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ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA) MYSORE - 570 013

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C O N T E N T S

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Biochemical Methods for Determination of Spoilage of Foods of Animal Origin : A Critical Evaluation

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Quality evaluation plays an important role in assessing the storage life of flesh foods during storage. Although several biochemical methods, based on the end-products of catabolism of proteins, carbohydrates, lipids and nucleic acids are available, the varied compositional nature of flesh as well as microfloral activities make none of the methods acceptable universally for all flesh foods. Several methods based on microbial metabolic end-products as well as their limitations are evaluated critically for their advantages and limitations. Assessment of freshness of irradiated flesh foods by biochemical methods during storage poses a challenge to food scientists and technologists, as gamma-radiation inactivates spoilage bacteria present in foods, thereby preventing putrefaction.

Keywords : Flesh foods, Biochemical methods, Mechanism of spoilage, Protein breakdown, Nucleotide degradation, Limitations.

Quality of flesh foods is evaluated during storage by sensory, microbiological and biochemical methods (Hillig 1963; Stroud et al. 1982; Ohashi et al. 1991). Though, microbiological methods for determining freshness of flesh foods are of value, the long incubation period required and varied composition of microflora do not make them attractive indices (Shewan 1977; Hobbs and Hodgkiss 1982). Sensory evaluation is important to determine the degree of freshness (Ryder et al. 1993). Being a subjective evaluation, it can not be used singly (Nunes et al. 1992). However, coupled with other methods, sensory evaluation forms an important quality index (Gill 1992). Even the biochemical methods, often employed to determine freshness of flesh foods, can not be singularly accepted as universal (Fields et al. 1968), due to the complex nature of flesh foods (Edwards et al. 1983). Hence, depending upon the composition of flesh with respect to its proteins, carbohydrates, lipids and nucleic acids, as well as coupled with composition of microflora, several biochemical methods have been devised to measure the degree of spoilage of flesh foods (Bryant et al. 1973; Castell et al. 1973; Vyncke 1978; Edwards et al. 1985; Fatima and Qadri 1985).

This review discusses the methodologies available to determine the freshness of flesh foods. The mechanisms involved in the formation of end-products arising from the metabolism of spoilage microorganisms are given to provide a background information, with a view to assess the limitations and advantages of the methods.

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Biochemical changes in seafoods

Lean fishes, such as cod and haddock comprise proteins (18-20%), fat (1%), carbohydrates (1%) and water (80%), whereas fatty fishes such as herring and mackerel have proteins (15-20%), fat (3-25%), carbohydrates (1%), and water (70%) (Hobbs and Hodgkiss 1982).

The metabolic activities of the microorganisms thriving on fish produces different compounds from the chemical constituents of fish, which could be used as indicators of freshness (Hashimoto 1965). Seafoods being rich in proteins, the breakdown products serve as useful parameters of freshness (Eskin et al. 1971). Different off-odorous compounds formed during storage are indeed contributed by the action of fish microflora on chemical constituents of the fishes (Shewan 1977).

Histamine produced by decarboxylation of histidine correlated well with the organoleptic properties of canned fish (Arnold and Brown 1978; Niven et al. 1981; Storey et al. 1984).

The phosphorylated compounds and their degradation products serve as good indices of quality of fishery products. The change from ATP to inosine followed by hypoxanthine is of both autolytic and bacterial origin. Hence, they could serve as reliable indices of freshness during storage of fish. Xanthine oxidase assay has also been used to assess the storage life of fish (Jones et al. 1964; Greene et al. 1990). A good correlation was found between concentration of hypoxanthine (Hx) and sensory scores in shrimp and snapper during prolonged storage in ice (Boyd and Wilson 1977; Fatima et al. 1981).

Total volatile basic nitrogen (TVBN)

Among the major compounds which contribute to the formation of total volatile basic nitrogen in spoiled fish are amines and ammonia. Ammonia, produced by deamination, decarboxylation and oxidation processes of amino acids by microbial enzymes was found to increase with spoilage of crab meat, haddock and herring (Vyncke 1978). Volatile bases produced from bacterial enzymatic decarboxylation of amino acids in tissue (Hashimoto 1965; Eskin et al. 1971) are also used to monitor terminal phases of deterioration of flesh foods (Thimann 1963). The method of determination of TVBN is as follows :

To an aliquot of the sample, an equal volume of 10% trichloroacetic acid (TCA) is added, allowed to stand for 15 min, and filter through a Whatman No. 1 filter paper, TCA extract, thus, obtained is used to determine TVBN by the method of Farber and Ferro (1956). One ml of the reagent [2% boric acid containing 0.1% solution of bromocresol green in alcohol (4%), and 0.1% alcoholic solution of methyl red (4%)] is added to the inner well of Convey unit. In the outer well, 1 ml of TCA extract is added, followed by addition of 1 ml saturated potassium carbonate (K_2CO_3). The unit is then closed immediately with a ground glass plate smeared with grease, and incubated at room temperature for 3 h. The reagent in the central well is then back-titrated with 0.002 N ammonium sulphate.

Measurement of TVBN would be a useful indicator of decomposition, but this would be of little use as an indicator of early freshness (Ohashi et al. 1991).

Trimethylamine (TMA)

Trimethylamine oxide (TMAO), which is found in many species of marine fish, is a part of the osmoregulatory system of gadoid and elasmobranch family (Love 1970). Trimethylamine (TMA) is formed by reduction of TMAO by bacterial TMAO reductase, coupled with oxidation of lactic acid to acetic acid and carbon dioxide (Regenstein et al. 1982). Triamine oxidase has been reported in cells of bacteria from spoiling fish muscle (Colby and Zatman 1973). All the genera in the family *Enterobacteriaceae*, with the exception of *Shigella* and *Erwinia* were able to reduce TMAO to TMA (Fields et al. 1968).

Dyer's method (1945) is employed for the estimation of trimethylamine nitrogen. Trichloroacetic acid (TCA) extract (4.0 ml) is placed in a stoppered test tube, and mixed with 1 ml formalin (4%), 3 ml saturated potassium carbonate and 10 ml distilled toluene. The tubes are shaken vigorously to mix, and the layers are allowed to separate. About 7-8 ml toluene is removed by a pipette, and placed in another tube. A pinch of anhydrous sodium sulphate is added to remove moisture. Toluene extract (2.5 ml) is then transferred to another tube, and treated with equal volume of 0.02% picric acid in toluene. The intensity of yellow colour developed is measured at 420 nm in spectrocolorimeter. The corresponding values of TMAN are read out from a standard curve, prepared by using trimethylamine hydrochloride. The values of TMAN are expressed as mg of TMAN per 100 g fillet.

Levels of TMA formed serve as an excellent quality index for cod, haddock, ocean perch, flounder and shrimp, which correlated well with sensory judgements (Fields et al. 1968; Shewan et al. 1971). However, TMA has no value in monitoring the loss of freshness in fish during the initial stages of storage in ice (Ryder et al. 1993).

Total volatile acids

Volatile fatty acids (VA) are derived either from fats upon hydrolysis of fat by lipolytic bacteria, or from proteins by deamination of amino acids (Thimann 1963), or from carbohydrates via glycolysis and Krebs cycle (Beddows et al. 1980) can serve as an useful index of quality.

Fish homogenate (30 g) made into a slurry with 90 ml water is treated with 15 ml 1 N H_2SO_4, and 24 ml phosphotungstic acid (15%). The precipitated proteins are discarded, and the filtrate (15 ml) is steam-distilled for 10 min. The steam-distillate (40 ml) is titrated against 0.01 M sodium hydroxide. VA number is expressed as ml of 0.01 N alkali required to neutralize the acids from 100 g fish (Venugopal et al. 1981).

A good correlation was found between volatile fatty acids formed with the spoilage of *budu* and Indian mackerel during storage (Beddows et al. 1980; Venugopal et al. 1981).

Volatile reducing substances (VRS)

Volatile reducing substances (VRS) include several compounds, such as acetyl methyl carbonyl (AMC), diacetyl and hydrogen sulphide (H_2S) (Dainty et al. 1985). The VRS method measures the entire odour complex, and it is independent of a single compound (viz., indole, H_2S), or a class of compounds such as volatile acids and volatile bases. The VRS value of more than 20 units indicates definite

spoilage of fish (Farber 1965).

The homogenate is prepared by blending 50 g of drained meat in a Waring blender for 3 min, and 0.2 ml of it is then pipetted into a 50 ml-Erlenmeyer flask for testing. Ten ml of N/50 $KMNO_4$ in NaOH is then pipetted into the reaction flask (45 min or 1 hr.). The reduction of the permanganate by volatile compounds from the sample is revealed by a change from the purple potassium permanganate to the green potassium manganate. This change can be measured either volumetrically or spectrophotometrically. A time standardization procedure should be evolved to ascertain this difference by plotting standard $Na_2S_2O_3$ needed for titration against reaction time (0-40 min).

After the reaction is complete, 5 ml of 6 N H_2SO_4 is pipetted into the reaction flask. The contents are stirred, and 3 ml of 20% KI is added. The liberated iodine is then titrated with N/40 $Na_2S_2O_3$, using starch solution as an indicator. The control of the unreacted permanganate is treated in the same manner as the sample (Farber and Ferro 1956).

μeq per 0.2 ml of sample = [Titration for control - titration for sample x 25]

where 25 = Micro equivalents (μ eq) per ml of N/40 $Na_2S_2O_3$

Lactic acid determination

Lactic acid accumulates during post mortem changes, because of the glycolytic conversion of storage glycogen in the fish muscle after the cessation of respiration (Tarr 1966). Lactic acid is derived from hydrolytic deamination of alanine, and succinic acid is formed by reductive deamination of aspartic acid (Nassos et al. 1985).

The method for determining lactic acid in tissue consists of homogenizing 400 g muscle tissue for 1 min in a homogenizer with a metal chopping disk. An extract is prepared from 50 g of the homogenized meat at 0.2 N HCl. The extract is esterified with boron trifluoride in propanol (BF_3–propanol), and the resulting derivatives of lactic acid is measured by gas chromatography. The lactic acid values are expressed as mg lactic acid per 100 g wet weight of lean tissue (wet weight of sample minus percent fat) (Nassos et al. 1983, 1984).

The measurement of lactic acid in fish flesh as an index of quality had little success due to wide variations, and difficulties in analysis (Eskin et al. 1971). Therefore, the measurement of lactic acid is included, as a part of a titration for total volatile acids.

Determination of indole

Indole, a product formed by the bacterial tryptophanase, has been used as an index of spoilage of fish. (Cheuk and Finne 1981).

Fish (40 g) is homogenized by adding 80 ml TCA solution in a Waring blender for 1 min. Ice-cold light petroleum (80 ml) is then added and blended for 1 min. Homogenate is transferred to 250 ml centrifuge bottle, and centrifuged for 10 min at 10,000 rpm. Supernatant is filtered through a Whatman No. 1 filter paper under slight suction. The filtrate is transferred to 250 ml separatory funnel. After the two layers have separated, the acid layer (lower) is transferred to a second 250 ml separating funnel. All light petroleum extracts are then combined into a separating funnel, and indole is extracted with 5 ml freshly prepared Ehrlich's reagent by vigorously shaking for 1 min.

Ehrlich's reagent is prepared by dissolving 9 g p-dimethylaminobenzaldehyde (PABA) in 45 ml concentrated HCl in 250 ml volumetric flask, and diluting to volume with ethanol (Cheuk and Finne 1981).

Ehrlich's reagent layer is read at 570 nm against a reagent blank. If the solution is not clear, it may be centrifuged before reading in spectrophotometer. Indole concentration is determined from the standard curve.

Standard curve is prepared as follows : Volumes from 0.5 to 4 ml (5 to 40 μg) stock indole solution are measured into 80 ml TCA in a separating funnel. Indole is re-extracted according to procedures described above, and a standard curve is constructed. Stock solution of indole is prepared in 100 ml light petroleum. A dilution of 1:10 is used as working solution.

Determination of indole in flesh foods has limited value, since only a fraction of total microflora can produce the enzyme, tryptophanase (Hillig 1963).

Determination of histamine in meat products

Histamine poisoning is a foodborne intoxication, resulting from the consumption of foods that contain high levels of histamine, as a result of L-histidine decarboxylation by bacteria (Arnold and Brown 1978; Taylor 1986). Biogenic amines such as cadaverine, and putrescine may potentiate the uptake of histamine (Lyons et al. 1983). Hence, their determination in flesh foods is essential to control scombrotoxic fish poisoning.

Spectrophotometric method of determining histamine developed by Vidal-Carou et al (1989) is described below :

The sample is ground, and homogenized to extract histamine with $HClO_4$. Five ml of perchloric acid extract is alkalinized to extract histamine with n-butanol. The extracted histamine is then transferred to HCl. Fluorescent complex formed with O-phthaldehyde is read at 350 nm excitation wavelength in a spectrofluorometer.

Histamine produced by decarboxylation of histidine (Niven et al. 1981; Storey et al. 1984) correlated well with the organoleptic properties of canned fish.

Determination of tyramine

The spectrofluorometric method of determination of tyramine developed by Santos-Buelga et al (1981) consists of grinding, alkalinization and homogenization of sample with anhydrous Na_2SO_4. Tyramine is extracted with ethylacetate in a glass column. Tyramine is transferred to HCl. Fluorescent complex formed with N-nitroso-β-naphthol is read at 450 nm excitation wavelength, and 540 nm emission wavelength in spectrofluorometer (Vidal-Carou et al. 1990).

Determination of diamines and non-volatile amines

Diamines and non-volatile amines (polyamines) have been suggested as freshness indicators by several researchers. There is a good correlation between levels of polyamines viz., putrescine, cadaverine and tyramine to freshness in various species of shell fish, salmon, tuna and beef (Edwards et al. 1985; Yamanaka et al. 1987).

Method of Yamanaka et al (1987) for determination of polyamines in muscle foods consists of homogenizing 5 g muscle with 20 ml of cold 3% perchloric acid in a homogenizer, and centrifuging at 10,000 rpm for 5 min in cold room. The procedure is repeated, and the supernatant fluid is removed and combined. Twenty ml. supernatant fluid is injected into high pressure liquid chromatography (HPLC) with a fluorescence detector (LC-6A G-1 system, Shimadzu Corporation, Kyoto). These five amines are separated on reverse phase type Shim-pack CLC ODS column at 50°C, converted into fluorescent derivatives by reaction with mixed solution of O-phthalaldehyde and mercaptoethanol, which is allowed to flow at a constant flow rate of 0.3 ml per min, and detected with a fluorescence detector (Shimadzu RF-530, Ex-348 non Em 450 nm). Mobile phase consists of solution A (0.1 M NaH_2PO_4+10 mM sodium octane sulphonate (pH 4.20), solution B (50% methanol in solution (pH 4.20). Gradient elution programme is controlled by a system controller (Shimadzu-SCL-6A) at the flow rate of 1.2 ml/min from 40% solution B in solution A over 25 min period, followed by the same composition for 5 min and after 30 min, 40% solution B in solution A. Each 5 mg of five standard amines are weighed, and dissolved in 100 ml 0.1 N HCl. Twenty ml of this mixed solution is injected into HPLC, and calibration curves are made with a Shimadzu Chromatopac C-R3A (Vidal-Carou et al. 1990).

2-Thiobarbituric acid (TBA) test for rancidity

Of the various methods reported for measuring the extent of oxidative deterioration of lipids in muscle foods, the TBA test (2-thiobarbituric acid) appears to be the most widely used (Melton 1983). The method is based on the spectrophotometric determination of the extracted malonaldehyde (Tarladgis et al. 1960). The method involving the steam distillate is the most popular for measuring TBA in muscle foods (Rhee 1978).

Samples are prepared by grinding thoroughly for TBA analysis. Five g meat and 5 ml 20% trichloroacetic acid in 2 M phosphoric acid, and 10 ml of 0.01 M 2-thiobarbituric acid (Eastman Kodak) are placed in a 50 ml centrifuge tube, heated in a boiling water bath for 30 min with occasional stirring, and then chilled the tubes in an ice bath for 10 min. The solid fat layer is removed by using spatula, and the pink coloured TBA complex is located in the aqueous layer. Fifteen ml of solvent containing a mixture of 2:1-isoamyl alcohol - pyridine is added. The tubes are shaken vigorously for 2 min, and then centrifuged for 15 min at 2400 rpm to break the emulsion formed during the extraction. The clear solvent extract is decanted into a cuvet and the colour measured at 538 nm against a solvent blank. The TBA value is defined as absorbancy at 538 nm against Beckman DV spectrophotometer and standard size of one cm cuvets (Tarladgis et al. 1964).

The method described by Turner et al (1954) is also based on the principle that malonaldehyde-like compounds react with 2-thiobarbituric acid to give pinkish red coloured complex, which shows maximum absorbance at 535 nm. It consists of grinding 5 g samples over coarse sand in a mortar, and extracting with 5 ml water. Four ml aqueous

extract are mixed with 4 ml TBA reagent, and heated in a water bath at 100°C for 45 min. Pinkish red colour developed is extracted with n-butanol, and measured at 535 nm on Bausch and Lomb spectronic 20. TBA values are expressed as mg malonaldehyde (MA) per kg material.

Standard curves for malonaldehyde are prepared by making appropriate TEP standard solution to give amounts, ranging from $1x10^{-9}$ to $1x10^{-8}$ Molar malonaldehyde in 5 ml. Determination is run by adding 5 ml TBA reagent I in each portion, and reading the absorbancy at 530 nm in a spectrophotometer, either after the sample has been kept at room temperature for 15 h, or after being heated in a water bath at temperatures of 37, 50, 70 and 100°C.

TBA reagent (0.01 M) is prepared by dissolving a weighed amount of the solute in slightly warmed molar phosphoric acid (Folch et al. 1957).

Carbonyl value

Method involving measurement of secondary changes e.g., formation of carbonyls is used to follow lipid oxidation. The method of Henick et al (1954) is based on the reaction of carbonyl compounds with 2:4-dinitro phenyl hydrazine to form corresponding hydrazones. These react with alkali to produce characteristic wine red colour.

Five g tissues are ground over coarse sand in mortar, and extracted with 25 ml distilled carbonyl-free benzene. To 2 ml benzene extract in test tube, are added 1 ml 4.3% trichloroacetic acid (TCA) in benzene, and 2 ml 0.5% 2:4-dinitro-phenyl-hydrazine (DNPH) reagent. The tubes are heated for 30 min at 50°C, and then cooled to room temperature. To 0.5 ml reaction mixture, is added 5 ml 4% alcoholic KOH, and the resulting colour is read at 430 nm after 10 min. Carbonyl values are expressed as mEq/Kg carbonyl-free benzene.

To 1000 ml of benzene, are added 5 gm 2:4-dinitrophenyl hydrazine (2:4 DNPH), and 1 gm trichloroacetic acid (TCA), and the mixture is then subjected to distillation prior to its use.

Peroxide value

Method involving primary changes such as formation of hydroperoxide (peroxide value and fatty acids) is more suited to measure low levels of oxidation in products stored at low temperature (Coxon 1987).

Colorimetric ferrous thiocyanate method of Wager et al (1947) is used to determine peroxide values in muscle foods. Oxidation by peroxide of Fe^{++} to Fe^{+++}, and reaction of the latter with NH_4CNS to produce characteristic red colour forms the basis of this method.

Ferrous thiocyanate reagent is prepared by dissolving 1 g ammonium thiocyanate (A.R.), and 1 ml H_2SO_4 (25% by weight) in 200 ml deaerated methanol, and the resulting solution is shaken with 0.2 g finely pulverized ferrous ammonium sulphate. Solution is decanted, and stored in a stoppered brown glass bottle. Fresh reagent is prepared every time.

Bligh and Dyer's (1959) method involves extraction of a 5 g tissue sample with chloroform-methanol mixture, and chloroform extract is used for estimation of peroxides. To 0.2 ml chloroform extract, is added 5 ml ferrous thiocyanate reagent and colour developed is read at 510 nm after 10 min. Peroxide values are expressed as mM per kg.

Determination of peroxide may not be useful as a measure of lipid oxidation in muscle foods during prolonged storage, especially if the muscle is ground (Melton 1983).

Free fatty acid (FFA) values

Colorimetric method of Duncombe (1963) for the determination of free fatty acids (FFA), which involves the reaction between free fatty acids, and copper (from copper reagent) to form the corresponding copper salts, is used. The copper salts of free fatty acids is treated with sodium diethyl dithiocarbamate. The characteristic golden yellow colour formed is quantitated spectrophotometrically.

Five ml chloroform solution of free fatty acids (FFA) are shaken with 2.5 ml copper reagent for 3 min. Copper reagent is prepared by mixing 9 ml of M triethanolamine, 1 ml of N acetic acid and 10 ml of 6.45% cupric nitrate solution. Reaction mixture is then centrifuged to separate the chloroform phase from aqueous phase, containing copper reagent. The latter is then completely removed by suction. To 2.5 ml chloroform solution, is added 2.5 ml 0.1% solution of sodium diethyl dithiocarbamate to develop the colour, which is measured at 435 nm. FFA values are expressed as nM per kg.

Determination of nucleotides from chill-stored muscle

Loss of freshness is related to the autodegradation of adenosine 5-triphosphate (ATP)

in fish tissue (Luong et al. 1989). Inosine monophosphate is known to contribute to the characteristic flavour of fresh fish, while its degradation product hypoxanthine imparts bitter off-taste odour, (Jones et al. 1964).

For determination of AMP, ADP, ATP and IMP, 25 g muscle is homogenized into 50 ml of 0.6 N perchloric acid at 0°C. A 50 ml aliquot of filtered homogenate is adjusted to pH 6.5 with 5 N potassium hydroxide at 0°C, and water added to a final volume of 7.5 ml potassium chlorate is removed by centrifuging at 0°C, Aliquot (7ml) of the neutralized diluted extracts are combined for analysis.

