothion, held in Geneva, from 10 to 14 December, 1990. It comprises of following 10 sections; 1 Summary and evaluation; conclusions; recommendations; 2 Identity; Physical and chemical properties; Analytical methods; 3 Sources of human and environmental exposure; 4 Environmental transport, distribution and transformation; 5 Environmental levels and human exposure; 6 Kinetics and metabolism; 7 Effects on experimental animals and *in vitro* systems; 8 Effects on man; 9 Effects on organisms in the environment; 10 Previous evaluation by international bodies followed by 446 references; Annexure I treatment of organophosphate poisoning in man; Annexure II No-observed effect levels in plasma, red blood cells and brain ChE, in animals treated with fenitrothion, resume given in two European languages.

The methods recommended by FAO/WHO Codex Alimentarius Commission 1989 are given in section 2. Section 3 gives data on production of fenitrothion and in this, India figures as one of the few fenitrothion manufacturing countries. In section 4, data on transport, distribution and transformation have been reviewed. Fenitrothion does not appear to be transported in significant amounts in soil or water. Its transformation is similar to that of parathion. Breakage of ester bond is the main degradation pathway. Non-enzymatic transformations like thermal decomposition, photolysis in air and water, hydrolysis in water are described. Bio-transformation in microorganisms is mainly through reduction of nitro group to amino group. In aquatic organisms like algae, crustaceans, fish and molluscs, it mainly undergoes demethylation and hydrolysis. The product 3-methyl-4 nitrophenol gets conjugated. There is no report of its accumulation in any organism. Contrary to it, in terrestrial organisms, demethylation is not an important pathway of its metabolism, instead, it undergoes oxidation to form oxon analogue, the ester bond of which gets hydrolysed. In plants, fenitrothion breaks up into several products including S-methyl fenitrothion and oxon, the two have higher mammalian toxicity than the parent compound; demethylation is the main detoxication mechanism in plants.

The systemic action of fenitrothion and high-persistence in balsam fir, white spruce and jack pine are significant. At high fenitrothion concentration, the dimethyl fenitrothion undergoes non-enzymatic alkylation in conifer seeds, giving rise to S-methyl fenitrothion. Similar results have not been reported for food plants, otherwise, residues of S-methyl fenitrothion residues in food will have to be regulated.

Fenitrothion does not appear to persist in environment or in significant quantities in food. It is excreted rapidly from the body, and does not appear to have any adverse effect on experimental organisms on long exposures. It is non-mutagenic. Its lower toxicity to mammals in comparison to methyl parathion appears to be due to its faster metabolism. Interestingly, it potentiates the toxicity of phosphamidon and malathion to mammals. It does not appear to have any significant adverse effect on man or organisms in the environment, provided it is used judiciously, taking all the normal precautions.

The last section deals with its Acceptable Daily Intake (ADI); No Effect Levels on rat, dog and man; FAO/WHO Codex Committee advised maximum residue limits (MRLs) in specific food commodities.

This report on fenitrothion would be very useful for all the research workers, who are engaged in research on pesticides, especially to insecticide toxicologists, pesticide residue/ formulation analysts, synthetic chemists, and graduate students. It will be useful for bureaucrats and policy makers in state plant protection agencies and in Ministries of Agriculture and Food, Health and Social Welfare. It was my proud privilege to

be able to go through this invaluable information on fenitrothion.

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Spices and condiments - Fourth Edition : by J.S. Pruthi, National Book Trust, India, A. 5, Green Park Road, New Delhi-110 016,1992; pp 288; Price : Rs. 46.

This is the fourth edition of the book "Spices and Condiments" first published in 1976. The very fact that the fourth edition has been brought out speaks volumes of the popularity of this book. The author has been a leading researcher on various technological aspects of spices, and his intimate knowledge of spices has helped in bringing out this commendable publication. However, the list of publications (bibliography) is very inadequate and incomplete. Although the author has referred in the text to CFTRI work in certain places, he has left out the bulk of useful publications from CFTRI, while preparing the bibliography. The author claims that he has revised and updated the information presented. However, on perusal of the book, one is tempted to think that no serious effort has been made in this direction. The author admits (in the preface to the first edition) that the publication is a non-technical or semi-technical one, though he has made in-depth coverage of technological aspects in certain cases (e.g. garlic, ginger, pepper). The book is very useful to the educated layman, as it provides general information on almost all spices viz., description and distribution, botanical names and varieties, vernacular names, quality, composition and uses. The present edition contains colour photographs of major spices grown in India. The PFA quality standards for spices form a useful annexure to the book. The glossary of technical and botanical terms should be very useful to the reader. The book is well written and highly readable. It should serve as a useful handbook on spices, and is a welcome addition to the literature on spices and herbs.

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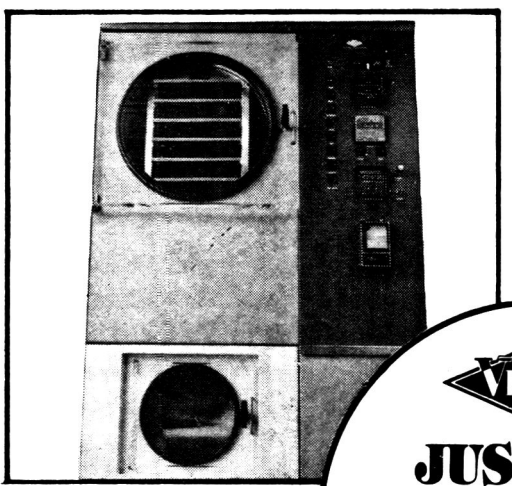

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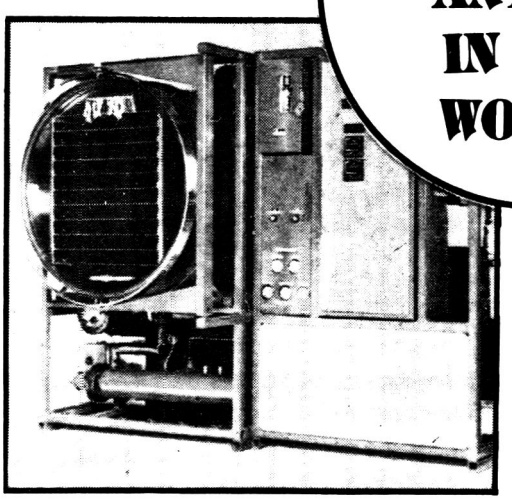

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