The combined extracts from fish of a group are passed through column (18.2 cm x 1.3 cm) of Dowex-I-X8 (Format) resin, 200-400 mesh. The columns are washed with water, until the optical density of the effluent fell to 0.01. Effluent and wash contain the purines, and nucleosides of the muscle. Nucleotides are retained on the column. Fractions of individual peaks are combined, and eluted spectrophotometrically at their ultraviolet absorption maxima (Jones 1960; Jones and Murray 1960).

Hypoxanthine concentration is a reliable indicator of storage in rock and yellow fin soles, since its concentration increases at a relatively constant rate during chilled storage (Boyd and Wilson 1977). Although hypoxanthine can be detected by either chromatography or enzyme electrodes, the interpretation of hypoxanthine as an indicator of freshness is problematical, since its concentration is dependent upon pre-death conditions (Mulchandani et al. 1989; Nguyen et al. 1991). To overcome these problems, K value, defined as (INO + Hx)/(IMP + INO + Hx) is proven applicable in assessing the freshness of several fish species.

Biosensor system for determining hypoxanthine ratio, an indicator of freshness

Tissue samples (1.5 g) are homogenized with 5 ml 10% trichloroacetic acid. After centrifugation for 10 min at 27,000 g, 9 ml of the supernatant is diluted 10-fold with 50 mM glycylglycine, and 5mM $MgCl_2$ buffer (pH 7.8). The sample is neutralized with 2M NaOH (200 µl) (unreacted fish extract).

A pre-wetted immunodyne membrane (1.5 x 1.5 cm) is stretched on the top of a hollow plastic cylinder (1 cm - diameter), and held in place by an O-ring. To a mixture of XD (0.35 units), and BSA (1.5 mg) in 10mM phosphate buffer (pH 7.0), glutaraldehyde (4µl, 25%) is added to initiate cross-linking, and 35 µl of the solution is layered on to the pre-wetted membrane, and allowed to cross-link at room temperature for 30 min. Membranes containing NP co-immobilized with XD are made by adding NP to the starting mixture.

To immobilize NT (5-nucleotidase), polystyrene centrifuge tubes (1 ml) are filled with 1 ml of 5% polyethylenimine solution, and incubated for 2 h at room temperature. The tube is emptied, and filled with 2.5% glutaraldehyde solution in 150 mM phosphate buffer (pH 7.8). After incubation for 3 h, the unreacted glutaraldehyde is removed, and the tube is then filled with 1 ml solution containing 20 IU of nucleotidase in 4 mM phosphate buffer, (pH 7.8), and incubated overnight at 4°C. NP is co-immobilized with NT by adding it to the starting solution.

The instrument consists of an amperometric electrode (platinum anode polarized at +0.7V with respect to silver/silver chloride cathode) inserted in a detection chamber (400 µl capacity), equipped with an injection port and current read out. The enzymic membrane disc is tightly attached to the sensing area of the electrode, and retained in place by an O-ring. One mole of Hx will be converted to 1 mole of uric acid, and 2 moles of H_2O_2 by immobilized XO.

Determination of H and K values

The breakdown products of adenosine triphosphate (ATP) are determined in order to evaluate the freshness of fish species. The K value calculated from the decomposition products of ATP has been suggested as an indicator of fish freshness (Gill 1992).

Hx is determined by injecting 100 µl of the unreacted fish extract into the detection chamber equipped with XO enzymic membrane ($Hx+O_2 \longrightarrow$ uric acid + H_2O_2). Total concentration of (Hx + INO + IMP) is determined by first reacting 500 µl of the unreacted fish extract with the immobilized NT tube for 5 min under continuous shaking NP (25 µl of 18.4 $IUml^{-1}$ in 150 mM phosphate buffer (pH 7.8) is added to the detection chamber, and 2 min later, the reacted fish extract (100 µl), containing INO and Hx is introduced to the detection chamber. The steady-state responses recorded after 2 min are used to calculate the H value.

The (Hx+INO) is determined by adding soluble NP to the detection chamber followed by the

unreacted fish extract. A second addition of NP followed by the injection of reacted extract (incubated with immobilized NT) facilitated (Hx + INO + IMP) measurement, and the K value is calculated (Luong and Male 1992).

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Studies on Growth, Pungency and Flavour Characteristics of Different Varieties of Onions During Bulbs Development

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Changes have been studied on growth, pungency and flavour characteristics in eight varieties of onions, during bulbs development at seven stages of growth, beginning one month after transplanting at an interval of 15 days till maturity. In general, fresh and dry weights, diam of bulb, total pyruvic acid, enzymatically produced and non-enzymatically produced pyruvic acid, sulphur content, and allinase activity increased throughout bulbs development. Varieties VL-3 and 102-1 have better size after 120 days, as compared to the other varieties.

Keywords : Onions, Growth, Pyruvic acid, Allinase, Sulphur, Pungency.

Onion (*Allium cepa* L.), a major crop of tropical countries is highly valued for its flavour and nutritional qualities. (Patil et al. 1991). Onion is said to possess stimulative, diuretic and expectorant properties, and is considered beneficial in flatulence and dysentery (Pruthi 1976). It is known to increase the secretion of bile (Sambaiah and Srinivasan 1991), and has anti-bacterial activity (Lewis et al. 1977). In spite of their widespread use as food, bulbs of onions, with high contents of moisture, are poor sources of calories, proteins, fats and carbohydrates (Chowdhury 1967). They however, contain moderate amounts of ascorbic acid, other soluble vitamins and minerals (Gopalan et al. 1989). Onions are used by the rich and the poor alike in their daily meals, although the way of consumption may vary from person to person.

The quality characteristics of bulbs are size, shape, colour, flavour, storage life, earliness of bulbing and dry matter (Rabinowitch and Brewster 1980). Onions are generally sold in graded diam classes in foreign countries (Rabinowitch and Brewster 1980). Onion pungency develops, when the enzyme allinase hydrolyses the flavour precursors S-alk (en)yl cysteine sulphoxides, during tissue bruising or maceration (Schwimmer 1971). The reaction products are pyruvate, ammonia, and the many volatile sulphur compounds, which are characteristics of onion flavour and aroma (Schwimmer and Weston 1961). Onion flavour, determined by measurements of flavour precursors, reaction products or allinase activity, vary in different genotypes (Bajaj et al. 1979). Little information is available regarding changes in growth, pungency and flavour characteristics during development of the onion bulbs of commercial cultivars in India. Such information is required, because pyruvic acid can serve as a reliable indicator for pungency, harvesting time, better storage characteristics, processing efficiency and positively correlated with good drying ratio.

Materials and Methods

Eight red onion varieties ('VL-1', 'VL-3', 'Arka Niketan' 'Agri found Light Red', '102-1', 'Punjab Red Round', 'Pusa Red', 'Hisar-2') were grown in rabi season of 1989-90 at the Vegetable Research Farm of the University, in a randomized block design in three replications. The recommended doses of fertilizers, and other agronomical practices were adopted to raise the crop under Hisar conditions (HAU 1981). Five onion plants from each replication were harvested at random, beginning one month after transplanting, at an interval of 15 days, till maturity. Bulbs were cut in dice of size 0.25 to 1.00 cm^2, after discarding the non-edible part, and dried at 60°C to a constant weight, before grinding to pass through a 80 mesh sieve. Bulb diam was calculated by using the relationship log diam mm = 1.02+0.364 log bulb fresh weight (g), as suggested by Robinowitch and Brewster (1980). Total sulphur was determined by the turbidometric method of sulphate estimation, as described by Chesnin and Yien (1951).

For allinase assay, samples were collected in a plastic bag, placed in the ice bucket and deep-frozen prior to analysis. Total, enzymatically released pyruvic acid by allinase, and non-enzymatically produced pyruvic acid in bulbs were estimated by the method of Hart and Fisher (1971). Soluble proteins in the enzyme extract were estimated by the method of Lowry et al (1951).

Results and Discussion

Growth behaviour : In all the cultivars, the fresh weight, dry weight and diam of bulbs increased throughout development, and differed only in the pattern of accumulation of all the growth indices studied at all stages (Table 1). However, the increases in bulb weights, and diam upto 30 days

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TABLE 1. CHANGES IN DIAMETER, FRESH AND DRY WEIGHTS OF ONION BULBS AT 30, 60, 90 AND 120 DAYS AFTER TRANSPLANTING

Varieties	Diameter, mm/bulb				Fresh weight, g/bulb				Dry weight, g/bulb			
	30	60	90	120	30	60	90	120	30	60	90	120
'VL-I'	11.09	18.06	34.20	49.50	0.50	4.47	24.24	72.31	0.067	0.48	2.71	7.85
'VL-3'	11.12	20.65	38.02	51.44	0.53	6.45	34.47	97.42	0.069	0.61	3.86	9.93
'Arka Niketan'	11.22	19.41	39.26	48.87	0.68	5.46	37.63	68.71	0.079	0.54	4.78	8.86
'Agrifound Light red'	11.14	19.10	36.31	47.51	0.54	5.20	30.41	64.24	0.062	0.52	3.91	8.74
'102-1'	11.14	21.88	40.64	52.48	0.57	7.59	41.50	83.53	0.063	0.77	4.91	10.09
'Punjab Red Round'	11.07	19.10	35.56	50.93	0.45	5.21	28.78	77.49	0.064	0.53	3.66	9.96
'Pusa Red'	11.17	22.70	39.99	48.64	0.59	8.37	39.65	68.02	0.088	0.84	5.44	9.38
'Hisar-2'	10.86	18.79	32.73	48.31	0.27	4.98	22.95	66.70	0.036	0.49	2.95	8.94
Mean	11.10	19.96	37.08	49.71	0.52	5.97	32.46	74.80	0.066	0.60	4.03	9.22

after transplanting were very low, as compared to rest of the stages. Since all the varieties were grown under similar agroclimatic conditions, the differences in deposition of fresh, and dry weights as well as diam at various stages, appear to be purely genetic ones. Kalra et al (1986) and Sharma and Nath (1991) reported that the average weight of onion bulb and the weight at maturity ranged from 30.5 to 90.0 g and 34.7 to 96.3 g, respectively, and the present results are in agreement with these reports. Bulb, weights varied from 44.75 to 130.50 g in *desi* red varieties (Khokhar et al. 1990). Both the dates of planting, and age of seedling had significant effect on weight and diam of individual bulbs, and ultimately on the yield (Mohanty et al. 1990). Robinowitch and Brewster (1980) reported that the bulb length/diam ratio decreased with increase in bulb weight as well as with plant density. Irradiation of onions reduces the level of malate present, indicating freshness (Piccini et al 1987).

Pungency and flavour properties : Total pyruvic acid, enzymatically and non-enzymatically produced pyruvic acid and specific activity of allinase increased throughout the development (Table 2, Fig. 1), thereby confirming the findings of Freeman (1975) and Bajaj et al (1980). However, values of pyruvic acid ranging from 30.3 to 52.4 μ moles/dry weight were also reported by Raina et al (1989). Schwimmer and Weston (1961) classified onions as of weak, intermediate and strong strength, on the basis of pyruvic acid concentration (2 to 4 μ moles, 6 to 10 μ moles and 15 to 20 μ moles on fresh weight basis, respectively). On the basis of these specifications, the varieties studied fall in intermediate pungency strength. Red onions are more pungent than white onions (Singh, 1989). Pungency differs with variety, stage of maturity, type of soil, soil moisture and growing temperature, while it was reported to be maximum just before tops begin to fall over (Kalra 1987). This is in agreement with our findings. The complete absence of enzymatically produced pyruvic acid, and specific activity of allinase during early period of bulb development, except in 'Arka Niketan' 'Pusa red' and 'Hisar-2' is also in agreement with the reports of Pintauro (1979) and Lancaster and Shaw (1991).

TABLE 2. CHANGES IN ENZYMATIC, NON-ENZYMATIC AND TOTAL PYRUVATE OF ONION BULBS AT 30, 60, 90 AND 120 DAYS AFTER TRANSPLANTING

Varieties	Enzymatically produced, μ moles			Non-enzymatically produced, μ moles				Total, μ moles			
	60	90	120	30	60	90	120	30	60	90	120
'VL-I'	0.36	2.98	4.14	2.09	2.15	5.89	6.22	2.09	2.51	8.87	10.36
'VL-3'	0.24	3.12	4.49	1.99	2.39	6.12	7.52	1.99	2.63	9.24	12.01
'Arka Niketan'	0.92	3.94	5.24	2.23	3.24	6.63	8.99	2.23	4.16	10.57	14.23
'Agrifound Light Red'	0.13	2.68	4.68	1.86	2.99	5.54	7.08	1.86	3.12	8.22	11.76
'102-1'	0.09	2.90	4.20	1.70	2.67	4.84	5.82	1.70	2.74	7.74	10.02
'Punjab Red Round'	0.42	3.79	5.01	2.16	2.00	5.09	6.86	2.16	2.42	8.88	11.87
'Pusa Red'	0.53	3.61	4.86	2.30	3.76	6.30	7.99	2.30	4.29	9.91	12.85
'Hisar-2'	0.66	3.41	5.12	2.42	3.54	7.02	8.48	2.42	4.20	10.43	13.60
Mean	0.42	3.31	4.72	2.09	2.84	5.93	7.37	2.09	3.26	9.23	12.09

Values per 100 g fresh weight

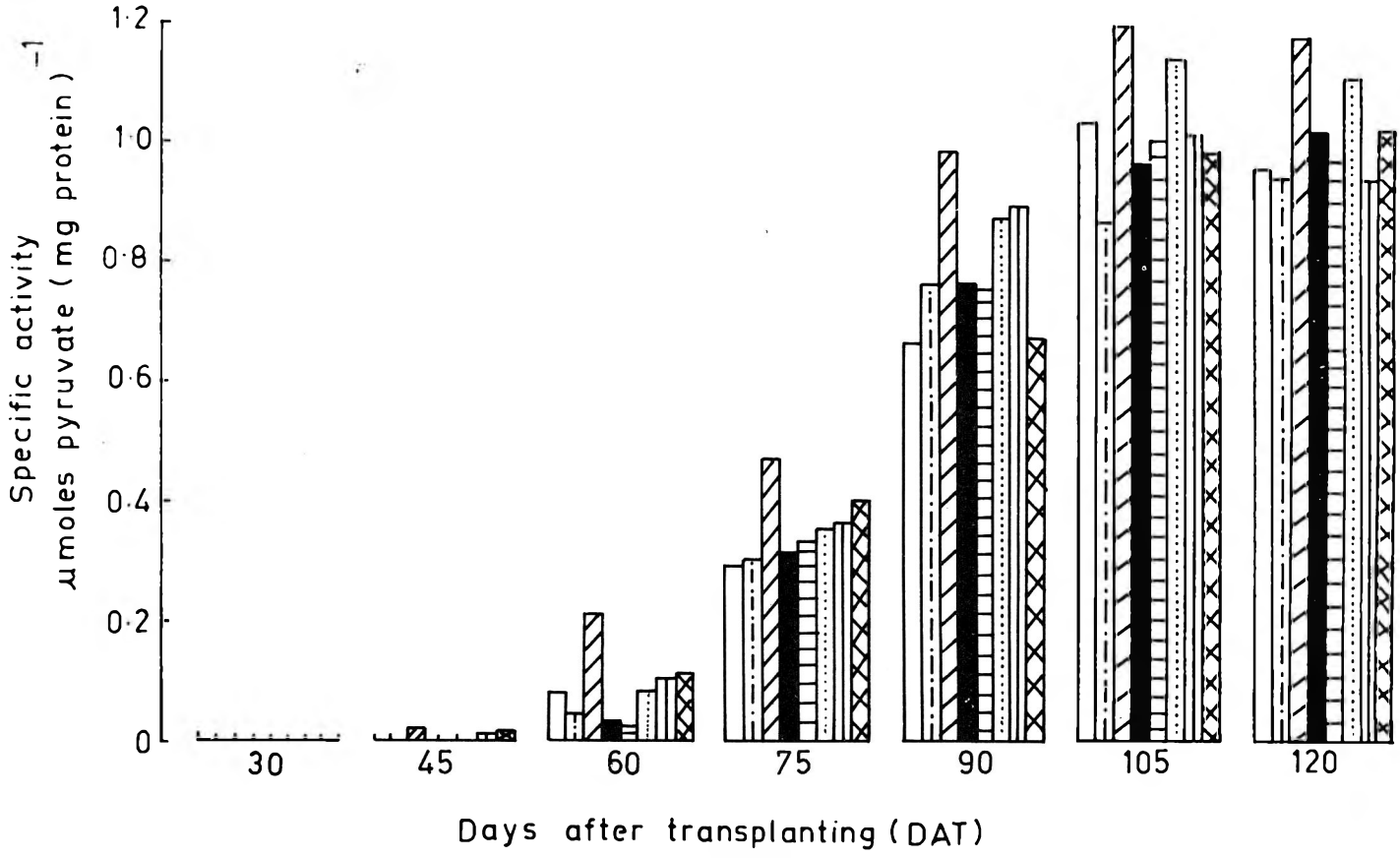

Fig. 1. Changes in allinase specific activity of onion during bulb development, ☐ 'VL-1', ▥ 'VL-3', ▨ 'Arka Niketan', ■ 'Agrifound Light Red', ▤ '102-1', ▩ 'Punjab Red Round' ◫ 'Pusa Red', ▩ 'Hisar-2'.

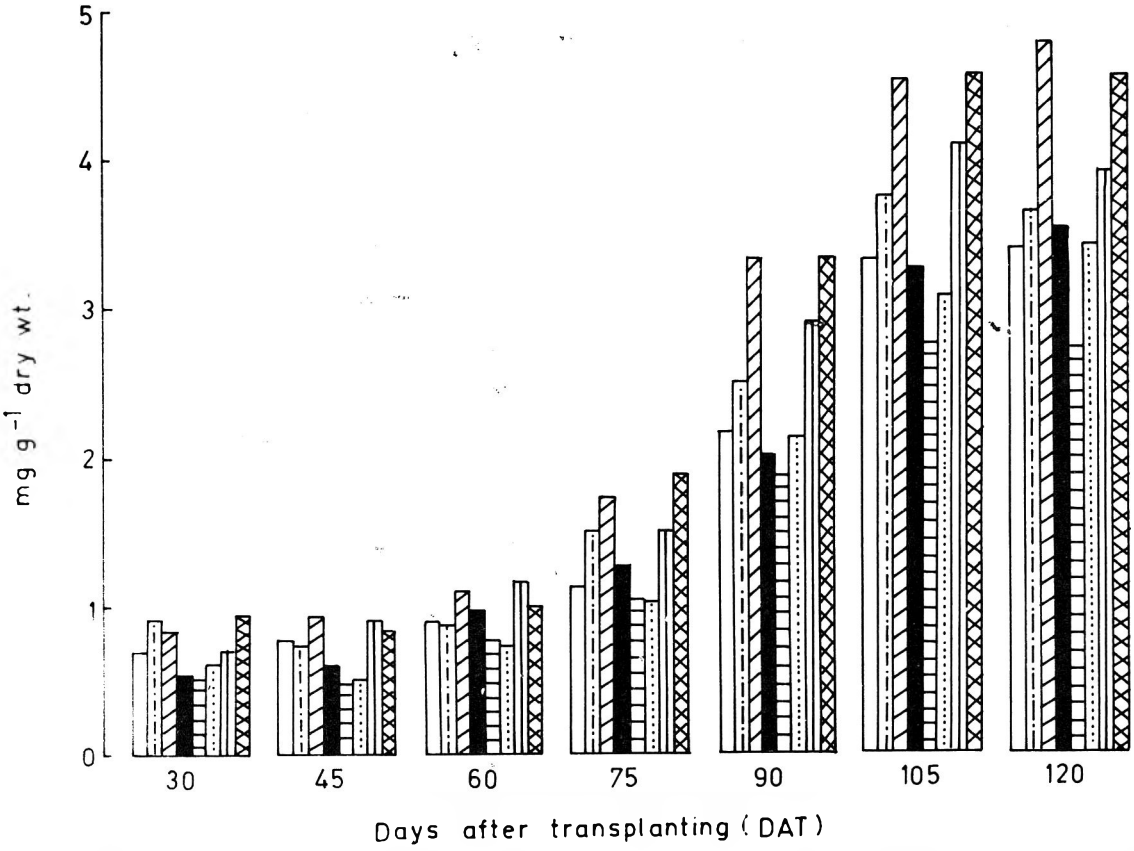

Fig. 2. Changes in sulphur content of onion during bulb development, ☐ 'VL-1', ▥ 'VL-3', ▨ 'Arka Niketan', ■ 'Agrifound Light Red', ▤ '102-1', ▩ 'Punjab Red Round' ◫ 'Pusa Red', ▩ 'Hisar-2'.

Total sulphur in bulbs also increased throughout the developments in all the varieties, except for a decline at 45 days after transplanting in a few varieties, and corroborated with all forms of pyruvic acid. Freeman and Mossadeghi (1971) observed a highly significant correlation between total sulphur and total pyruvates (r=0.91). Sulphur occurs in cysteine, methionine, cystine and other sulphur containing metabolites, which have important metabolic roles in function/synthesis of co-enzyme A, ferredoxins, S-adenosyl methionine, lipoic acid, biotin, sulpholipid, thiamine pyrophosphate derivatives, particularly non-protein amino acid sulphoxide precursor substrates of allinase of onion (Schwimmer 1971; Rao 1991). The sulphur contents at various stages of bulbs development varied from 0.49 to 4.76 mg g^{-1} dry weight (Fig.2), whereas Freeman (1975) reported sulphur concentration of 0.429 to 0.532% in onion bulbs. Kalra (1987) viewed that grower can potentially manipulate the onion flavour by choice of variety, sulphate nutrition, and water regimes. Further, the variations in sulphur contents in bulbs may be attributed to the differences in sulphur uptake, intra plant distribution and metabolism (Anderson 1978).

It may be concluded that during the two months period of bulbs development, the bulbs increased in fresh and dry weights, total pyruvates, non-enzymatically as well as enzymatically produced pyruvates, and total sulphur to the extent of 12.53, 15.36, 3.70, 2.59, 11.03 and 4.07-fold, respectively. Moreover, the varieties investigated had considerable differences in growth, pungency and flavour characteristics, not only at maturity, but also prior to maturity. Varieties 'VL-3' and '102-1' had better sizes. However, varieties 'Arka Niktan' and 'Hisar-2' have been found to contain higher pyruvic acid and sulphur after 120 days, as compared to the other varieties, and therefore, appear to have more pungency and better storage quality.

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Physico-chemical Composition and Processing Characteristics of Pearl Millet Varieties

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Thirty eight pearl millet cultivars, comprising local varieties, popular and new hybrid lines, were evaluated for physico-chemical composition and processing characteristics, in addition to the correlation of the physico-chemical properties with processing qualities. Wide variations were observed in thousand-kernel weight (3.2-17.6 g), and volume (2.2-12.9 ml), and hardness (49.3-98.5 BU) of native seeds of the varieties, in addition to considerable variations in proteins (8.1-13.9%), starch (57.4-70.3%), fat (3.4-7.4%), and ash (1.2-2.4%) contents. The yield of pearled grains and brokens of millet varied from 80.0 to 96.8%, and 0.9 to 30.3%, respectively. The yield and expansion ratio of popped grains ranged from 8.3 to 77.1%, and 2.3 to 11.3%, respectively. Wide variations in the amylase activity (567-3141 maltose units) of malted millets were observed. The grain hardness correlated positively with protein content ($r=0.35$, $p<0.05$), amylase activity ($r=0.38$, $p<0.05$), but negatively with milling breakage ($r=-0.37$, $p<0.05$). Protein content showed a significant positive correlation with popping yield ($r=0.36$, $p<0.05$), and amylase activity ($r=0.45$, $p<0.01$), but negative correlation with milling breakage ($r=0.34$, $p<0.05$).

Keywords : Pearl millet, Grain hardness, Proteins, Milling, Malting, Popping.

Pearl millet [*Pennisetum americanum* (L.) Leeke], is one of the staple foods in India and Africa. It is produced in India to a tune of about eight million metric tonnes (FAO 1991). Millet, being a dryland crop, has an increased potential growth in India and other tropical countries. It is a rich source of proteins and fat, as compared to other cereals (Kurien et al. 1961). However, almost all the millets produced in India are used in the preparation of conventional foods, partly because of the non-availability of ready-to-use and convenient food products (Rooney and McDonough 1987). The major constraints for widespread utilization of millet are its coarse fibrous seed coat, coloured pigments, characteristic astringent flavour, and poor keeping quality of the processed products (Desikachar 1975). Pearling or debranning of millet overcomes some of these constraints, and also improves its nutritional quality as well as consumer acceptability (Reichert 1979; Pawar and Parlikar 1990; Akingbala 1991). Popped pearl millet finds usage in weaning and supplementary foods (Malleshi et al. 1986), whereas malted millets have been used in brewing (Pal et al. 1976; Novellie 1977). Roller-dried and extrusion-cooked millet products are also successfully prepared (Almeida-Dominguez et al. 1993). The present paper reports some of the physico-chemical, milling, popping and malting characteristics of recently released, 38 pearl millet varieties, and elucidates the relationship between physico-chemical properties, and the processing qualities.

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Materials and Methods

Thirty eight pearl millet strains, comprising local varieties, popular hybrids, and new hybrid lines, were procured from different locations in India (Table 1).

Physico-chemical properties : The samples were exposed to atmosphere for one week to equilibrate to same moisture level, before determination of physical properties. One thousand kernels of the whole grains were counted in a numigral grain counter (Tecator Co., Hoganas, Sweden), and their weights were determined. Thousand-kernel volume was measured using air comparison pycnometer (Model 930, Beckman, USA). Based on weight and volume, the density was calculated. For hardness measurement, 50 g sample was crushed in a hardness and structure tester (Brabender Co., Duisburg, Germany) with 1.5 and 0.5 mm clearance successively, and the torque developed during grinding was recorded on a chart (Capanzana and Malleshi 1989). The area covered under the peaks was measured at respective clearances, and the same was presented as hardness in brabender units (BU). Their sum represented the total hardness. The hardness of thirty randomly selected kernels was also determined individually (Kumar et al. 1991) in a hardness tester (Kiya Seisakusho Ltd., Tokyo, Japan).

Samples were pulverized in cyclone mill (Udy, UD Corporation Boulder, Colorado, USA), and their ether extractives were estimated (AACC 1983), using petroleum ether (60-80°C). The defatted flour was analyzed for proteins and total ash contents

(AACC 1983). Starch was estimated by the enzymic digestion method of Chiang and Johnson (1977). All the analyses were done in duplicate, and average values are reported.

Milling (decortication) : The samples were milled in a McGill mill (Century Elec., St. Louis, USA), initially just for 30 sec under 1.4 kg pressure, and the glumes were separated by aspiration. Deglumed samples (120 g), with original moisture content of about 10%, were sprayed with 4% additional water, mixed, equilibrated for 5 min, and milled again for 3 min at 1.4 kg pressure in the McGill mill. The milled grains and bran fractions were sieved through 20 and 30 mesh sieves, respectively. The −20 fraction of endosperm and +30 fraction of bran were pooled, and termed as brokens (milling breakage). The milled grains, brokens, and bran were equilibrated, and weighed to determine the milling yield (Malleshi and Desikachar 1985).

Popping and malting : The grains were sprayed with 6% additional water, tempered for 5 h in a closed container, and popped for 30 sec in an electrically heated, and hand-operated coffee roaster (local make), using sand heated to 250°C (Malleshi and Desikachar 1981). The % yield (by weight), and bulk volume of the popped product were calculated. The ratio of the volume of popped grains to that of native grains was taken as the expansion ratio. Seeds were steeped in distilled water for 16 h, and germinated for 48 h at 25°C in a BOD incubator. Sprouted seeds were sorted out, dried in an air oven at 50°C, and brushed gently to remove the rootlets. The devegetated malt was ground in a Udy cyclone mill, and the amylase activity of malt was assayed (Malleshi and Desikachar 1979).

Statistical analysis : The mean and the standard error (SE) were calculated for each parameter, based on 38 cultivars. The correlation coefficients among the various physical properties, chemical constituents with milling, popping and malting characteristics for 14 parameters were determined (Snedecor and Cochran 1962).

Results and Discussion

Physico-chemical properties : The varieties were arranged in the descending order of their thousand-kernel weight in Table 1. The thousand-kernel weight and volume of the cultivars ranged from 3.2 to 17.6 g and 2.5 to 12.9 ml, respectively. The cultivars 'IP 10436' and 'IP 10438' were exceptionally bold, having thousand-kernel weight of 17.6 and 16.0 g, respectively, while the thousand-kernel weight of majority (29 varieties) ranged from 6.0 to 11.6 g, and the values for 7 low weight varieties ranged from 3.2 to 5.9 g. Density of the grains ranged from 1.29 to 1.54 g/ml. The variations in total hardness were between 49.3 and 98.5 BU, while the Kiya hardness varied from 1.3 to 5.2 kg/grain. Wide variations in thousand-kernel weight, volume and hardness among different cultivars of pearl millet have also been reported by Akingbala (1991). Protein content of the cultivars ranged from 8.1 to 13.9%, and starch from 57.4 to 70.3% (Table 2). The fat content of millet varieties varied from 3.4 to 7.4%, whereas the ash content ranged from 1.1 to 2.4%. The samples with higher protein contents generally had lower proportions of starch. Mean values of the chemical constituents were similar to those reported by Kurien et al (1961) and Subramanian et al (1986).

There was a good correlation between physical properties and chemical constituents (Table 3), the hardness being highly correlated with protein content. Similar relationship between hardness and protein in maize cultivars has been reported by Dorsey-Redding et al (1991). No correlation was observed between grain hardness with starch, fat and ash contents. However, density showed significant positive correlation with starch content. Hardness also correlated significantly with thousand-kernel weight and volume.

Milling, popping and malting characteristics : Large differences existed in the yield of pearled (head) grains, and the milling breakage (Table 2). While the yield of pearled grains containing brokens varied from 80.0 to 96.8%, the milling breakage was of the order of 0.9 to 30.3%. Considerable variations in decortication characteristics among pearl millet cultivars may be related to the endosperm texture, as reported by De Francisco et al (1982), and also borne out by the present data, showing negative correlation of milling breakage with protein and ash contents (Table 3). Milling breakage correlated positively, and significantly with thousand-kernel weight. This indicates that bolder grains and varieties of lower protein contents break easily during milling.

The yield and expansion ratio of popped grains varied from 8.3 to 77.1% and 2.1 to 11.3, respectively (Table 2). Eleven cultivars had more than 50% popping yield with mean expansion ratio of 7.5. Popping was also positively correlated with protein and ash contents (Table 3), indicating that well popped millet could be obtained from the cultivars

TABLE 1. PEARL MILLET CULTIVARS AND THEIR PHYSICAL CHARACTERISTICS

Cultivars	Source	1000 kernel		Density, g/ml	Hardness		Kiya, kg/grain
		Weight, g	Volume, ml		Brabender (BU) 0.5 mm	Brabender (BU) Total (1.5+0.5 mm)	
'IP 10436', 'Hybrid S 5 Line'	ICRISAT	17.6	12.93	1.36	42.5	90.2	2.7
IP 10438, Hybrid S 5 Line'	ICRISAT	16.0	12.31	1.29	37.2	78.5	3.2
'863AxPMBPN 35', 'New hybrid line	Gulbarga	11.6	8.32	1.39	48.0	98.5	5.2
'IP 8027', 'Local'	Ghana[a]	11.4	8.24	1.38	36.5	74.1	3.2
'81AxPMIN 87 33', 'New hybrid line'	Gulbarga	11.3	7.79	1.45	40.9	93.4	4.8
'843AxPMBPN 48', 'New hybrid line'	Gulbarga	10.8	7.63	1.41	42.8	86.1	3.4
'IP 8032', 'Local'	Ghana[a]	9.6	7.26	1.32	42.7	87.0	3.4
'MBH 157', 'Hybrid'	Gulbarga	9.4	6.43	1.46	42.2	86.3	2.2
'841AxPMBPN 87 68', 'New hybrid line'	Gulbarga	9.4	6.63	1.41	39.8	83.0	3.5
'CO 6', 'Variety'	Coimbatore	9.3	6.44	1.44	46.2	96.7	4.4
'81AxPMBPN 9', 'New hybrid line'	Gulbarga	9.3	6.37	1.45	38.8	82.8	3.7
'IP 12070', 'Local'	Nigeria[a]	9.3	6.81	1.36	30.3	63.8	2.8
'ICMP 451', 'Hybrid'	Gulbarga	9.1	6.43	1.41	43.3	86.1	3.4
'81AxPMBPN 3'7, 'New hybrid line'	Gulbarga	9.1	6.33	1.43	29.6	73.4	4.9
'IP 14890', 'Local'	Cameroon[a]	9.0	6.43	1.39	32.8	64.6	1.3
'IP 12848', 'New hybrid line'	Gulbarga	8.9	6.12	1.45	44.6	87.3	4.6
'81Axwhite bajra', 'New hybrid line'	Gulbarga	8.8	6.05	1.45	40.8	84.7	4.3
'81AxPMBPN 6', 'New Hybrid line'	Gulbarga	8.8	6.05	1.45	39.0	82.1	4.3
'IP 8638', 'Local'	Sudan	8.7	6.61	1.31	33.0	66.0	2.1
'841AxPMBPN 1', 'New hybrid line'	Gulbarga	8.6	6.02	1.42	36.5	73.7	2.9
'841AxPMBPN 88 60', 'New hybrid line'	Gulbarga	8.4	5.63	1.49	35.6	73.6	2.8
'HHB 67', 'Hybrid'	Hisar	8.2	6.09	1.34	45.3	86.7	3.2
'MH 179', 'Hybrid'	Gulbarga	8.1	6.03	1.34	43.7	88.2	3.1
'HHB 68', 'Hybrid'	Hisar	7.9	5.80	1.36	47.0	91.0	3.4
'WC C 75', 'Hybrid'	Gulbarga	7.8	5.71	1.36	38.3	77.6	3.3
'81AxPMBPN 21', 'New hybrid line'	Gulbarga	7.7	5.23	1.47	43.7	85.0	4.7
'841AxPMBN 1'0, 'New hybrid line'	Gulbarga	7.7	5.19	1.48	34.6	67.2	3.2
'81AxG 73 k 77', 'New hybrid line'	Gulbarga	7.1	4.93	1.44	34.4	73.6	4.8
'HHB 60', 'Hybrid'	Hisar	6.9	4.63	1.49	51.8	90.0	3.5
'IP 5738', 'Local'	Nigeria[a]	6.3	4.61	1.36	34.3	66.3	2.6
'HHB 50', 'Hybrid'	Hisar	6.0	4.26	1.41	46.4	84.2	3.6
'81AxPMBPN 48', 'New hybrid line'	Gulbarga	5.9	4.36	1.35	39.4	78.7	3.9
'Local (Gulbarga)', 'Local'	Gulbarga	5.6	4.02	1.39	41.9	68.9	3.4
'IP 3595', 'Local'	Tamil Nadu[a]	4.9	3.50	1.39	41.4	67.2	2.6
'IP 3471', 'Local'	Tamil Nadu[a]	4.8	3.58	1.34	38.5	59.8	2.4
'Local (Mysore)', 'Local'	Mysore	4.3	3.00	1.43	45.0	83.1	3.1
'IP 11902', 'Local'	Sierra Leone[a]	3.9	2.52	1.54	43.4	59.7	2.9
'IP 11835', 'Local'	Eastern Ghats[a]	3.2	2.25	1.42	38.3	49.3	2.6
Mean		8.4	6.01	1.40	40.3	78.6	3.4
S.E.[b]		0.46	0.34	0.009	0.82	1.84	0.14

[a] Obtained through ICRISAT, Hyderabad. [b] Standard error

with higher protein content. The variation in popping yield could be genetic or influenced by harvesting conditions (Kumar et al. 1991). Varieties, which exhibited high popping yield also showed high expansion ratio. Popped millet could be used as a snack or in preparation of ready-to-eat nutritious food formulations. Popping is especially suitable for pearl millet, since the high-temperature-short-time (HTST) treatment may inactivate lipase, and enhance its keeping quality.

Among the 38 cultivars studied, about 13% viz., 'Local Mysore', 'HHB 50', 'HHB 67' and 'HHB

TABLE 2. CHEMICAL COMPOSITION[a] AND PROCESSING CHARACTERISTICS OF PEARL MILLET CULTIVARS

Cultivars	Proteins, %	Starch, %	Fat, %	Ash, %	Milling		Popping		Malting
					Head grains[c], %	Brokens, %	Yield, %	Expansion ratio	Amylase activity[d], MU
'IP 10436'	12.6	61.2	5.4	1.8	86.1	11.3	33.3	6.3	16668
'IP 10438'	12.6	67.1	4.0	1.7	80.0	9.6	45.4	8.0	1845
'863A x PMBPN 35'	10.6	65.7	5.4	1.7	89.9	7.9	27.3	2.4	1308
'IP 8027'	8.1	68.7	5.2	1.4	91.3	12.2	43.9	8.7	652
'81A x PMIN 87 33'	13.9	63.2	5.9	1.5	95.2	3.9	68.8	5.3	1849
'843A x PMBPN 48'	11.8	66.0	6.9	1.7	89.9	18.0	39.0	4.3	1330
'IP 8032'	12.0	64.5	5.0	1.7	94.3	19.2	53.5	11.3	1934
'MBH 157'	9.9	70.3	4.7	1.3	91.5	26.0	26.9	2.8	1892
'841A x PMBPN 87 68'	9.7	67.5	4.8	1.1	89.1	15.3	31.8	2.7	1646
'CO 6'	13.6	65.8	5.9	1.4	91.6	0.9	77.1	11.3	2179
'81AxPMBPN 9'	9.8	69.0	6.1	1.6	96.8	10.4	72.2	5.6	1350
'IP 12070'	9.1	64.2	5.5	1.8	89.9	14.9	48.7	6.6	2192
'ICMP 451'	11.7	64.4	5.5	1.3	92.1	16.9	33.3	2.7	2082
'81A x PMBPN 37'	9.4	68.5	6.1	1.6	94.7	10.5	29.8	3.0	1596
'IP 14890'	9.9	65.0	7.3	1.5	87.3	30.3	37.5	6.8	1981
'IP 12848'	10.8	68.0	5.7	1.5	94.8	8.6	47.7	3.2	1062
'81A x white bajra '	9.4	68.2	5.6	1.4	95.1	3.6	53.6	3.8	1833
'81A x PMBPN 6'	11.0	65.0	5.4	1.8	90.8	13.7	53.6	4.2	1943
'IP 8638'	10.0	62.1	5.9	1.8	86.0	22.0	20.5	3.8	2052
'841A x PMBPN 1'	10.5	66.7	5.0	1.6	90.3	29.9	11.6	2.1	567
'841A x PMBPN 88 60'	10.9	66.2	4.5	1.6	90.0	20.8	39.1	4.0	1634
'HHB 67'	10.9	63.4	6.1	1.8	95.7	4.2	17.0	2.4	2679
'MH 179'	10.9	65.8	5.9	1.6	90.3	16.0	37.9	3.4	2015
'HHB 68'	12.5	57.4	7.4	2.0	96.3	3.5	31.6	3.2	2649
'WC C 75'	11.1	65.7	6.6	1.5	89.7	9.1	63.6	10.0	2057
'81A x PMBPN 21'	9.6	64.6	6.9	1.7	89.9	11.1	47.4	2.9	1553
'841A x PMBPN 10'	9.2	70.0	4.9	1.2	91.6	15.9	8.3	2.3	1481
'81A x G 73 k 77'	12.5	65.2	6.5	1.7	93.8	3.5	68.2	4.2	1833
'HHB 60'	12.0	63.4	7.2	1.8	92.2	3.5	40.8	3.1	3086
'IP 5738'	10.1	63.7	5.1	1.8	92.1	1.7	40.0	9.0	1760
'HHB 50'	12.2	59.0	7.3	1.8	90.4	1.7	8.3	2.9	3141
'81A x PMBPN 48'	9.5	67.8	5.5	1.5	94.2	10.6	18.4	3.1	1464
'Local (Gulbarga)'	9.7	68.7	6.5	1.3	91.2	1.8	13.0	2.1	2328
'IP 3595'	13.8	64.7	5.5	2.1	86.9	0.9	61.5	6.3	1866
'IP 3471'	13.5	61.0	7.2	2.4	87.7	0.9	72.1	7.4	2201
'Local (Mysore)'	13.4	64.5	6.3	1.9	89.9	6.1	48.5	5.7	2848
'IP 11902'	9.8	66.3	3.4	2.1	90.4	0.9	70.7	7.4	1519
'IP 11835'	9.6	67.1	4.1	2.1	93.8	0.9	57.4	6.2	1634
Mean	11.0	65.4	5.7	1.7	91.1	10.5	42.0	5.0	1861
S.E.[b]	0.24	0.45	0.15	0.04	0.54	1.34	3.11	0.42	90.55

[a] Moisture free basis. [b] Standard error. [c] Includes brokens [d] Maltose units.

68' exhibited very high amylase activity (2600-3141 maltose units-MU), and therefore, may be classified as good malting strains. About 60% cultivars showed high (1570-2378 MU), and other 22% cultivars showed moderate amylase activity (1062-1550 MU). The cultivars '841 A x PMBPN 1' and 'IP 8027' are poor malting strains. Amylase activity was significantly correlated with the hardness. This indicates that harder cultivars, and cultivars with high protein contents are good for malting. Pearl millet is comparable to wheat with regard to its amylolytic activity, and possesses favourable ratio of alpha to beta-amylase (Pal et al. 1976). Beer produced with 20% millet malt as adjunct was reported to be readily acceptable (Singh and Tauro 1977). However, high oil content and poor keeping

TABLE 3. CORRELATION AMONG PHYSICAL, CHEMICAL AND PROCESSING CHARACTERISTICS OF PEARL MILLET CULTIVARS. (N = 38)

Characteristics	Volume	Density	BH, 0.5mm	BH, total	KH	Proteins	Starch	Fat	Ash	Head grains	Brokens	Amylase	Popping yield	Expansion ratio
100-kernel weight	0.99[b]	-0.28	-0.06	0.49[b]	0.14	0.07	0.03	-0.14	-0.34[a]	-0.31	0.35[a]	-0.28	-0.09	0.09
Volume		-0.38[a]	-0.07	0.46[b]	0.09	0.09	-0.01	-0.14	-0.29	-0.35[a]	0.33[a]	-0.25	-0.11	0.13
Density			0.17	0.03	0.29	-0.13	0.33[a]	-0.12	-0.17	0.34[a]	-0.08	-0.14	0.19	-0.27
Brabender hardness, (BH, 0.5 mm)				0.66[b]	0.27	0.42[b]	-0.28	0.23	0.10	0.17	-0.37[a]	0.38[a]	0.01	-0.15
Brabender hardness, (total)					0.55[b]	0.35[a]	-0.14	0.26	-0.34[a]	0.23	0.00	0.15	-0.06	-0.17
Kiya hardness						0.08	0.13	0.13	-0.23	0.44[b]	-0.36[a]	-0.13	0.16	-0.23
Proteins							-0.57[b]	0.29	0.39[a]	-0.18	-0.34[a]	0.45[b]	0.36[a]	0.25
Starch								-0.49[b]	-0.59[b]	0.11	0.26	-0.58[b]	-0.01	-0.08
Fat									0.12	0.15	-0.14	0.49[b]	-0.04	-0.16
Ash										-0.16	-0.41[a]	0.27	0.33[a]	-0.23
Head grains											-0.23	-0.00	0.07	-0.21
Brokens												-0.37[a]	-0.38[a]	-0.17
Amylase activity													-0.04	-0.02
Popping yield														0.64[b]

[a] Significant at 5% level ($p<0.05$). [b] Significant at 1% level ($p<0.01$). BH : Brabender hardness, KH : Kiya hardness.

quality of millet malt are the constraints for its utilization in brewing industry (Opoku et al. 1983).

The present study revealed wide varietal variations with regard to physico-chemical characteristics, and processing quality of 38 pearl millet cultivars, and identified the suitability of some millet cultivars for milling, popping and malting. 'Local Gulbarga', 'CO 6', 'IP 5738', 'HHB 50', 'IP 3595', 'IP 3471' 'IP 11902' and 'IP 11835' are suitable for milling, whereas cultivars 'CO 6', '81 A x PMBPN 9', 'IP 3471' and 'IP 11902' are suitable for popping. Majority of the cultivars are suitable for malting. Protein content appears to be the dominant chemical constituent that influences the grain hardness, milling, popping, and malting characteristics. This parameter, therefore, could serve as one of the basic quality indicators in millet breeding programme.

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Economic Utilization of Cabbage Wastes through Solid State Fermentation by Native Microflora

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Cabbage waste, collected from the local market, was treated by solid state fermentation at 30°C, utilizing their commensal microflora. During the course of fermentation, the total heterotrophic bacteria showed a marginal decline from $9.4x10^8$/g to $1.7x10^8$/g after 8 days. The moisture content increased upto 92.9%, and the initial acidic pH shifted from 5.5 to 10.0 after 8 days. Total and reducing sugars were 1.8% and 0.1%, as compared to initial 6.3% and 2.0%, respectively. Protein content increased from 1.8% to 3.1%. *Pseudomonas* sp dominated other flora during fermentation. Alpha-amylase (38,600 U), protease (450 U) and cellulase (352 U) per kg of cabbage waste could be recovered after natural fermentation under uncontrolled conditions. Results indicate that there is a good potential for utilization of cabbage waste as solid substrate for industrial enzyme production, protein enrichment, and consequent prevention of pollution in urban areas.

Keywords : Cabbage waste, Solid state fermentation, Enzyme production.

Cabbage, is extensively cultivated in different parts of the world (Steinkraus 1983), and consumed as a leafy vegetable after cooking. It can also be used for dehydrated vegetable products (Bhat et al. 1974). A critical survey of fruit and vegetable markets of the towns, and cities in South India reveals that cabbage wastes constitute a dominant proportion among the solid wastes generated, and thus become a source of environmental pollution. A clean environment, free of pollutants, and disease-free community, in cities and towns could be achieved only through efficient cabbage waste management, either through safe disposal or recycling of wastes through utilization. Bio-conversion through solid state fermentation would be the right method of choice (Yang 1988), if economic utilization of cabbage waste, is desired.

Cabbage waste is fed to cattle in rural areas, while it gets rotted in urban areas, causing environmental pollution due to the emanating foul odour. The cabbage waste, a rich source of organic matter (Steinkraus 1983), can be efficiently utilized for various purposes, such as upgradation of its protein value by solid state fermentation or its use as solid substrate in solid state fermentation for production of industrially important microbial metabolites. Therefore, the present work was undertaken to study the use of cabbage waste as a bioresource material for the production of exoenzymes of commercial significance through solid state fermentation by native flora of cabbage waste, and the results are reported here.

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Materials and Methods

Fresh cabbage (*Brassica oleracea* var capitata) wastes, collected from the local market, were weighed, cut into small pieces (0.85x0.35 cm mean size), after removing the mud, and debris with brush and/or knife. The pieces were oven-dried at 40°C for 6 h to remove free water associated with the samples. Such pre-treated material was used as solid substrate for fermentation by commensal microflora.

The pre-treated cabbage wastes (100 g) were transferred into 1000 ml beakers. The mouth of each beaker was covered with sterile cotton cloth to prevent aerial contamination, and incubated at 30°C for a total period of 8 days. The cover also helped in preventing rapid loss of moisture during fermentation. Samples were withdrawn at 24 h intervals, and subjected to various analyses. The results reported are the mean of three sets of experiments.

The moisture content was estimated by drying the fresh sample at 105°C for 6 h (Pearson 1973). Carbohydrates as total sugars (Dubois et al. 1956), reducing sugars (Miller 1959), proteins (Lowry et al. 1951) and pH were determined after homogenizing the sample with distilled water in an electric blender at 1:10 ratio. Total heterotrophic bacterial population, associated with the cabbage wastes, during the course of solid state fermentation, was enumerated using nutrient agar medium (HI-Media), employing pour plate technique. Inoculated plates were incubated at 30°C for 3-5 days. After enumeration, the colonies developed on the plates were subcultured on nutrient agar slants, purified

and identified to various genera based on their morphological, and biochemical characteristics according to Buchanan and Gibbons (1974).

Enzymes associated with the fermenting cabbage wastes were extracted, by adding 100 ml phosphate buffer (pH 7.2) to 10 g sample, and the contents were allowed to stand at room temperature (30±2°C) for 60 min, with intermittent stirring. Later, the solubilized enzyme in the buffer was recovered, following a simple contact method of enzyme extraction (Kumar and Lonsane 1987). The extract was centrifuged at 8000 rpm for 20 min at 4°C, and the supernatant was used for enzyme assay.

Alpha-amylase activity was determined by the method described by Medda and Chandra (1980). One unit of alpha-amylase activity, expressed as dextrinizing activity, is defined as the amount of enzyme required to bring about the hydrolysis of 0.1 mg of starch at optimal pH, and temperature in 10 min of incubation. Cellulase activity was determined by employing modified filter paper assay (Mandels et al. 1976). Filter paper activity is the mg of glucose produced, when 0.5 ml of enzyme solution acts on 50 mg of Whatman No. 1 filter paper at pH 7.2, 50°C, for 1 h. Protease activity was assayed according to Elmar (1984). One unit of protease activity is defined as the amount that could liberate the peptide fragments equivalent to 1 mg of bovine serum albumin under the assay conditions.

Results and Discussion

Fresh cabbage waste contained (%) 90.2 moisture, 6.3 total sugars (carbohydrates), 1.8 proteins, 0.1 crude fat, 1.0 crude fibre, and 0.6 mineral matter. Results in Table 1 indicate that there was considerable change in the composition of biochemical components due to microbial activity, during solid state fermentation. Reduction in total heterotrophic bacterial population, from $9.4x10^8$/g (0 h) to 1.7×10^8/g (8 days), indicate that there was an elimination of some groups, owing to a decline in the available nutrients during the course of fermentation. The change in initial pH, from 5.5 to 10.0 at the end of fermentation, might be due to the release of certain amines and ammonia, as a result of microbial activity on the available protein fractions of cabbage wastes. This alkaline pH could have also contributed to the elimination of intolerant microbes, and hence the marginal decline in total heterotrophic bacterial population.

Moisture content of the fermented cabbage increased from 88.0% (0 h) to 92.9% (8 days). The increase in the moisture content of the substrate during fermentation might be due to the production of metabolic water (Yang 1988). Similar increase in moisture content of the final product, (4-11.5%) during solid state fermentation of sweet potato residue inoculated with *Pichia burtonii* has been reported (Yang 1988). Decline in total sugars and reducing sugars during fermentation provided evidence for the utilisation of the carbohydrate

TABLE 1. CHANGES IN THE BIOCHEMICAL AND BACTERIOLOGICAL CHARACTERISTICS OF CABBAGE WASTES DURING SOLID STATE FERMENTATION BY NATURAL FLORA

Attribute	Incubation period, days				
	0	2	4	6	8
		Biochemical			
pH	5.5	7.0	8.0	9.0	10.0
Moisture, %	88.0	90.9	91.3	92.0	92.9
Total sugars, %	6.3	4.9	3.8	2.4	1.3
Reducing sugars, %	2.0	1.0	0.4	0.2	0.1
Proteins, %	1.8	2.5	2.7	2.9	3.1
		Bacteriological			
Total heterotrophic bacterial population/g ($x10^8$)	9.4	8.5	6.5	4.6	1.7
Bacillus sp, %	38.0	32.0	28.0	22.0	18.0
Pseudomonas sp, %	44.0	58.0	66.0	68.0	74.0
Vibrio sp, %	10.0	6.0	4.0	4.0	2.0
		Enzymes, U/kg SS			
Amylase	0	26,000	38,600	36,600	36,200
Protease	0	280	400	450	200
Cellulase	0	17	280	324	352

SS - Solid substrate

fractions by the commensal microflora. Since no attempt was made to analyze secondary metabolites other than enzymes, the fate of bioconversion of carbohydrates could not be ascertained. Protein content increased progressively during the course of fermentation from 1.8% to 3.1% (Table 1). Yang (1988) observed increase in protein content on nitrogen supplementation of sweet potato residue during fermentation, and suggested that solid state fermentation is a method for protein enrichment of sweet potato residue with *Saccharomyces* sp. Hence, the rise in residual protein in the fermented cabbage wastes could be accounted with the dead and live microbial cell proteins.

Bacillus sp, *Pseudomonas* sp and *Vibrio* sp were dominant during the initial stages of fermentation. However, *Pseudomonas* sp dominated other species as the fermentation proceeded further. This might be due to the capability of this species to utilize available biochemical sources through efficient hydrolytic enzyme systems, and to adapt to unfavourable conditions, such as alkaline pH, as compared to other co-existing microbial flora. A maximal of 38,600 U of alpha-amylase, 352 U of cellulase, and 450 U of protease, per kg of solid substrate were obtained from the fermented cabbage wastes.

Substrates traditionally used in solid state fermentation include rice, wheat, millet, barley, corn and soybeans (Hesseltine 1972; Yang 1988). However, fruit and vegetable wastes might also be good solid substrates, for solid state fermentation process, for they are abundant in the environment as unwanted garbage. The present study suggests that cabbage wastes could be aptly utilized, as solid substrate for the industrial production of microbial enzymes at a cheaper cost.

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Studies on the Protein Quality and Storage Stability of Textured Soya Proteins

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Textured soya protein (TSP) chunks, procured from different commercial sources, were studied for proximate composition, protein efficiency ratio (PER) and trypsin inhibitor (TI) activity. Storage studies of TSP in different flexible packaging materials indicated a significant decline of PER from 1.94 to 1.72, 1.82 and 1.77 at the end of 12 months storage in three of the packaging materials. However, the product remained acceptable from the point of view of sensory qualities.

Keywords : Textured soya protein, Protein efficiency ratio, Storage studies, Nutritive value, Sensory quality.

Extrusion cooking process, which is widely employed in the West for preparation of meat extenders for human consumption, destroys several antinutrients including trypsin inhibitor (TI) activity (Manohar Kumar et al. 1978; Liener 1994). Yet, the commercial TSP products retain 5 to 20% of TI activity originally present in the soyabean (Rackis and Gumbmann 1982). The underprocessed soyabean extender has been reported to cause gastro-intestinal disorder (Gunn et al. 1980), whereas processing with extra heat might damage the nutritional and functional properties of protein. Hence, there is a need for maintaining optimal processing conditions.

TSP products are of relatively recent origin in India. The Indian armed forces showed interest in using TSP as a meat extender or partial substitute for meat in their ration. In fact, textured soy nuggets have been used for improving the quality of loaves after supplementing with poultry meat (Thind et al 1991). Apart from its low cost, TSP offers several other advantages to the armed forces from logistic point of view, particularly in transportation and storage. However, the product should have a long shelf life, and its specification formulated in order for the armed forces to accept TSP as a ration item. Although there had been extensive studies reported from the West, there is paucity of information on quality parameters of TSP produced in India. Therefore, commercial TSP, chunks were investigated for nutritional quality, and storage behaviour in selected packaging materials. The results are reported in this paper.

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Materials and Methods

TSP chunks, procured from five different commercial sources and designated as A, B, C, D and E, were used for chemical analysis. The product A was used for the storage studies.

Chemical analysis : The TSP products were analyzed in duplicate for their proximate composition by AOAC (1984) methods. The TI activity was estimated in triplicate by the method, as described by Smith et al (1980), using benzoyl-DL-arginine paranitroanilide (Sigma Chemical Co., USA), as the substrate.

Storage studies : Product A, freshly obtained from factory, was packed in 1 kg quantities in four types of flexible pouches, i.e., (i) paper-foil-polyethylene (PFP : trilaminate of 40 GSM paper-foil-and 37.5 μ polyethylene) with 0.02 mm foil thickness, (ii) PFP with 0.012 mm foil thickness, (iii) PFP with 0.009 mm foil thickness, and (iv) a co-extruded film of polyester-nylon-surlyn (PNS:85 μ thickness). The packages were stored under ambient conditions (19-30°C; relative humidity 47-78%) in an insect-proof shelf for a period of one year. The product was analyzed for sensory qualities once in three months (data not presented for third month), and protein efficiency ratio (PER) after 6 and 12 months of storage.

Bioassay : PER of the defatted soya flour (DSP) and the different brands of TSP, both fresh, and stored, was determined by the rat growth method of Osborne et al (1919), as described by ISI (1974). Male weanling albino rats (21±1 days old; 35±5 g weight) of 'Wistar' strain, bred in our animal house, were used in groups of 10 animals each for the products under study, along with a reference standard group on casein diet. Feed and water were

provided *ad libitum* during the 28 day-experimental period. Individual rat weight and feed consumption were recorded weekly, and the observed PER (weight gain g^{-1} protein) was calculated at the end of the feeding period. The PER values reported are means of ten values. For comparing the PER values of defatted soya flour and different brands of TSP, before and after storage, the observed PER values were statistically analyzed for significance of differences, and presented along with the corrected PER. The latter, was calculated from the former by fixing PER of casein at 2.5 and using the formula,

$$\text{Corrected PER} = \frac{\text{Observed PER of experiment group} \times 2.5}{\text{Observed PER of casein group}}$$

Diet formulation : The TSP products were separately soaked in 4-5 volumes of hot water (about 60°C), containing about 1% sodium chloride, for 20-30 min. The water was drained off, and the chunks were hand-squeezed to remove excess water. The material was passed through a meat mincer (bore size 3 mm diam) to obtain a uniform particle size. Calculated amounts of this material were added to other dietary components (refined groundnut oil : 9%, shark' liver oil fortified with α-tocopherol acetate at 0.1% level : 1%, vitaminized starch : 1%, mineral mix : 4% and corn starch to make up to 100%), as per ISI (1974), to obtain a 10±0.1% protein diet on dry weight basis. In addition to this, diets similar in protein contents were also prepared from DSF, and casein for comparison. The diets were prepared afresh every week, and stored in air-tight containers in a deep freezer at −18°C. The diets were thawed to room temperature, before feeding to the animals.

Sensory evaluation : Since TSP is intended to be used as a meat extender by the armed forces, it was evaluated for sensory qualities in the form of a spiced curry, similar to meat curry, which is popular among the military personnel. Assessment of several attributes (colour, aroma, taste and texture), and overall acceptability of the curry was carried out on a scale of 9 points by experienced panelists (13-15 persons) drawn from amongst the scientific staff (Ranganna 1986). A grade of 9 represented excellent or like extremely, and 1 extremely poor or dislike extremely.

Preparation of curry : A ground spice mix, consisting of (g) chopped onions 75, tomatoes 75, garlic 2, ginger 5, cardamom poppy seeds 10, green chillies 2, red chilli powder 5, coriander powder 15, and turmeric powder 1, was added to onion (75 g), being fried in fat (a mixture of 30 g groundnut oil and 20 g *vanaspathi)* to a golden brown colour. The frying was continued with constant stirring, until the oil started oozing out of the paste of spices. Then, 75 g chopped tomatoes were added, and the frying continued for another 10 min. The TSP chunks (100 g), hot water-soaked and gently-squeezed as described earlier, were then added along with about 100-150 ml of hot water and further cooked for 15 min, after addition of salt to taste. Finely powdered 0.5 g each of cloves, cinnamon, and cardamom were added at this stage and cooking continued for another 2 min, when the curry was ready for sensory evaluation.

Statistical analysis : The data were statistically analyzed using Duncan's multiple range test.

Results and Discussion

Evaluation of nutritional and antinutritional qualities of TSP : The commercial TSP products had moisture contents of 4.0-8.0% (Table 1). The

TABLE 1. NUTRITIONAL AND ANTINUTRITIONAL PROPERTIES OF TSP

Source	Moisture, %	Fat, %	Proteins, %	Carbo-hydrates, %	Crude fibre, %	Total ash, %	Energy value, Kcal/100g	PER observed	PER corr-ected	Feed efficiency ratio	TIA
DSF	8.1	-	53.3	-	-	-	-	1.89 ± 0.10[a]	1.44	0.19 ± 0.01*	22.7
A	5.3	0.27	53.4	30.5	3.3	7.2	338	2.51 ± 0.18[b]	1.99	0.24 ± 0.02	7.6
B	4.0	0.29	51.6	33.9	3.2	7.0	345	2.20 ± 0.16[c]	1.68	0.22 ± 0.02	5.4
C	7.4	0.28	52.7	29.3	3.3	7.0	331	2.24 ± 0.20[c]	1.71	0.23 ± 0.03	5.2
D	8.0	0.30	53.1	28.2	3.2	7.2	328	2.27 ± 0.27[c]	1.79	0.25 ± 0.03	6.8
E	6.4	1.00	55.3	27.8	3.2	6.4	341	2.65 ± 0.13[b]	2.02	0.28 ± 0.01	7.4
BIS spcn.	6.0 (Max)	-	45.0 (Min)	-	3.5 (Max)	7.0 (Max)	340 (Min)	-	-	-	-

Values are mean ± SD in the case of observed PER and feed efficiency; TIA : Trypsin inhibitor activity expressed as mg pure trypsin inhibited per g material; a,b,c : values not sharing the same superscript are significantly ($p<0.05$) different; * : Significantly different ($p<0.05$) from the rest. BIS spcn. : Bureau of Indian Standards Specifications.

moisture levels in two samples did not meet Bureau of Indian Standards (BIS) (ISI 1982). The residual fat contents of all the samples were negligible, as expected, since the raw material used for manufacturing of TSP was the defatted soy flour. The protein ranged from 51.6-55.3%, and was higher than the minimum specified by BIS (ISI 1982). The crude fibre and total ash contents were found to be well within the limits of specifications. The calorific value, however, was slightly lower in some samples than the minimum limit prescribed by BIS. This could be attributed to the low fat content of the product.

The PER (both observed and corrected) of DSF (Table 1) was found to be about 60% of that of casein (casein PER = 2.5). The PER and feed efficiency of TSPs, on the other hand, were markedly higher, as expected, due to the inactivation of the TI activity during texturization. The low TI levels of these products, 5.4-7.6 mg g^{-1}, were not apparently different, and agreed with reported values (Liener 1994). These levels of TI are not likely to cause any adverse effect in humans (Bodwell and Hopkins 1985). The PER values obtained for the TSPs used in the present study, were in the range of 1.68-2.02, as against 2.12 reported by Kies and Fox (1971). Further, Jansen et al (1978) reported a PER of 1.98 for whole soya, 1.89 for glanded cotton seed, and 1.51 for glanded cotton seed - whole corn blend (30:70 ratio). They found that extrusion temperature had a great bearing on the PER values. Bjorck and Asp (1983) also observed that the protein quality was affected by several processing parameters, including the type of extruder, temperature, pressure, moisture content of feed, compression ratio and residence time.

A relationship between the amount of TI inactivated by heat, and the quality of the protein has been reported by Rackis and McGhee 1975. This was also apparent in the present study, when PER and TI activities of DSF were compared with those of various TSP products, but not when these parameters were examined amongst TSPs. Thus, apart from the TI activity, there might be other factors that could affect the protein quality. For example, the loss of lysine has been reported to increase with temperature and decrease with increase in moisture content of the feed (Bjorck and Asp 1983).

Storage stability : The PER of the product stored for six months (Table 2) was 7-9% lower than that of the fresh product, irrespective of the type of packaging material used. This marginal reduction in PER did not reach the level of significance ($p<0.05$), except in one case. At the end of one year, a further drop of about 2-6% in PER was observed in the case of all packaging materials, except for PFP with a foil thickness of 0.02 mm, and a higher barrier property (Gosh et al. 1977). It was noticed that this assumed significance ($p>0.05$), when the decline in PER exceeded 10-15%.

Thermal treatment during texturization, apart from destroying TI activity, and consequent improving protein quality, could also cause or initiate certain other changes, such as breakdown of water-soluble proteins, thereby leading to a decrease in available lysine, cysteine and cystine (Chauhan and Bains 1985, 1988; Jeunink and Cheftal 1979). This could further progress during storage, thereby affecting protein quality. In some cases, the protein quality and PER are known to increase on extrusion cooking (Dublish et al. 1988).

TABLE 2. CHANGES IN PER AND SENSORY SCORES OF TSP DURING STORAGE FOR 12 MONTHS

Packaging material	PER				Sensory scores							
	6 M		12 M		Aroma		Taste		Texture		OAA	
	Obs	Cor	Obs	Cor	6 M	12 M	6 M	12 M	6 M	12 M	6 M	12 M
Fresh	2.37 ± 0.17	1.97			7.5 ± 0.7		7.5 ± 1.0		7.6 ± 0.7		7.4 ± 0.6	
PFP 0.02 mm	2.18 ± 0.26	1.85	2.29 ± 0.26	1.94	7.5 ± 1.1	7.2 ± 1.2	7.5 ± 1.1	7.7 ± 0.7	7.6 ± 0.9	7.3 ± 0.9	7.4 ± 1.0	7.9 ± 0.7
PFP 0.012 mm	2.15 ± 0.39*	1.82	2.02 ± 0.20**	1.72	7.5 ± 1.1	6.7 ± 1.9	7.5 ± 1.1	6.7 ± 1.7	7.4 ± 1.1	7.0 ± 1.4	7.3 ± 0.8	6.7 ± 1.7
PFP 0.009 mm	2.19 ± 0.08	1.85	2.14 ± 0.20**	1.82	7.5 ± 1.1	7.3 ± 1.3	7.5 ± 1.1	7.1 ± 1.2	7.2 ± 1.2	7.0 ± 1.2	7.2 ± 1.2	7.2 ± 0.9
PNS	2.19 ± 0.08	1.85	2.08 ± 0.24**	1.77	7.4 ± 1.1	7.3 ± 1.2	7.2 ± 1.1	7.0 ± 1.2	7.6 ± 1.2	7.0 ± 1.0	7.4 ± 1.0	7.0 ± 1.0

Values are mean ± S.D.; Obs : observed; Cor; corrected; OAA : overall acceptability; M : months

*, ** : Significantly different from the values for the fresh at $p<0.05$ and $p<0.01$ level, respectively.

It is also possible that an early Maillard damage could occur to the protein during storage of TSP, similar to that reported for milk powders during storage (Hurrell 1984). However, 10-15% reduction in PER, observed after 12 months storage in the present case, is not expected to affect the overall protein status of the defence personnel, since TSP would form only a part of the mixed protein diet.

Data on sensory scores are presented in Table 2. There was practically no change in colour (data not presented), and aroma of the curry prepared from TSP, irrespective of the packaging material, and the duration of storage. Although there appears to be a slight fall after 12 months' storage in other attributes, such as taste and texture on the Hedonic scale, and also in the acceptability or quality scores, the differences are not statistically significant.

The problem of controlling off-flavour and successfully simulating a desired flavour (e.g., meat flavour) has retarded the wide spread use of soya proteins as an extender. However, the proposed curry familiar to the military messes was found to be good, and acceptable on sensory evaluation at the end of one year. The advantage of inclusion of TSP in the military ration is that it could be used as a meat extender, whenever meat is in short supply at places like field areas. Thus, the results demonstrated that TSP can be stored for one year in PFP with 0.02 mm foil thickness, without affecting the protein and sensory qualities. Storage in PFP of lower foil thickness or in another packaging material (PNS) with poor oxygen barrier property affected (marginally) protein nutritional quality, though the product remained good and acceptable.

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Development of Instant *Kadhi* Mix

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Instant *kadhi* mix was prepared by cooking *Bengal*gram flour (1 kg) with curd (8 kg), water (8 l), salt (30 g), and turmeric powder (4 g). It was spray-dried using inlet air temperature of 180-200°C, and outlet air temperature of 100-110°C, and the powder was blended with spice mix and fried onions. It reconstituted within 3 min, when boiled with 5 times water. Acidity of the curd and *Bengal*gram flour to curd ratio was found to influence sensory acceptance, reconstitution characteristics and drying behaviour. Instant *kadhi* was most stable at 0.33 a_w. It remained stable for 12 months in paper (42 GSM)-Al. foil (0.02 mm)-polyethylene (37.5 μ) (PFP) and metallized polyester/HDLD polyethylene composite film laminate pouches at room temperature, and 37°C. In polyethylene (75μ) pouches, it remained stable for six months, but in polypropylene (PP) and composite films of low density polyethylene (LD/LD), polyester/LD/ionomer (90 μ), high density/low density/high density (HD/LD/HD) (90 μ) polyethylene, instant *kadhi* mix remained stable for 9 months at room temperature, and 6 months at 37°C. During storage, peroxide value, thiobarbituric acid value, and free fatty acids increased, while total carotenoids decreased considerably with a concomitant decrease in sensory scores. Microbiological quality of instant *kadhi* mix remained unchanged during storage.

Keywords : Instant *kadhi* mix, *Bengal*gram flour, Curd, Spray drying, Storage changes, Packaging materials, Reconstitution, Proximate composition.

Traditionally, *kadhi* is prepared from *Bengal*gram (*Cicer arietinum*) flour, and sourish curd or butter milk, both with and without vegetables and/or seasoning. It is eaten with cooked rice or *chapatis*, and is common all over India. The specific degree of sourness of the curd/butter milk is of vital importance in obtaining the best quality *kadhi*. Therefore, it is essential that such curd/butter milk is made available to prepare the instant *kadhi* mix at any given time. Moreover, preparation of *kadhi* at high altitudes, and snowbound areas is not feasible, due to difficulty in preparing the curd during winter months. Also, butter milk is available as a by-product in butter and cheese manufacturing industries, and its utilization is posing serious problems (Joshi et al. 1994). Development of instant *kadhi* and other popular traditional Indian preparations may provide a means of their effective utilization. Therefore, efforts were made to optimize the processing conditions for preparing instant *kadhi* mix by spray-drying, and evaluate its storage stability in different packaging materials.

Materials and Methods

Curd was procured from Mysore milk dairy, and it contained 3.2-3.5% fat, 11-12% total solids, and 0.8% acidity as lactic acid. Good quality dehusked *Bengal*gram *dhal* (5 kg), coriander seeds (1kg), turmeric (500 g), red chillies (500 g), and cumin seeds (1 kg) were cleaned, and ground individually in an ultracentrifugal mill (Model-Retsch R-1, Haan, Germany), using 1 mm sieve. Mustard seeds were cleaned, and mixed as such without grinding. Good quality onions were peeled, and fried in *vanaspati* (hydrogenated fat) at 150°C, and subsequently dried in a cabinet dryer (Model-SDA, Type E, Kilburn, McNeill and Magor Ltd., Calcutta) to a moisture level of about 5%.

Preparation of kadhi : One kg *Bengal*gram flour (*besan*) was mixed with 8 kg curd, 8 l water, 4 g turmeric powder, and 30 g salt. The slurry was cooked for 15 min, and spray-dried (Filtron, Pune), using inlet air temperature ranging from 180°-200°C, outlet air temperature 100-110°C, feeding rate 2.2 kg/h as per the flow diagram (Fig. 1). The spray-dried *kadhi* powder (3.5 kg) was blended with fried onion (0.5 kg), masala powder (0.5 kg) and *vanaspati* (0.5 kg). The samples (100 g/pouch) were

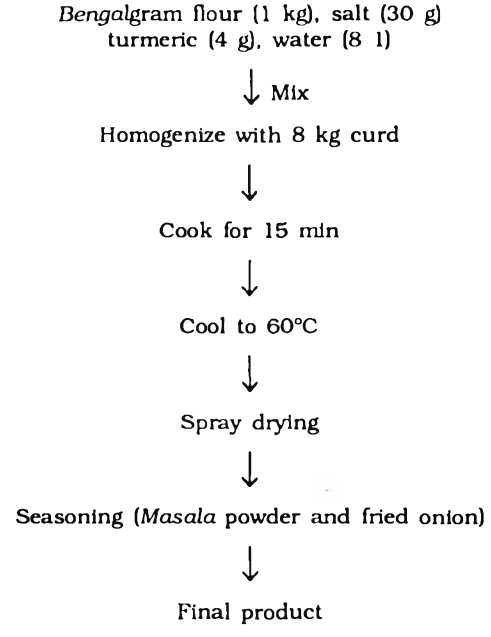

Fig. 1. Process flow chart for making instant *kadhi* mix

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packed in various packaging materials, viz., (1) low density polyethylene (LDPE, 75 μ); (2) polypropylene (PP, 75 μ); (3) composite films of low density polyethylene (LD/LD, 75 μ); (4) polyester/low density polyethylene/ionomer (PET/LD/ionomer, 90 μ; (5) high density polyethylene/low density polyethylene/ high density polyethylene (HD/LD/HD, 90 μ); (6) laminates of metallized polyester/HDLD (90 μ); and (7) paper (42 GSM)-Al. foil (0.02 mm)-polyethylene (37.5 μ) (PFP) for storage at room temperature (15-34°C), and 37°C for evaluating storage stability.

Reconstitution : Instant *kadhi* mix (100 g) was reconstituted in 500 ml boiling water, and stirred continuously for 3 min.

Analysis : Moisture, proteins, fat and total ash were determined by standard AOAC (1984) methods. Storage changes in instant *kadhi* were monitored by determining peroxide value (PV), and free fatty acids (FFA), as per AOCS (1973) methods, while thiobarbituric acid value (TBA), and total carotenoids were determined, as per the methods of Tarledgis et al (1960) and Arya et al (1979), initially and after every three months. The apparent viscosity of *kadhi* slurry was determined, using Brookfield viscometer (spindle No. 3, 50 rpm) at 40°C. The fat in curd was estimated by Gerber method (ISI 1981). The total acidity values in curd and *kadhi* were determined, as per ISI (1981) method and expressed as % lactic acid. Microbiological analysis was performed by the method described by Harrigan and McCance (1976). Sensory quality of instant *kadhi* mix was evaluated by a minimum of 10 judges by grading for colour, flavour, consistency, taste and overall acceptability on a 9-point Hedonic scale with 9, as excellent in all respects, and 1 for completely unacceptable quality.

To study the effect of water activity (a_w) on the stability of instant *kadhi* mix, the samples (200 g each) were stored for 30 days in petri dishes in desiccators, containing saturated salt solutions of known a_w: Lithium chloride (0.11), magnesium chloride (0.33), sodium bromide (0.57), and sodium nitrate (0.73).

Results and Discussion

The sensory quality of instant *kadhi* mix and spray-drying characteristics of the *kadhi* were found to be influenced by the curd acidity, and the proportion of *Bengal*gram flour to curd used in the *kadhi* preparation. The overall acceptance scores of *kadhi* mix prepared, using curd samples, having total acidity of 0.6, 0.8, 1.0, 1.1 and 1.3% were 6.4±0.3, 7.9±0.1, 6.4± 0.3, 6.7±0.2 and 6.2±0.2, respectively, on a 9-point Hedonic scale. Thus, overall acceptance of *kadhi* mix was highest, when curd acidity was 0.8%. Above and below this level, the overall acceptability of *kadhi* and *kadhi* mix tended to decrease. Also, when curd acidity increased to 1.0%, drying of *kadhi* became difficult and *kadhi* powder became sticky, and difficult to reconstitute with water. Above 1.3% acidity, *kadhi* powder tended to form insoluble lumps during reconstitution. Also, *kadhi* could be dried without causing any scorching or dispersibility problems at inlet air temperature of 200°C, as compared to maximum inlet air temperature of 175±5°C, used for drying of cheese (Kumar and Tewari 1990), and 180°C, for drying of curd (Jayaraman et al. 1991).

To optimize the ratio of *Bengal*gram flour to curd, instant *kadhi* powder was prepared, using *Bengal*gram flour and curd in different proportions. The overall acceptability scores of instant *kadhi* powders having 1:10, 1:8,1:7, 1:4, 1:3 *Bengal*gram flour to curd ratio were found to be 7.8±0.2, 8.2±0.1, 7.9±0.3, 7.4±0.1 and 6.6±0.2, respectively, indicating 1:8 *Bengal*gram flour to curd ratio to be the most suitable for *kadhi* preparation. However, instant *kadhi* prepared, using minimum of 4 parts of curd for every part of *Bengal*gram flour had only marginally lower scores and high acceptability. A lower proportion of curd in instant *kadhi* preparation is desirable in keeping the cost of the product *kadhi* low, while maintaining the overall acceptance scores above 7 on a 9-point Hedonic scale. The viscosity of *kadhi* prepared from 1:4 *Bengal*gram flour to curd ratio was 5500 cps, as compared to 1500 cps of *kadhi*, prepared from 1:8 *Bengal*gram flour to curd ratio. *Kadhi* samples having viscosity beyond 6000 cps could not be pumped for atomization during spray-drying operation.

Instant *kadhi* mix prepared with 1:8 *Bengal*gram flour to curd ratio had 17.9±0.5% proteins, 24.1±0.3% fat, 3.3±0.1% moisture and 5.7±0.1% total ash, while the one prepared with 1:4 *Bengal*gram to curd ratio had 15.8±0.2% proteins, 20.6±0.3% fat, 3.5±0.1% moisture and 7.1±0.1% total ash. High ash content in instant *kadhi* preparation having 1:4 *Bengal*gram flour to curd ratio was mainly due to higher level of salt, which was added on the basis of *Bengal*gram flour.

Effect of water activity (a_w) : Sorption isotherm of instant *kadhi* mix, and the changes in PV, and FFA in instant *kadhi* mix, when stored at different a_w for one month, are shown in Fig. 2. Instant *kadhi*

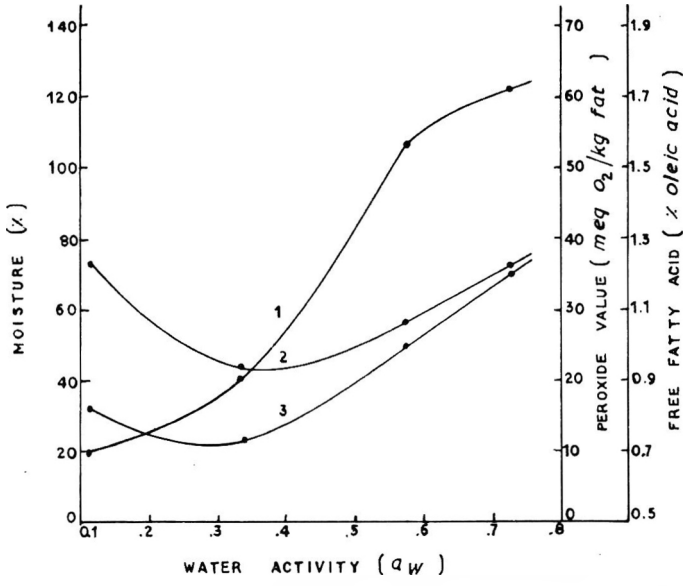

Fig.2. Effect of water activity on moisture, peroxide value (PV) and free fatty acid (FFA) contents of instant *kadhi* mix after 30 days of storage at room temperature (15-34°C).

mix equilibrated to 1.97, 3.90, 10.55 and 12.28% moisture at 0.11, 0.33, 0.57 and 0.73 a_w, respectively. No microbial spoilage was observed, when instant *kadhi* mix was stored upto 0.73 a_w for one month at room temperature (15-34°C). However, PV and FFA increased at all a_w during storage. The rate of increase was lowest at 0.33 a_w, and the rate of formation of PV and FFA increased considerably both below and above 0.33, indicating maximum product stability around 0.33 a_w. This is in conformity with the published data, as water forms hydrogen bonds with hydroperoxides, and thus prevents their undesirable decomposition at lower a_w (Labuza 1978). Water also hydrates transition metal ions, which are known to catalyze autooxidation of fats (Labuza 1978). Normally, increase in FFA in stored products results from the lipolysis of fats (Clayton and Morrison 1972). However, in instant *kadhi* mix, lipolytic enzymes get inactivated during cooking and spray-drying operations, and increases in FFA during storage mostly result from the hydrolysis of triglycerides in the presence of water. Higher FFA contents at lower a_w have also been reported in stored *halwa* mix (Arya and Thakur 1986), and *trisnacks* (*sakarpara*) (Thakur and Arya 1990).

Effect of packaging materials : The role of various packaging materials on the storage stability of instant *kadhi* mix at room temperature (15-34°C), and 37°C (Tables 1 and 2) was investigated by following changes in PV, FFA, TBA, total carotenoids, colour, aroma, taste and overall acceptability. Though the samples were analyzed after every three months, only the results of 6 and 12 months are presented. It may be observed that the rates of auto-oxidative deterioration, as measured by changes in PV and TBA values and total carotenoids, were highest in samples stored in LDPE (75 μ) and least in PFP-laminate pouches. The peroxide value of *kadhi* mix increased from 3.5 meq O_2/kg fat to 6.3-8.5 after 6 months, and 6.9-10.2 meq O_2/kg fat after 12 months storage at room temperature in various packaging materials. The increases of PV in samples stored at 37°C were slightly higher, the corresponding values being 6.9-10.2 and 7.5-12.3 after 6 and 12 months of storage, respectively. Increases in TBA values were relatively smaller.

The TBA values after 12 months storage ranged from 0.21 to 0.28 at room temperature, and from 0.22 to 0.30 at 37°C, as compared to 0.11 in the freshly prepared sample. On storage, total carotenoids in instant *kadhi* mix decreased considerably, and losses ranged from 21.1 to 35.3% and 29.1 to 57.5% at room temperature and 33.8 to 41.4% and 35.3 to 70.9% at 37°C after 6 and 12 months of storage, respectively. The losses in carotenoids were highest in LDPE and least in PFP and metallized PET/HDLD laminate pouches. FFA in instant *kadhi* mix increased on storage in all the packaging materials evaluated. Increases were considerably higher in LDPE and PP films than in PFP laminate pouches. After 12 months of storage, the FFA values in LDPE and PP pouches were 0.94 and 0.91, respectively, compared to 0.71 in PFP pouches at room temperature (15-34°C), while the corresponding values ranged from 0.83 to 1.11 at 37°C. Though moisture content in instant *kadhi* samples also increased during storage, the increases in FFA did not correlate with the changes in moisture. Maximum increases in FFA occurred during the first 3 months of storage, when the moisture increases were relatively smaller.

There was practically no change in the microbiological status of instant *kadhi* mix during storage. The total plate counts of freshly prepared instant *kadhi* mix ranged between 180-800 colonies g and those of 12 months stored samples 120-600 colonies/g. Yeast and mould counts in fresh and stored samples were below 100 colonies/g. Samples stored in different packaging materials did not differ in total plate and yeasts plus mould counts upto one year storage. All the samples were free from *Salmonella*, *S. aureus* and *E. coli*.

Changes in colour, taste, flavour and overall

TABLE 1. CHANGES IN PEROXIDE VALUE (PV), FREE FATTY ACID (FFA), THIOBARBITURIC ACID VALUE (TBA) CAROTENOIDS AND MOISTURE IN INSTANT *KADHI* MIX STORED IN DIFFERENT PACKAGING MATERIALS AT ROOM TEMPERATURE (RT) AND 37°C

Storage period, months	Packaging material	Moisture, %		PV, meq O_2/kg fat		FFA, % oleic acid		TBA, mg malonaldehyde per kg sample		Carotenoids, µg/g	
		RT	37°C	RT	37°C	RT	37°C	RT	37°C	RT	37°C
0		3.3	-	3.5	-	0.47	-	0.11	-	26.1	-
6	LDPE	4.2	3.5	8.5	9.1	0.87	1.00	0.21	0.26	16.9	15.3
	PP	4.1	3.5	6.3	8.0	0.77	0.86	0.21	0.19	18.0	17.3
	LD/LD	4.2	3.4	7.0	8.7	0.75	0.90	0.19	0.22	19.0	18.1
	PET/LD/Ionomer	4.3	3.5	7.8	10.2	0.70	0.98	0.22	0.22	20.3	18.3
	HD/LD/HD	3.8	3.4	8.3	11.0	0.58	0.90	0.22	0.23	20.6	19.7
	Met. PET/HDLD	3.5	3.3	6.2	7.4	0.71	0.87	0.19	0.24	20.1	19.6
	PFP	3.5	3.3	6.3	6.9	0.57	0.81	0.21	0.20	20.0	19.9
12	LDPE	7.2	4.8	10.2	12.3	0.94	1.11	0.28	0.30	11.1	7.6
	PP	7.2	4.9	8.6	9.1	0.91	0.96	0.27	0.27	14.1	10.5
	LD/LD	5.9	5.0	8.9	10.3	0.87	0.99	0.27	0.27	15.0	12.5
	PET/LD/Ionomer	6.6	4.6	8.7	10.0	0.86	0.99	0.26	0.28	16.9	14.8
	HD/LD/HD	4.8	4.8	9.0	10.3	0.77	0.99	0.23	0.28	16.6	13.4
	Met. PET/HDLD	4.3	4.4	7.6	8.7	0.79	0.86	0.21	0.25	18.1	16.9
	PFP	4.1	4.3	6.9	7.5	0.71	0.83	0.22	0.22	18.5	16.5

All results are mean of two values.

acceptability of the reconstituted *kadhi* samples stored at room temperature (15-34°C), and 37°C in various packaging materials are given in Table 2. Initially, the instant *kadhi* mix had an overall acceptability score of 7.9 on a 9-point Hedonic scale and, therefore, a score of 7.0 was taken as limit of shelf-life in storage experiments. As is evident, packaging material played a major role in determining the shelf-life of instant *kadhi* mix during storage. The changes in sensory scores were least in PFP and metallized PET/HDLD stored samples, and maximum in LDPE stored samples. The chemical changes also showed the same trend (Table 1). Instant *kadhi* mix stored in PFP and metallized

TABLE 2. CHANGES IN COLOUR, AROMA, TASTE AND OVERALL ACCEPTABILITY OF INSTANT *KADHI* MIX STORED IN DIFFERENT PACKAGING MATERIALS AT ROOM TEMPERATURE (RT) AND 37°C

Packaging material	Storage period, months	Colour		Aroma		Taste		Overall acceptability	
		RT	37°C	RT	37°C	RT	37°C	RT	37°C
LDPE	6	7.4	7.4	7.1	6.8^b	6.8^b	6.6^a	7.2^b	6.8^a
	12	6.5^a	6.1^a	5.6^a	6.3^a	5.6^a	6.1^a	5.5^a	5.4^a
PP	6	7.4	7.6	7.1	7.0	6.9^b	6.9^b	7.3^b	7.2
	12	6.6^a	6.6^a	6.3^a	6.2^a	6.1^a	6.2^a	6.6^a	6.0^a
LD/LD	6	7.0^b	7.2	7.0	7.3	6.7^b	7.4	7.1	7.4
	12	6.6^a	6.7^b	6.0^a	6.3^a	6.3^a	6.6^a	6.1^a	6.1^a
PET/LD/Ionomer	6	7.0^b	7.5	7.0	7.2	7.0^b	7.4	7.2	7.5
	12	6.6^a	7.0^b	6.3^b	6.4^b	6.7^b	6.8^b	6.7^b	6.8^a
HD/LD/HD	6	7.3	7.6	7.0	7.4	7.2	7.5	7.6	7.6
	12	7.0^b	6.8^b	6.1^a	6.8^b	6.7^a	6.9^b	6.5^a	6.8^a
Met PET/HDLD	6	7.6	7.8	7.3	7.5	7.3	7.6	7.7	7.7
	12	7.1^b	7.2	6.8	7.0	7.2^b	7.1	7.1	7.1
PFP	6	7.6	7.8	7.4^b	7.6	7.4	7.7	7.8	7.7
	12	7.4	7.4	6.9^b	7.4	7.5	7.6	7.4	7.4

Initial sensory scores for colour, aroma, taste and overall acceptability of instant *kadhi* mix were 7.8, 7.6, 7.8 and 7.9, respectively. SD in case of colour, aroma, taste and overall acceptability ranged from ±0.1 to ±0.3, ±0.2 to ±0.4, ±0.1 to 0.5 and ±0.2 to 0.5, respectively, and b- significantly different from initial value at 99 and 95% confidence, respectively.

PET/HDLD remained stable during the entire 12 months storage period, both at room temperature (15-34°C) and 37°C. On the other hand, instant *kadhi* mix, when packed in LDPE pouches, had shelf-life of only 6 months at room temperature. In PP, PET/LD/ionomer and HD/LD/HD pouches, *kadhi* mix remained stable for 9 months at room temperature and 6 months at 37°C.

Autooxidation of lipids and carotenoids is the major cause of sensory changes in instant *kadhi* mix during storage. Consequently, rate of storage deterioration in instant *kadhi* mix, and its shelf-life are expected to be governed by the availability of oxygen in the pack, which in turn, will be governed by the oxygen permeability of the packaging material. The PFP and metallized PET/HDLD having relatively low oxygen permeability (<100 ml/M²/24 h) exhibited longer shelf-life (>12 months). On the other hand, LDPE, LD/LD, HD/LD/HD, PP and PET/LD ionomer having relatively higher oxygen permeabilities (>1000-7700 ml/M²/24 h) could provide protection only for 6-9 months. Though packaging materials (PFP, metallized PET/HDLD) having lower water vapour transmission rates (WVTR, 0-5 g/M²/24 h/37°C-95% RH) generally exhibited longer shelf-life, WVTR of the packaging materials studied did not prove critical in determining the shelf-life of instant *kadhi* mix under experimental storage conditions.

Thus, the shelf-life of instant *kadhi* mix is mainly influenced by storage temperature, water vapour transmission rates, and oxygen permeability of the packaging materials. The PFP and metallized PET/HDLD laminates, having negligible WVTR and oxygen permeability, provided maximum protection against oxygen and moisture ingress, and therefore, samples stored in these packaging materials had the least chemical and sensory changes.

It is evident that highly acceptable instant *kadhi* mix, capable of reconstitution in 3 min by boiling with water, can be prepared by spray-drying of cooked *kadhi*, and blending the dried product with spices, fried onions and *vanaspati*. The ratio of *Bengal*gram flour to curd and the acidity of the curd have been found to play a critical role in drying operation and acceptance of *kadhi* mix. Autooxidation of lipids and carotenoids is the major cause of storage deterioration, and the nature of packaging material mainly determines the shelf-life of instant *kadhi* mix. Instant *kadhi* mix remained stable for one year, when packed in PFP, and metallized PET/HDLD pouches.

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Effect of Storage Conditions on Popping Quality of Sorghum

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Sorghum (*Sorghum bicolor* L. Moench) grains, var. 'Gwalior White', were stored at 50 to 80±3% relative humidities at 24±8°C for 6 and 12 months. Samples stored at 80±3% RH showed the highest popping, popping volume, expansion volume, flake size and organoleptic qualities. Grain hardness had significantly negative correlation with flake size, expansion volume, popping %, and grain moisture.

Keywords : Sorghum, Storage, Relative humidity, Popping quality, Organoleptic quality.

The technique of popping is commonly employed to corn (Pordesimo et al. 1990), and to some extent to sorghum (Savithri and Rao 1985). Recent studies on popped sorghum indicated that certain varieties could be popped to manufacture the product with good organoleptic qualities (Arora 1991; Savithri and Rao 1985). It has also been reported that the genotypes with good popping quality had corneous endosperm texture, greater grain hardness values and lower amount of starch, as compared to genotypes with poor popping quality (Arora 1991). The physico-chemical properties of grains change during storage (Ratna 1992). The present investigation was, therefore, undertaken to evaluate the popping quality of grains after storage at different RH levels for 6 and 12 months, and to evaluate organoleptic qualities of the popped sorghum.

Freshly harvested sorghum grains ('Gwalior White') were cleaned of dirt and grit before drying in an oven at 50 to 60°C for 4 to 8 h to kill initial insect infestation, if any (Cotton and Wilbur 1974). Grains at 8% moisture content (300g) were stored at four different RH levels viz., 50±3%, 60±3%, 70±3% and 80±3%, in two replicates each, in desiccators (dimensions; ht 300 mm, depth 105 mm, diam 200 mm) at 24±8°C. RH levels were maintained by using potassium hydroxide solution (Soloman 1951). The samples were drawn at 6 and 12 months of storage for analysis. Moisture was determined by AACC (1962) method. Hundred kernel volume was estimated by water displacement method (Arora 1991), and grain hardness by using Kiya hardness tester (Kulkarni et al. 1987). Endosperm texture was measured by visual examination of longitudinal half kernel (Rooney and Miller 1982). Popping was done, using common salt as heating medium, in an open iron pan, containing sample (5 g), and common salt (granule size : 40 mesh) at a ratio of 1:20 at 160–180°C for 40-60 sec (Arora 1991). The number of completely popped grains per sample were recorded, and expressed as popping % (Arora 1991). Popping volume was measured by rapeseed displacement method (Arora 1991). The respective formulae for expansion volum[1] and expansion volume[2] were as follows:

$$\text{Expansion volume}^1 = \frac{\text{Total popped volume (ml)}}{\text{Original weight of raw kernels (g)}}$$

$$\text{Expansion volume}^2 = \frac{\text{Total popped volume (ml)}}{\text{Original weight of raw kernels (g)}}$$

Unpopped kernel ratio was calculated taking the ratio of number of popped kernels to number of total kernels, and multiplied by hundred (Pordesimo et al. 1990) Flake size was defined as an index of individual kernel expansion, and computed as ratio of total popped volume to number of popped kernels (Pordesimo et al. 1990). Organoleptic qualities of samples were evaluated by a trained panel of 5 members. Sensory qualities were judged by scoring on a 10-point scale (Amerine et al. 1965). The panelists evaluated different samples for colour, flavour, texture, taste, softness, cloggyness, appearance, and overall acceptability. Cloggyness referred to the clogging of spaces between the teeth by popped grain. Each character was assigned a maximum score of ten marks.

The grain moisture increased to 9.33, 11.00, 13.00 and 15.20% at RH 50±3, 60±3, 70±3 and 80±3%, respectively, at 24±8°C after 6 months of storage, and remained almost constant till one year. Sorghum had floury endosperm. Hundred kernel mass and hundred kernel volume did not change appreciably during storage at various RH levels (Table 1). Grain hardness decreased from 5.9 to 5.43 kgf at RH 80±3% at 6 months of storage. Of

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TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS, POPPING QUALITY AND ORGANOLEPTIC QUALITY OF STORED SORGHUM SAMPLES

Attribute	Storage period, months								CD at 5%	
	0	6				12			1	2
		RH%				RH%				
		50	60	70	80	50	60	70	80	
Physico-chemical characteristics										
100 kernel weight, g	2.26	2.43	2.15	2.41	2.33	2.41	2.30	2.34	2.53	0.07	0.08
100 kernel volume, ml	2.00	2.00	1.90	2.00	2.00	2.10	1.90	2.00	2.10	0.19	0.22
Grain hardness, kgf	6.20	6.03	5.40	5.37	5.27	6.13	5.63	5.20	4.80	0.80	0.92
Moisture,%	8.00	9.33	11.00	13.00	15.20	9.33	11.00	13.00	15.20	0.08	0.09
Popping quality characteristics											
Popping, %	76.67	74.33	75.33	85.00	81.33	79.00	76.67	83.33	88.33	3.80	4.39
Popping volume, ml	10.67	10.50	11.17	11.67	13.83	10.08	10.83	12.50	16.00	0.35	0.41
Expansion volume[1], ml/g	4.70	4.30	5.18	4.83	5.93	4.17	4.70	5.33	6.31	0.22	0.26
Expansion volume[2], ml/g	4.72	4.75	4.65	5.40	5.98	4.33	4.65	5.37	6.67	0.22	0.25
Unpopped kernel ratio	23.33	25.67	24.67	11.67	18.66	21.00	21.67	16.67	11.00	3.60	4.15
Flake size, ml/popped kernel	0.18	0.20	0.21	0.20	0.22	0.16	0.17	0.19	0.23	0.00	0.00
Organoleptic quality scores											
Colour	6.56	-	-	-	-	3.68	6.87	6.62	8.50		
Flavour	6.75	-	-	-	-	4.93	6.62	6.56	7.68		
Texture	6.68	-	-	-	-	4.06	6.56	6.43	7.00		
Taste	6.62	-	-	-	-	5.00	6.56	6.62	7.68		
Softness	6.62	-	-	-	-	4.62	5.75	6.50	8.15		
Cloggyness	6.56	-	-	-	-	5.10	6.25	6.31	7.43		
Appearance	6.43	-	-	-	-	3.68	6.50	6.50	8.37		
Overall acceptability	6.81	-	-	-	-	4.31	6.12	6.75	8.25		

CD_1 : Between storage period, CD_2—: Between relative humidity

Scoring scale : Very poor 1-2, poor 3-4, Fair 5-6, good 7-8, very good 9-10

all the stored samples, the samples stored at RH 80±3% for 12 months gave the highest popping (88.33%), popping volume (16.0 ml), expansion volume[1] (6.32 ml/g), expansion volume[2] (6.67 ml/g), and flake size (0.23). However, popping quality of samples stored at RH 70±3%, and 80±3% improved after 6 and 12 months of storage. No significant improvement in popping quality of samples stored at RH 50±3% and 60±3% could be observed at 6 and 12 months storage (Table 1). On the basis of present study, it is not possible to explain the reason for the observed changes. Further investigations on changes in starch granules of the grains stored at different RH may give possible explanations.

Data on organoleptic evaluation of popped kernels also showed that samples popped after storage at RH 80±3% scored maximum for all sensory quality characteristics (Table 1). Correlation studies, at 5% level of significance, indicated significantly negative correlation between grain moisture and grain hardness (-0.842); significant positive correlation (0.77 to 0.78) between grain moisture and expansion volume[1&2]; and significant negative correlation of grain hardness with popping % (-0.75), flake size (-0.72), and expansion volume[1&2] (-0.88 and -0.83). Thus, increase in grain moisture and decrease in grain hardness of sample stored at RH 80±3% resulted in improved popping quality of 'Gwalior White' sorghum.

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Comparative *In Vitro* Binding of Minerals by the Fibres from Pulses, Cereals and Vegetables

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In vitro binding of Ca^{++}, Cu^{++}, Zn^{++} and Fe^{++} by 27 plant fibres, commonly consumed in India, has been studied. Binding of Cu^{++} was in the range 8.69 to 41.3%. Cereal fibres showed maximum binding for Zn^{++} (10-12%), while leafy vegetables, such as colocasia and *shepu* showed maximum binding for Ca^{++} (57 and 86%, respectively). No significant binding of Fe^{++} was observed in any of the fibres. Binding of Zn^{++} was mainly due to lignin, while Cu^{++} binding was due to cellulose and lignin.

Keywords : Plant fibres, *In vitro* binding, Calcium, Copper, Zinc, Iron, Cellulose, Hemicellulose, Lignin.

Importance of dietary fibre in prevention and treatment of non-communicable diseases is well known (Behall et al. 1987). However, high fibre diets may constitute a risk of leading to a negative mineral balance (Kelsay 1981). Indians consume a variety of cereal-pulse based diets, the intakes of dietary fibre in Indian diets being 20 to 40 g/day (Joshi et al. 1991). It is, therefore, necessary to study the comparative mineral binding ability of fibres from cereals, pulses and vegetables. This would help in understanding the relative risk of mineral binding by various dietary ingredients, and in giving dietary recomendations. *In vivo* studies of comparative performance of a large number of individual fibre in binding minerals are difficult. It was, therefore, felt more appropriate to collect this data through *in vitro* studies. Earlier work has indicated that fibres from individual foods are specific in their bile acid binding (Agte and Joshi 1991). The objectives of the present work were (i) to study *in vitro* binding of Ca^{++}, Cu^{++}, Zn^{++} and Fe^{++} by 27 plant fibres of the food items commonly consumed in India, (ii) to evaluate the effect of the level as well as component of fibre on the mineral binding, and (iii) to examine, if there is any association between binding of minerals with the components of fibre.

The fibre residues from 27 commonly consumed Indian foods were prepared, using the modified method of Hellendoorn et al (1975), which simulated gastrointestinal conditions. The binding experiment was conducted as per Ismail-Beigi et al (1977). The supernatant was tested for Ca^{++}, Fe^{++}, Cu^{++} and Zn^{++} contents, on an atomic absorption spectrophotometer (Model 2308, Perkin Elmer, Connecticut, U.S.A.), using specific cathode lamps. Sample blanks were also run simultaneously, where the above buffer without ions was added to 50 mg sample, to take care of the native ions released from the sample (Ismail-Beigi et al. 1977). The difference between the concentration of ions in the supernatants of the sample, and the control (buffer containing ions without fibre residue) indicated the amount of metal bound by the sample.

The binding of 27 residues for Zn^{++} was confirmed, using labelled $^{65}Zn^{++}$ (t1/2 244 days, B^+ 0.3 MeV, r 1.15 MeV + 0.51 MeV). For this, the buffer containing 1 µg $^{65}Zn^{++}$ /ml, with activity of 0.47 mci per ml, was added to each tube. The rest of the procedure was, as described earlier. The counts were taken on a Geiger counting system, (Model GCS 14A, Electronics Corporation of India, Hyderabad) for 500 sec. The difference in the counts/min of the supernatant, and that yielded by control solutions indicated the amount of metal bound to the sample.

During initial studies, the fibre residues showed high values for % binding of Cu^{++}. In order to further study these aspects, the binding of 8 fibres was tested using the buffer containing Cu^{++} (1 ppm), Ca^{++} (5 ppm), Fe^{++} (2 ppm), Zn^{++} (2 ppm) with 25, 50, 75 and 100 mg fibre, respectively. This is considered to cover the actual ratios, where the range of Cu^{++} intakes may be 0.5 to 5 mg/day, and that of fibre being 20 to 40 g/day (Chiplonkar et al. 1993). Binding is expressed as % binding. Care was taken to use double distilled water, and to use analar grade standards as well as reagents. Any possible contamination present was taken care of by running the blanks.

Table 1 gives % binding of each of 4 ions by 27 fibres. In order to study the effect of binding, all the results for individual ions were ranked. It is also of interest to see, if these plant fibres, as

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TABLE 1. BINDING VALUES (%) OF ZINC, COPPER, CALCIUM AND IRON

Vegetable	Botanical name	Zinc		Copper	Calcium	Iron
		a	b			
Ambat chuka	*Rumex vaxicarius*	9	7.96	19.56	26.22	0
Lettuce	*Lactuca sativa*	1	0.17	39.13	0	0
Cabbage	*Brassica oleracea*	0	n.a.	30.43	0	0
Capsicum	*Capsicum annum*	0	n.a.	17.39	0	13.10
Spinach	*Spinacia oleracia*	28	n.a.	17.39	6.06	0
Amaranth	*Amaranthus gangeticus*	0	0	4.34	32.45	0
Chawli	*Amaranthus viridis*	0	0	13.04	30.27	0
Fenugreek leaves	*Trigonella foenum graecium*	0	0.07	36.95	11.19	0
Colocasia leaves	*Colocasia antiquorum*	0	2.26	43.47	57.26	0
Radish leaves	*Raphanus sativus*	0	4.34	23.91	0	0
Shepu	*Peucedanum gravedens*	33	28.90	19.56	85.74	0
Greengram	*Phasaeolus auraus*	9	9.18	23.91	32.79	0
Rajma	*Phasaeolusvulgaris*	5	5.43	21.73	29.21	7.09
*Bengal*gram	*Cicer ariatinum*	5	1.63	23.91	0	0
Cow pea	*Vigna catjung*	1	2.74	21.73	6.27	3.03
Moth beans	*Phaseolus aconitifolius*	0	n.a.	8.69	17.91	3.10
Redgram	*Cajanus cajan*	0	1.32	13.04	0	0
Greengram *dhal*	*Phsaeolus aureus*	0	0	15.21	8.09	0
Peas	*Pisum sativum*	0	n.a.	17.39	12.40	0
Blackgram	*Phaseolus mungo*	3	7.04	17.39	12.06	2.02
Cauliflower	*Brassica oleracea*	0	0	19.56	2.7	7.37
French beans	*Phaseolus Vulgaris*	0	0	15.21	0.35	0
Cluster beans	*Cyamopis tetragonaloba*	0	0	10.86	0	0
Wheat	*Triticum aestivum*	22	21.58	32.60	8.29	0
Ragi	*Eleusine coracana*	16	16.20	41.30	8	0
Jowar	*Sorghum vulgare*	21	23.73	34.78	0	6.06
Rice	*Oryza sativa*	11	10.03	34.78	0	0

a : Estimated by Atomic Absorption Spectrophotometer, b: estimated by using radio isotope, n.a. : not available

Comparison between a and b by paired t test indicated the difference to be non-significant (t=0.639). Each value represents mean of 2 independent determinations.

a class, behave similarly for each ion. Analysis of variance (Snedecor and Cochran 1967) indicated that there was significant difference in % binding of the 4 classes, namely pulses, cereals, leafy vegetables and other vegetables, for Cu^{++} ($F= 5.04$, $p<.01$), Zn^{++} ($F= 3.38$, $p< .05$) and Fe^{++} ($F= 4.69$, $p<.05$), while there was no significant difference in binding between these classes in case of Ca^{++} ($p>0.05$). Binding of Cu^{++} was observed for all the 27 fibres studied. The % binding was in the range 8.69 to 41.3. There is no report available on the *in vitro* binding of dietary fibre residues from the Indian food materials for trace minerals including Cu^{++}. The evidence from *in vivo* studies is also controversial (Kelsay et al. 1979a; Turnland et al. 1985).

Zn^{++} showed negligible binding with leafy vegetable fibres with the exception of *shepu* (33%) and spinach (28%). This may be due to the high contents of oxalates in these two vegetables (Agte et al. 1984). Data also indicated that fibre residues from cereals bound Zn^{++} in the range of 10-22%. Further, the presence of fibre, along with high oxalate containing foods, may be considered as a risk factor in the maintenance of Zn^{++} balance. Binding with Fe^{++} is generally seen to be low. The buffer used in the experiment contained ascorbic acid, and this could possibly explain the low binding of Fe^{++}. Studies on most of the human balance experiments indicate that fibre alone does not affect the iron balance (Sandstead et al. 1978), if phytate content is low.

Binding of Ca^{++} was in the range of 0-57%, except for *shepu*, which showed 87% binding. Other leafy vegetables, like colocasia, amaranth, *ambat chuka*, fenugreek and *shepu*, showed considerably higher rank for Ca^{++} binding than that by spinach.

Table 2 gives % mineral binding values of eight fibre residues at four levels of fibre. There was no binding of any mineral at 25 mg level of fibre. Binding % increased as the level of fibre residue increased for Fe^{++}, Cu^{++}, Zn^{++} and Ca^{++}. Literature reports involving *in vivo* studies indicate that these ions may affect the bioavailability of each other by competing for the same sites or carrier molecules (Agte et al. 1994; Solomons 1983). The response of binding of isolated ions may, therefore, be different than the actual situation, wherein a variety of ions are available. Studies on binding of ions in a buffer containing a mixture of ions,

TABLE 2 : PERCENT BINDING VALUES OF IRON, COPPER, CALCIUM AND ZINC

Vegetable	Fe			Cu			Ca			Zn		
	50 mg	75 mg	100 mg	50 mg	75 mg	100 mg	50 mg	75 mg	100 mg	50 mg	75 mg	100 mg
Fenugreek	0	0	31.2	0	37.0	51.9	0	0	21.6	0	25.6	42.5
Ragi	0	0	7.3	5.3	38.6	51.3	0	24.7	40.7	0	28.4	58.7
Wheat	0	0	9.5	4.0	36.0	50.7	0	0	0	0	26.8	50.8
Jowar	3.6	18.3	22.4	33.3	57.0	62.8	8.6	49.0	51.3	24.3	52.4	62.7
Peas	0	0	38.2	4.5	36.4	51.0	0	0	6.3	0	24.0	47.0
Cauliflower	25.0	47.0	53.7	4.6	36.4	54.3	0	2.1	12.3	0	32.8	43.7
Moth beans	0	0	16.5	34.5	58.1	62.0	0	33.6	34.1	29.0	49.7	65.0
Redgram	0	0	10.5	2.6	35.9	51.3	0	40.6	58.9	0	27.2	44.7

No binding at 25 mg level

indicated the low fibre- high ion, low ion-high fibre, and intermediate situations. Human balance studies on interaction of fibre with mineral absorption from the composite Indian diets support these findings (Agte et al. 1994).

The components of fibre residues were further tested as independent factors for their ability to bind each of the mineral, using multiple regression analysis (Snedecor and Cochran 1967). Results indicated a significant association of lignin and Zn^{++} binding (r^2= 0.56, F3,20=8.48; t=4.46, p<.01), but not for cellulose and hemicellulose. These results are also supported by an *in vitro* study, in which hemicellulose and cellulose components from soy flour, tapioca starch and wheat flour, did not affect Zn^{++} availability (Shah and Belonje 1983). In case of copper, both cellulose and lignin (t=3.3, 4.8, p=<.01) played a role in binding.

The present study indicated binding of Cu^{++} by all the fibres. Cereal fibre showed maximum binding of Zn^{++}, while leafy vegetables showed negligible binding. This suggests a risk of negative Cu^{++} and Zn^{++} balances on consuming high levels of cereal fibre and low levels of Cu^{++} and Zn^{++}. No Significant binding of Fe^{++} was observed in case of all fibres indicating Fe^{++} availability in the presence of ascorbic acid.

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Production of Mango Vinegar by Immobilized Cells of *Acetobacter aceti*

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Vinegar was produced from mango pulp by the twin processes of fermentation and oxidation, using *Saccharomyces cerevisiae* and *Acetobacter aceti*. *S. cerevisiae* was recycled to improve fermentation rate, while *A. aceti* was immobilized on wood shavings for semi-continuous vinegar production. The final vinegar produced had 5.3% acidity as acetic acid. A conversion efficiency of 60% was achieved.

Keywords : Vinegar, Mango, *Saccharomyces cerevisiae*, *Acetobacter aceti*, Cell recycling, Immobilization, Acetic acid.

Fruit vinegar is produced mostly from grapes throughout the world, while in India, it is generally produced from sugarcane (Tewari et al. 1991), and *Jamun* (*Syzygium cuminii* L.) (Anon 1976). Mango is one of the major fruit crops and is grown in large tracts of the country (Majumdar and Sharma 1990). Despite being a rich source of sugars (Karla and Tandon 1983), it has not been used for vinegar preparation. Vinegar is mostly produced by natural batch fermentation, but it is a very slow process, and takes more than five weeks for its completion (Ebner 1982). By immobilization of bacterial cells, fermentation time can be reduced drastically (Flint 1987; Hang et al. 1989). The present study was, therefore, undertaken to find out the feasibility of mango vinegar production by cell immobilization.

Saccharomyces cerevisiae var. ellipsoideus Montrachet strain, obtained from Department of Food Science, New York State Agricultural Experimental Station, NY, was maintained on Yeast Extract Potato Dextrose agar slants (Collins and Lyne 1985). *Acetobacter aceti* NCIM 2094, obtained from National Chemical Laboratory, Pune, was maintained on Acetobacter agar slants (Collins and Lyne 1985). The cells were grown in Acetobacter broth for 48 h at ambient temperature (30°-37°C). Actively growing cells were concentrated by centrifugation, and re-suspended in phosphate saline buffer (pH 7.0). *Acetobacter* cells were immobilized by the methods of entrapment and adhesion. In the former method, cells were immobilized on calcium alginate gel beads (2 mm size), as described by Hang et al (1989). Each bead contained approximately 10^{10} cells. In case of adhesion, sterile wood shavings (1 mm thick and 6-8 cm long) were dipped in cell concentrate for 48 h, till a film of bacteria was formed (Ebner 1982). The wood shavings contained approximately 10^8 cells/g.

Mango wine was prepared by inoculating homogenized ripe mango (cultivar 'Dashehari') pulp with *S. cerevisiae* at 10% inoculum level and incubating at 25±5°C. After the desired fermentation process, as indicated by ethanol production, and sugar consumption, the wort was siphoned after 15 days. The sediment containing cells was recycled, and used as the inoculum at 10, 20 and 30% levels for preparing the next batch of wine/wort.

Five hundred ml of wort was inoculated with 10% immobilized calcium alginate beads or wood shavings or free cells. The flasks were kept at 30±5°C, till a constant acidity was achieved. The wort was analyzed regularly for ethanol (Caputi et al. 1968), total acids, and sugars (AOAC 1975). *Acetobacter* population was estimated by pour plate method on Acetobacter agar (Collins and Lyne 1985). The sensory quality of the mango vinegar was assessed by a panel of 7 judges using Hedonic scale for colour, flavour and taste.

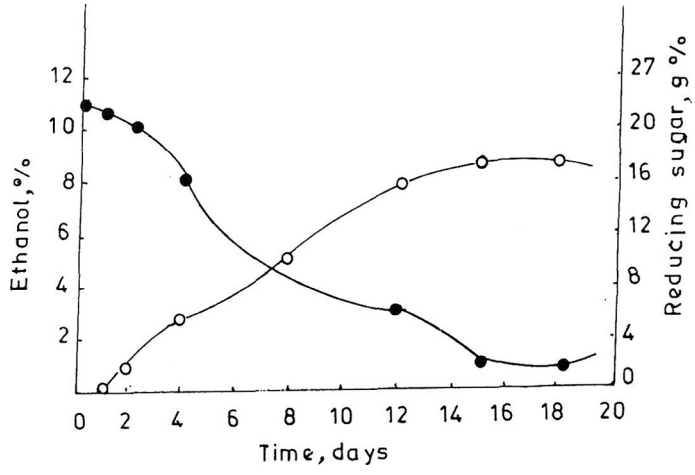

Fig. 1. Kinetics of mango fermentation by *S. cerevisiae*. O:Ethanol produced, ● : Reducing sugar

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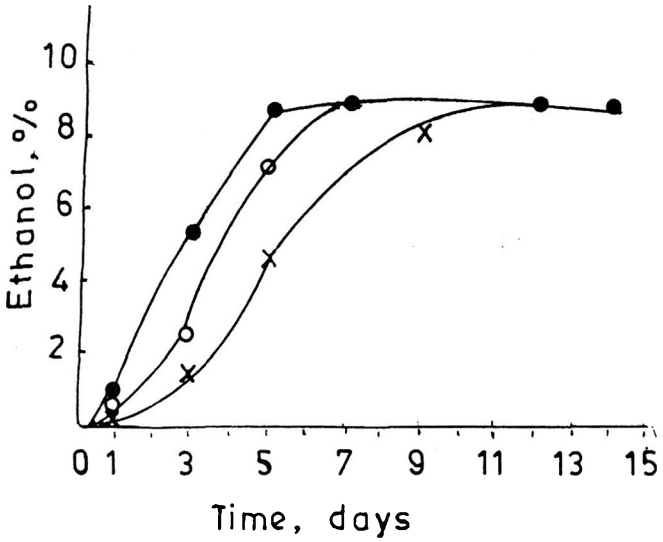

Fig. 2. Effect of recycling on mango pulp fermentation. ● : 30%, cell pulp recycling, O : 20% cell pulp recycling, X : 10%, cell pulp recycling

The batch fermentation of mango pulp yielded 8.8% ethanol after 15 days of fermentation. The total sugar content decreased from 22% to around 1% (Fig. 1). Efforts were made to further reduce the fermentation period by cell recycling technique, where a high initial yeast level was maintained either by centrifugation or sedimentation (Bardiya 1980). The fermentation was complete in 12 days with 10% recycled inoculum, whereas it took 7 days, when the cell mass was doubled. Higher initial cell mass, i.e., 30% inoculum, further reduced the fermentation time to five days (Fig.2). Similar reduction in fermentation time for bioethanol production from cane molasses and sugar beet has been reported earlier (Bardiya 1980; Goyal et al. 1984).

Fermented wort, when inoculated at 10% inoculum level with free *Acetobacter* cells, took 28 days for conversion of ethanol into acetic acid. The acetification time was reduced to 22 and 7 days, respectively, by using immobilized beads and wood shavings. Further, recycling of wood shavings reduced the acetification time to 5 days. Maximum productivity of 1.06% acetic acid/day was obtained with wood shavings. In the adhesion method, higher fermentation efficiency was achieved, because of increased surface area, and air spaces within the wooden threads (Ebner 1982). On the contrary, slower fermentation rate with immobilized gel beads was due to the fact that viability of some cells was lost during entrapment in the gel. Moreover, the transport of reactants in the gel was limited, because of the diffusion gradient between the gel matrix and the cells (D'Souza 1989). In the adhesion method, the cells adhering to wood thread surface were in direct contact with the liquid phase and distinctly separated also. This eliminated the gradient difference problem associated with the entrapment technique.

After the completion of acetification, raw vinegar was racked at 10±2°C for 2 months. Vinegar was then filtered through a Whatman No 1 filter paper, and pasteurized at 0.7 kg/cm² for 5 min. before bottling. The mango vinegar was stored at ambient temperature in sterilized crown-corked 200 ml glass bottles. The vinegar thus produced had a final acidity of 5.3% as acetic acid. It was light yellow in colour with pleasant fruity flavour, and was organoleptically superior, and acceptable. The mango vinegar had acceptable quality parameters in terms of colour, odour, and taste. The conversion efficiency achieved was 60%. The recovery of vinegar was 15% of the total pulp taken. The cost of preparing mango vinegar may seem to be high, but it gets compensated by the quality in terms of colour and taste. Its cost of production can be lowered, if the residue left after ethanol production is used as a source of single cell protein in animal feeds (Goyal et al. 1984).

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Fermentation Prospects of Two Phylloplane Bacteria in Traditional *Hawaijar* Made from Boiled Soybean (*Glycine max* L.)

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Bacillus subtilis and *Xanthomonas* sp. were predominantly isolated from the phylloplane of fig (*Ficus hispida* L.) leaves. Fermentation prospects of these two bacteria showed that *B. subtilis* was more suitable than *Xanthomonas* sp. in terms of appearance, texture, odour, flavour and acceptability for the preparation of *Hawaijar*, a fermented soybean preparation.

Keywords : *Hawaijar*, Fig (*Ficus hispida* L.), Phylloplane bacteria, *Bacillus subtilis*, *Xanthomonas* sp., Soybean fermentation.

Hawaijar, a fermented soybean product, is traditionally consumed in Manipur. The fermented bean is either eaten with little salt to glorify the monotonous rice dishes or incorporated in various curry preparations to add flavour, and to soften the recipes. It is also used in place of fermented fishes, when the latter is not allowed due to religious taboos.

Hawaijar is prepared by wrapping visually healthy boiled soybeans in the leaves of common fig plant (*Ficus hispida* L.), and incubating for 3-4 days at ambient temperature in the kitchen. It results in the production of a gray tan coloured bean with sticky substances in between the beans. The desired flavour and taste can not be perceived, if the boiled seeds are not wrapped with these leaves. Wrapping of boiled soybeans in straw also serves the purpose (USDA 1958), since the straw contains the organisms responsible for soybean fermentation. Japanese *Natto* and Thai *Thua-nao* also undergo fermentation of boiled soybeans with inoculation of *Bacillus natto* (*B. subtilis*, according to Buchanon and Gibbons 1974). No attempt has so far been made to identify the organism responsible for such fermentation from fig leaves. Therefore, in the present studies, the phylloplane bacterial microflora of *Ficus hispida* were isolated, identified, and their role in soybean fermentation was investigated.

Fresh leaves of *Ficus hispida* were collected monthly from three localities in Imphal during 1990 in absolute alcohol surface-sterilized polythene bags. Dilution plate method (Deak and Timar 1988) was used for isolation of phylloplane bacteria of *F. hispida*. Fifty circular discs (0.5 cm diam) of leaves were cut with flame sterilized cork borer, and washed in peptonised steriled distilled water (Deak and Timar 1988). One ml of such leaf-wash was inoculated aseptically into nutrient agar plate and incubated at 38±1°C for 48 h, and colony forming units were counted and identified (Buchanon and Gibbon 1974; Deak and Timar 1988).

Only two bacteria were isolated from the leaves of *F. hispida* throughout the year. The monthly total CFU-cm^2 of each bacterium and meteorological variables are shown in Table 1. The Gram positive, motile, catalase positive, endospore forming rods were identified as *Bacillus subtilis*. It hydrolyzes starch, liquifies gelatin at 22°C, shows positive Voges Proskauer reaction, does not grow in anaerobic condition, and withstands steam cooking for 20 min. Another isolate, Gram negative, motile and catalase positive rod, and yellowish colony with smooth margin in nutrient agar medium, was identified as *Xanthomonas* sp.

TABLE 1. MONTHLY CFU-cm^2 OF *BACILLUS* AND *XANTHOMONAS* FROM PHYLLOPLANE OF FIG LEAVES AND METEOROLOGICAL VARIABLES.

Month	Mean temp, °C	Mean RH, %	Mean rain fall, mm	*Bacillus* 10^4 cfu cm^{-2}	*Xanthomonas* 10^3 cfu cm^{-2}
February	13.0	67.1	143.0	2.20	6.25
March	19.4	61.4	38.0	3.30	8.25
April	21.0	54.3	22.0	ND	0.37
May	21.0	70.0	44.0	0.85	1.65
June	25.0	74.3	236.5	1.53	1.25
July	25.0	84.3	148.5	1.13	6.20
August	25.0	84.3	196.0	1.20	6.50
September	24.2	82.9	44.0	1.30	7.50
October	17.8	80.0	66.0	0.85	7.50
November	12.2	82.9	77.0	0.62	7.50
December	10.6	68.5	60.5	0.38	8.10
January	9.8	67.1	55.0	0.16	8.05

ND : Not done

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The role of these bacteria on soybean fermentation was tested by inoculating about 10^4 cells each of *B. subtilis* and *Xanthomonas* sp. separately into 100 g boiled soybean seeds in a 250 ml conical flask aseptically. A control series with no bacterial cells was also taken. Five replications were taken for each treatment, and incubated at 38±1°C for 38 h in an incubator. The seeds were examined visually for every 12 h. At the end of 48 h, the seeds were subjected to sensory evaluation by a group of healthy students, teachers and persons, who were traditional makers of *Hawaijar* in Manipur. The team was asked to assess the seeds on a 10-point scale, viz., poor 1-2, fair 3-4, good 5-7, and excellent 8-10, in terms of appearance, texture, flavour and smell. The seeds treated with *B. subtilis* scored an average of 7-8 points, as in case of home made traditional *Hawaijar*. In contrast, the seeds treated with *Xanthomonas* species scored a poor scale of 1-2, thereby indicating that they were unacceptable and unsuitable for fermentation. Control beans were as good as freshly boiled ones.

Solid substrate fermentation of oriental foods for enhancement of flavour has been known for many centuries (Moo-Young et al. 1983; Steinkraus 1983). Similarly, the fermented soybean (*Hawaijar*) prepared in the traditional way is being consumed since time immemorial in Manipur State. However, there is always a potential danger of contamination with toxic organisms with occasional stray instances of food poisoning after consumption of these products. This may either be due to contamination with alien microorganisms or use of unhealthy seeds. Hence, scientific preparation of *Hawaijar* by incorporating starter culture of *Bacillus subtilis* isolated from *Ficus hispida* will have good potential.

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Occurrence of *Salmonella* Serovars in Foods of Animal Origin with Special Reference to Antibiogram and Enterotoxigenicity

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A total of 920 samples comprising poultry and poultry products (500), frozen fish (120), and milk as well as milk products (300) were examined for the presence of *Salmonella*. Highest level of *Salmonella* contamination of 10.52% was detected in dressed frozen chicken, while *Salmonella* was not isolated from any of the samples of chicken-sausage, quail meat, raw milk or cheese. Antibiogram of the isolates indicated maximum resistance to triple sulphas (86.66%), followed by sulphonamide compounds (66.66%), trimethoprim (53.33%), ampicillin, streptomycin and nitrofurantoin (33.33% each), tetracycline (22.66%), chloramphenicol, gentamicin and kanamycin (6.6% each). All the isolates were sensitive to neomycin. Among 18 isolates, 11 (61.1%) were enterotoxigenic, as indicated by rabbit-ligated-ileal-loop technique.

Keywords : *Salmonella*, Enterotoxin, Antibiogram, Poultry meat, Milk products, Frozen fish.

Changing food habits of the society have substantially increased the demand for and consequently, production of ready-to-cook and ready-to-eat foods of animal origin (Chatterjee 1985). These foods are prone to contamination with bacterial pathogens (Singh 1981; Paturkar et al. 1992). Salmonellosis has been reported to outnumber all other bacterial food poisoning outbreaks (Sinell and Kolb 1981). *Salmonella* are also known to occur in meat and meat products (Narasimha Rao, 1983; Reddy and Mandokhot 1988a, 1988b). Among the foods, poultry products are predominantly involved in causing foodborne salmonellosis (Bryan 1980). Besides, milk and milk products as well as fish products have been incriminated in outbreaks of human salmonellosis (D'Aoust 1989). However, the information available with reference to the occurrence of *Salmonella* in poultry, fish and dairy products is very scanty in India (Gupta and Verma 1993). In the present study, an attempt has been made to find out the level of *Salmonella* contamination in poultry, fish and dairy products, with emphasis on their antibiogram and enterotoxigenicity.

Samples of frozen fish, poultry products, raw milk and milk products were collected from various retail outlets in New Delhi, Pantnagar, Haldwani, Bareilly, Moradabad and Nainital. Samples were collected under aseptic conditions, and brought to the laboratory in an ice-box. The samples of raw milk/meat were analyzed within 3 h of collection, while frozen meat samples were stored in freezer, and examined within a week of collection.

Pre-enrichment of different types of samples was done in buffered peptone water (Edel and Kampelmacher 1973). Frozen chicken carcasses were washed vigorously in buffered peptone water in plastic bags, the carcasses removed and the wash was used (D'Aoust et al. 1982). To each of the 25 g finely minced samples of poultry/fish meat, 225 ml buffered peptone water was added (Vassiliadis et al. 1984). Eggs were washed in running water, followed by 95% alcohol, and dried. The egg content was removed aseptically, and mixed with 250 ml buffered peptone water. One litre of raw milk was stirred at 100 rpm for 10 min, using folded sterile gauge (10x15 cm), wrapped on sterile steel wire, and the gauge was transferred to 150 ml buffered peptone water. To the remaining milk, 20 mg brilliant green was added (Wells et al. 1971). To 25 g finely ground cheese and chocolate, 225 ml sterile reconstituted non-fat-dry-milk containing 0.002% (w/v) brilliant green was added, and blended thoroughly in a blender. Milk powder (25 g) was reconstituted in 50 ml buffered peptone water, and kept at room temperature for 30 min to avoid osmotic shock. This was followed by further dilution with 175 ml buffered peptone water (Poelma et al. 1981). Incubation for pre-enrichment was done in all the cases, at 37°C for 18 h.

After pre-enrichment, 0.1 ml of the pre-enriched broth was transferred to 10 ml of Rappaport-Vassiliadis (RV) medium (Vassiliadis 1983). After 24 and 48 h incubation at 43°C, sub-cultures were made on modified brilliant green agar plates (HiMedia), and the plates were incubated at 37°C for 24 h. From the plates, showing *Salmonella* like

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growth (Edwards and Ewing 1972), 3 suspected colonies were picked up, and transferred to nutrient agar (HiMedia) slants. The isolates were identified on the basis of their morphological, and biochemical characteristics according to Edwards and Ewing (1972). The serotyping of the cultures was done at the National *Salmonella* Centre, Kasauli, and I.V.R.I., Izatnagar, India. Enterotoxigenicity of the isolates was tested in the cell-free supernatant by rabbit-ligated-ileal-loop (RLIL) technique (Sedlock and Deibel 1978). A preparation yielding a dilatation index (fluid accumulation in ml/cm gut segment) of 0.4 or above was considered as enterotoxic.

Antibiograms of the isolates were determined by disc diffusion method, as described by Bauer et al (1966). Two ml of brain heart infusion broth (HiMedia) medium was inoculated with the test organism, and incubated at 37°C for 6 h. Thereafter, a uniform lawn of the culture was prepared on Muller-Hinton agar (HiMedia) plates with the help of sterile swab, soaked with the culture. Standard discs of 11 common antibiotics (ampicillin, chloramphenicol, gentamicin, kanamycin, neomycin, nitrofurantoin streptomycin, tetracycline, trimethoprim, triple sulphas, and sulfonamide compounds), procured from M/s HiMedia, Bombay (India), were placed onto the surface of agar plates. The zone of inhibition of growth was measured, following overnight incubation of plates at 37°C, and the resistance pattern was determined according to the manufacturer's instructions.

The distribution of *Salmonella* serovars in various foods of animal origin is shown in Table 1. Among these foods, frozen chicken carcasses accounted for the highest level of contamination (10.52%), resulting in the isolation of 4 serovars viz., S. Typhimurium, S. Saintpaul, S. Indiana and S. Stanley. The level of contamination is less than the level of 19% reported by Zurcher and Hadorn (1984). A lower level of *Salmonella* contamination observed in the present study is in accordance with the findings of Izzi and Quesada (1981). The presence of *Salmonella* in dried milks and chocolates (Table 1) is of serious public health concern, as these products are not heat-processed prior to consumption. A lower level of *Salmonella* contamination (1.66%) was recorded in samples of frozen fish, similar to that reported by Izzi and Quesada (1981). Considering the consumption pattern of fish, the presence of *Salmonella* even in low numbers is of great public health significance. Fish and shell-fish products have been incriminated in a large number of outbreaks of human salmonellosis (D'Aoust 1989).

TABLE 1. DISTRIBUTION OF *SALMONELLA* SEROVARS IN FOODS OF ANIMAL ORIGIN

Products	Sample numbers: Analyzed	Positive for *Salmonella*	*Salmonella* serovars isolated	
Poultry				
Dressed frozen chicken	76	8	*S. Typhimurium*	(3)
			S. Saintpaul	(3)
			S. Indiana	(1)
			S. Stanley	(1)
Chicken-n-ham	46	2	*S. Derby*	(1)
			S. Newport	(1)
Chicken sausage	23	-	-	-
Egg	320	2	*S. Newport*	(2)
Quail meat	35	-	-	-
Milk and milk products				
Raw milk	36	-	-	-
Dry milk powder	83	3	*S. Havana*	(3)
Milk chocolate	126	1	*S. Stanley*	(1)
Cheese	55	-	-	-
Fish				
Frozen fish	120	2	*S. Senftenberg*	(1)
			S. Amsterdam	(1)

- : Nil. Figures in parentheses indicate the number of isolates obtained.

Isolates of *Salmonella* obtained from the foods of animal origin belonged to 9 different serovars as follows : 3 each of *S.* Typhimurium, *S.* Newport, *S.* Havana and *S.* Saintpaul, 2 of *S.* Stanley and 1 each of *S.* Indiana, *S.* Derby, *S.* Amsterdam and *S.* Senftenberg. Of these serotypes, *S.* Newport and *S.* Indiana have been reported to be associated with a large number of foodborne outbreaks of human salmonellosis (Beckers et al. 1985).

Drug resistance studies revealed maximum resistance to triple sulphas (86.66%), followed by sulphonamide compounds (66.66%), trimethoprim (53.33%), ampicillin, streptomycin and nitrofurantoin (each 33.33%), tetracycline (22.66%), chloramphenicol, gentamicin and kanamycin (6.6% each). All the isolates were found to be sensitive to neomycin. Absence of resistance to neomycin and low levels of resistance to chloramphenicol, gentamicin, and kanamycin may be attributed to relatively little use of these antibiotics in animal treatment in India. Out of 18 isolates, 2 showed resistance to 2 antibiotics, 5 to 3 drugs, 3 to 4 drugs, 4 to 5 drugs, 2 to 6 drugs, and 2 to 7 drugs.

Enterotoxigenicity of cell-free supernatants from 18 *Salmonella* isolates revealed that 11 (61.1%) isolates were enterotoxigenic. These enterotoxigenic serotypes included *S.* Stanley, *S.* Saintpaul, *S.* Newport and *S.* Typhimurium. The presence of

enterotoxigenic *Salmonella* in foods of animal origin is of significance, as these can induce cholera-like profuse diarrhoea in human beings (Axon and Poole 1973). From the public health point of view, it is important that measures should be introduced at the time of processing of products to reduce the levels of *Salmonella* contamination.

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Effect of Lactic Acid, Ginger Extract and Sodium Chloride on Electrophoretic Pattern of Buffalo Muscle Proteins

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SDS-PAGE technique was used to study the effect of anti-microbial agents, such as lactic acid, ginger extract, and sodium chloride on buffalo meat (LD muscles), stored at two different temperatures (i) ambient (26±2°C) and (ii) chill temperature (4±1°C). There was an increased proteolysis of muscle proteins at ambient temperature, compared to chill temperature, as evidenced by reduction in the number of protein bands separated by electrophoresis. Lactic acid, ginger extract and sodium chloride treatment of meat enhanced proteolytic activity in meat during storage. Ginger-extract can be used as a good source of proteolytic enzyme for tenderization of buffalo meat.

Keywords : Lactic acid, Ginger extract, Sodium chloride, Proteolysis, Meat tenderization, Electrophoretic analysis.

Polyacrylamide gel electrophoresis was used by some workers (Aberle and Markel 1966; Jong and Tatsumi 1983) to study meat proteolysis under different temperatures and pH, and also used for the detection of adulteration of meat (Moorjani et al. 1974). Yates et al (1983) studied the effect of higher temperature on release of catheptic enzymes which increased tenderness of meat. Addition of organic acids, like acetic and lactic, inhibits microbial growth, controls spoilage, and extends the shelf-life of meat and quail meat (Ockerman et al. 1974; Corlett and Brown 1980). Addition of lactic acid bacteria is known to improve the colour and texture (Sankaran et al. 1986). Lactic acid is often used for controlling microbial growth on meat (Gill and Newton 1982; Murali et al. 1985). Ginger (*Zingiber officianalis*) is one of the popular spices in Indian cuisine (Govindarajan 1982), and contains a powerful proteolytic enzyme that can be used for tenderizing meat (Lee et al. 1986). Lactic acid and ginger, along with sodium chloride, are likely to bring about certain changes on muscle proteins. The changes in the proteins of meat treated with lactic acid and ginger extract, singly and in combination with sodium chloride, were, therefore, studied by the sodium dodecyl sulphate polyacrylamide gel electrophoretic (SDS-PAGE) technique.

Longissimus dorsi muscles (LD muscles) of buffalo carcasses were procured, soon after slaughter, from the local slaughter units. The test solutions were sprayed on meat cuts at the rate of 20 ml/kg. The test solutions consisted of 2% lactic acid, 20% sodium chloride, 2% lactic acid + 20% sodium chloride, ginger extract, and ginger extract + 20% sodium chloride. The treated and untreated (control) meat cuts were stored at (i) ambient temperature (26±2°C), and at (ii) chill temperature (4±1°C) for 24 h and five days, respectively. Ginger extract was prepared by blending 100 g Mysore variety ginger cubes with 100 ml chilled distilled water for 1-2 min. Meat samples from both treated and control LD muscles were collected for analysis. Minced meat (5 g) was mixed with 50 ml of 0.01 N sodium phosphate buffer (pH 7.0), containing 1% sodium dodecyl sulphate (SDS) plus 1% 2-mercaptoethanol, and incubated at 37°C for 2 h. The mixture was then centrifuged at 4000 rpm for 30 min. An aliquot of supernatant was dialyzed overnight at room temperature (26±2°C), against 0.1 N sodium phosphate buffer, containing 0.1% SDS and 0.1% 2-mercaptoethanol. About 50 µl of dialyzed solution was used for SDS-PAGE (Weber and Osborn 1969). Staining with coomassie blue was done following the method described by Fairbanks et al (1971). The molecular weight of each protein band was calculated by extrapolating the mobility values from a standard curve obtained by the log of molecular weights of proteins. Five replicates were used for each set of experiment.

Effect of lactic acid and sodium chloride on meat protein pattern at ambient temperature (26±2°C) : In case of meat samples, treated with 2% lactic acid (Fig. 1C) or 20% sodium chloride (Fig. 1D), 13 bands of both low and high molecular proteins, with the molecular weights ranging from 14.1 to 104.7 Kilodalton (KD), were seen, as against 16 bands in control (Fig. 1A) with KD values in the range of 14.4 to 112.2. The decrease in the number of bands in lactic acid and sodium chloride-treated meat samples may probably be due to denaturation and proteolysis of treated meat samples. In case of meat samples, treated with the combination of 2% lactic acid + 20% sodium chloride, 15 bands

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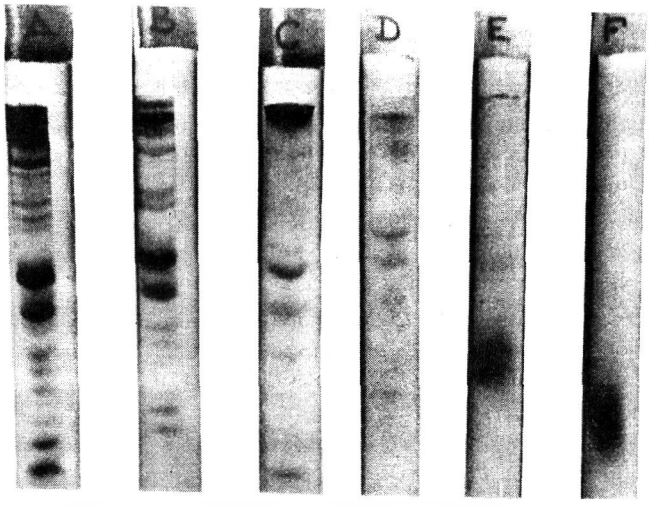

Fig.1. Effect of antimicrobials on electrophoretic pattern of muscle proteins stored at 26±1°C
A : Untreated (control), B : 2% Lactic acid + 20% NaCl, C : 2% Lactic acid, D : 20% NaCl, E: Ginger extract, F : Ginger extract + 20% NaCl

with molecular weights ranging from 10.0 to 104.6 KD values were observed (Fig.1B) at the end of storage period. The combined treatment seems to bring about less changes in the meat protein pattern.

Effect of ginger extract and sodium chloride at ambient temperature (26±2°C) : Meat samples, treated with ginger extract showed only four bands, with the molecular weights in the range of 12.5 to 39.8 KD (Fig. 1E), as against 16 bands in control at the end of 24 h storage. Meat treated with ginger extract + 20% sodium chloride solution showed only one thick band of 10 KD value (Fig 1F), indicating high degree of proteolysis due to the treatment with ginger. This is in agreement with the findings of Lee et al (1986), who observed

Fig.2. Effect of antimicrobials on electrophoretic pattern of muscle proteins stored at 4±1°C.
A : Untreated (control), B : 2% Lactic acid + 20% NaCl, C : 2% Lactic acid, D : 20% NaCl, E: Ginger extract, F : Ginger extract + 20% NaCl

increased proteolysis in meat due to the effect of ginger extract on meat proteins.

Effect of lactic acid and sodium chloride at chill temperature (4±1°C) : Meat samples treated with 2% lactic acid or 20% sodium chloride solution showed 15 bands with KD values ranging from 14.13 to 114.8 in both the cases (Fig. 2 C and D), as compared to 16 bands in control. The combined treatment of 2% lactic acid + 20% sodium chloride caused 16 bands with molecular weights of 14.1 to 114.8 KD, at par with untreated samples (Fig. 2 A and B). It, thus, appears that the proteolytic activity of muscles was very slow at chill temperature, as compared to that at ambient temperature. This is further confirmed by the negligible reduction in the number of protein bands, even after the storage of meat samples for five days at chill temperature.

Effect of ginger extract and sodium chloride at chill temperature (4±1°C) : Meat treated with ginger extract showed 6 bands in the range of 14.1 to 48.9 KD values (Fig. 2E). Samples treated with ginger extract + 20% sodium chloride solution showed 5 bands (Fig. 2F), with 10.4 to 36.3 KD values, thereby indicating more pronounced proteolytic activity, which renders the meat more tender. However, the proteolysis of meat under the influence of anti-microbials was more pronounced at ambient temperature, as compared to chill temperature. A drastic reduction in the number of protein bands in muscles treated with antimicrobials and the resulting increased proteolysis could also be due to the formation of protein complexes, that are insoluble in the buffer system used. Thus, the present data indicate that the ginger can be used as a new source of proteolytic enzyme for buffalo meat tenderization, which is generally tough, especially when the animals are slaughtered at the end of their productive age.

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Studies on Cake Doughnut Premix : Packaging and Keeping Quality in Flexible Packs

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Moisture sorption data of cake doughnut premix indicated that a moisture content of 9.7% (as is basis), equilibrating to 64% RH, is critical with respect to storage stability of the product. Shelf-life of the premix with 9.0% initial moisture and packed in double polypropylene, and metallized polyester/polyethylene was 50 and 30 days, respectively, at 90% RH, 38°C and more than 150 days at 65% RH, 27°C. Shelf-life at accelerated condition could be increased to 110 and 85 days, respectively, in the above packs by reducing the initial moisture content of the product to 7.7%.

Keywords : Cake doughnut premix, Shelf-life, Packaging materials, Sorption studies, Accelerated and ambient conditions, Moisture content.

Work on the development of ready premixes of traditional foods is on the increase in recent years (Desikachar 1986). Besides, the ease of preparation and consumer acceptability of ready mixes, the functional and attractive packaging to provide adequate shelf-life is of importance for popularizing such products in the market (Bhupendar Singh et al. 1990). Doughnut, a popular snack food in Western countries, is becoming popular in India among urban population. In view of the lack of knowledge about the formulation or preparation methodology, the product is rarely prepared in Indian households. Hence, a doughnut premix having desired characteristics has been developed at CFTRI (Unpublished data). The results of moisture sorption characteristics, and keeping quality of the premix in flexible packs are presented in this communication.

Cake doughnut premix and packaging : Cake doughnut premixes with 9.0 and 7.7% moisture were prepared by mixing 100 g refined wheat flour (*maida*), 37 g sugar powder, 10 g hydrogenated vegetable fat, 4 g egg powder, 4 g skimmed milk powder, 4 g defatted soya flour, 1 g salt, and 2 g baking powder. In case of premix with 7.7% moisture, *maida* was dried at about 50°C, till the moisture reduced from 13.8 to 11.5%. Flexible packs (130 x 80 mm size), made up of (i) 45 micron (180 gauge) TQ polypropylene (PP), (5.3), double pack of one inside the other and (ii) 12 micron (50 gauge) metallized polyester/37.5 micron (150 gauge) polyethylene laminate (MPP) (2.0) were used. The water vapour transmission rates (WVTR) (expressed as g/sq. cm/ day under 90% RH gradient at 38°C) of the above materials, which decide the extent of moisture protection, were determined according to ISI (1960) method.

Chemical analysis : Moisture, extractable fat acidity, and total carbondioxide of the premix samples were determined according to AACC (1993) methods, while alcoholic acidity was carried out, as per ISI (1979) method. The sensory evaluation of the doughnut was carried out by a panel of six trained judges, who were familiar with the product, using standard composite scoring test. The products were graded as follows : excellent 45-50, very good 40-44, good 35-39, fair 30-34, and poor > 29. The data were analyzed according to Duncan's multiple range test (Duncan 1957).

Sorption studies : Humidity-moisture relationship of the product was studied at 27°C, as per the static method (Rockland 1960). The samples were periodically weighed, till they attained a constant weight or showed signs of mould growth, whichever was earlier. After the equilibrium, the samples were assessed for changes in free flow property, and visible change in colour, odour as well as mould growth. The equilibrium moisture content (EMC) of the product at different RH was calculated as follows : % EMC = % initial moisture content ± % loss or gain of moisture at a particular RH.

Fig 1 shows humidity-moisture relationship (sorption isotherm) of the product at 27°C. The isotherm was of a typical sigmoid type curve exhibited by starchy products, and showed a steep rise above 70% RH, indicating rapid changes in the quality of the product above this RH. The product with initial moisture content of 9.6% was in equilibrium with 57% RH (ERH), and free flowing. At 64% RH, the product had a equilibrium moisture content of 9.7%, and showed tendency for lumping.

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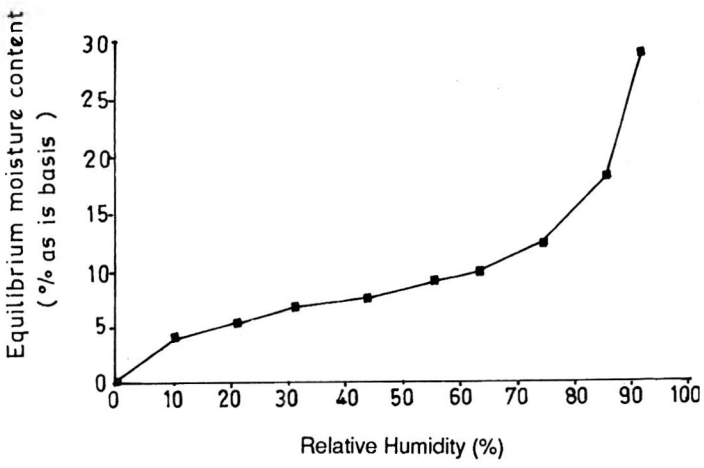

Fig. 1. Humidity-moisture relationship of cake-doughnut premix

At 75% RH and above, it was lumpy, and developed mould growth in 15 days.

From the sorption studies, it is inferred that a moisture content of 9.7% (as is basis) equilibrating to 64% RH is critical, as the doughnut premix is prone to rapid physico-chemical changes, leading to deterioration in quality, above this level of moisture. Further, the results suggest that the product with initial and critical moisture contents of 9.0% and 9.7% has hardly 0.7% moisture tolerance. This means that it needs good moisture barrier for giving adequate shelf-life. Hence, a premix with 7.7% moisture was also prepared, and used for storage studies.

Packaging and storage studies : Product (200 g) was filled in pouches, heat sealed, weighed individually, and exposed to (i) accelerated storage condition of 90 ± 2% RH, 38 ± 1°C and (ii) Indian standards condition of 65 ± 2% RH, 27 ± 1°C (ISI 1960). Premixes containing 9.0 and 7.7% moisture were studied individually. During storage, the individual packs were weighed periodically, and the doughnuts prepared from the stored premixes were subjected to sensory evaluation along with the control premix stored in refrigerator. The changes in chemical parameters and overall acceptable quality of the products packed in double polypropylene (PP), and metallized polyester/ polyethylene laminate (MPP) packs are presented in Table 1.

Accelerated condition of 90% RH and 38°C : The moisture content in doughnut mix (m.c 9.0%) packed in double PP and MPP reached critical level of 9.7% in 50 and 30 days, respectively. On the other hand , this critical level was reached in 110 and 85 days in the above packs, when the initial moisture content of the mix was 7.7%. Therefore, a longer shelf-life could be obtained even under accelerated condition of storage by reducing the initial moisture content of the product.

The changes in alcoholic acidity in the products, during storage at 90% RH and 38°C, were rapid in both the packs, when the moisture content was 7.7%. The initial alcoholic acidity of 0.06% increased to 0.21-0.23 (three and a half times more) during 150 days of storage in both types of the products. The maximum alcoholic acidity for *maida* specified by ISI (1979) standards is 0.1%. As *maida* is the major ingredient in doughnut mix, alcoholic acidity value of 0.1% for the doughnut mix can be considered to be the maximum limit. This limit of 0.1% was reached in both double PP and MPP in about 60 days (Table 1). The extractable fat acidity also increased progressively, as the storage period increased for both the mixes packed in two different packaging materials. The total carbondioxide content decreased rapidly during storage in both the packs. There was almost 50% decrease in total carbon dioxide content (from 0.32 to 0.15) during 50 days of storage. At longer storage periods of 120 days, the decrease was more than 85%.

With regard to the doughnut quality, the specific volume decreased progressively, as the storage period increased (Table 1). At the end of 120 days of storage, the specific volume decreased from 1.8 to 1.3, indicating that the softness of the products had reduced considerably, due to the loss of about 85% carbondioxide from the baking chemicals. The overall quality of doughnut prepared from stored packs were not significantly different upto 30 days of storage period, and were quite acceptable and satisfactory upto 90 days of storage. But, a slight bitter taste had developed at the end of 120 days. Doughnuts prepared from samples stored for 180 days had a distinct bitter note, and were unacceptable.

Ambient condition of 65% RH and 27°C : The moisture changes in both the packs containing premix with 9.0% moisture content, were practically negligible even at the end of 180 days of storage (Table 1). As the product ERH 57% was nearest to storage RH of 65%, it was expected that changes in moisture would be practically minimal. However, the premix with 7.7% moisture content, corresponding to 48% ERH, was expected to pick up moisture during storage at ambient conditions. Actually, the moisture content of this premix increased from 7.7% to 8.4%, when stored for 180

TABLE 1. CHEMICAL AND QUALITY CHANGES IN CAKE DOUGHNUT PREMIX (7.7 AND 9.0% MOISTURE CONTENT) IN DIFFERENT PACKS

Packing materials and storage conditions	Storage period, days	Moisture, %		Alcoholic acidity, %		Extractable fat acidity, %		Total carbon dioxide, %		Doughnut quality			
										Total score, Max.50		Specific volume, cc/g	
		M1	M2	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
Initial	0	7.7	9.0	0.06	0.06	27	27	0.23	0.32	45.0^{A}	45.2^{A}	1.8	1.8
Double pack	30	-	9.4	-	0.08	-	64	-	0.17	-	42.0^{B}	-	1.7
of PP,	60	-	9.8	-	0.10	-	91	-	0.15	-	37.5^{C}	-	1.5
90% RH,	90	9.4	10.1	0.12	0.13	138	122	0.11	0.07	37.8^{B}	$36.0^{C,D}$	1.3	1.3
38°C	120	9.9	10.4	0.19	0.13	167	142	0.08	0.05	33.2^{C}	37.8^{C}	1.3	1.3
	150	10.2	10.6	0.21	0.17	195	160	0.04	0.03	28.0^{D}	33.8^{D}	1.2	1.3
	180	10.8	-	0.21	-	218	-	0.03	-	-	-	-	-
										SEM ± 0.86 (20 df)		SEM ± 0.97 (30 df)	
Double pack	60	-	9.0	-	0.07	-	62	-	0.16	-	42.2^{B}	-	1.6
of PP,	120	8.2	9.0	0.10	0.10	92	93	0.14	0.14	$43.8^{A,B}$	42.0^{B}	1.7	1.6
65% RH,	150	8.3	9.0	0.11	0.11	96	110	0.12	0.11	$42.2^{B,C}$	42.2^{B}	1.6	1.6
27°C	180	8.4	9.1	0.12	0.12	106	124	0.11	0.10	40.3^{C}	38.2^{C}	1.6	1.6
										SEM ± 0.68 (20 df)		SEM ± 0.65 (25 df)	
MPP,	30	-	9.7	-	0.08	-	64	-	0.19	-	42.5^{A}	-	1.6
90% RH,	60	-	10.2	-	0.10	-	94	-	0.15	-	38.7^{B}	-	1.5
38°C	90	10.1	10.7	0.13	0.13	135	120	0.11	0.09	37.8^{B}	36.8^{B}	1.4	1.3
	120	10.8	11.4	0.19	0.15	165	140	0.07	0.07	33.0^{C}	36.7^{B}	1.3	1.4
	150	11.4	11.8	0.22	0.17	194	156	0.05	0.05	28.0^{D}	32.8^{C}	1.3	1.3
	180	12.1	-	0.23	-	216	-	0.03	-	-	-	-	-
										SEM ± 0.15 (20 df)		SEM ± 1.02 (30 df)	
MPP,	60	-	9.0	-	0.06	-	61	-	0.23	-	$43.2^{A,B}$	-	1.6
65% RH,	120	8.1	9.0	0.10	0.10	81	93	0.14	0.18	$41.8^{B,C}$	42.0^{B}	1.7	1.6
27°C	150	8.2	9.0	0.11	0.12	94	109	0.12	0.15	42.2^{B}	42.2^{B}	1.6	1.6
	180	8.4	9.1	0.12	0.12	106	126	0.11	0.11	39.7^{C}	41.0^{B}	1.6	1.7
										SEM ± 0.70 (20 df)		SEM ± 0.71 (25 df)	

Each observation except for total score is mean of 3 values and total score is mean of 6 values. Mean of same column followed by different superscripts differ significantly ($p<0.05$) according to Duncan's multiple range test. M1 - Premix with 7.7% moisture and M2 - Premix with 9.0% moisture.

days either in double PP or MPP. However, as the maximum moisture content attainable will be 9.7% at 64% ERH, these products can have a very long storagelife.

Changes in alcoholic acidity at 65% RH and 27°C were not as rapid as in accelerated condition, but there was a gradual increase. Alcoholic acidity value of 0.1% was reached in 120 days in both the packs and in both the types of mixes. Increase in extractable fat acidity was also gradual. Extractable fat acidity values of 124 and 126 were reached in 180 days at ambient condition, as compared to 90 days in accelerated storage condition. A similar trend was also observed in case of premix having 7.7% initial moisture, during the storage period. Changes in total carbondioxide were also gradual, and were not as pronounced as those at accelerated storage condition. It took more than 120 days for 50% reduction in total carbondioxide. Also, there was no perceptible change in the total scores, and specific volume of doughnuts prepared from premixes stored for 120, 150 and 180 days. These data indicated that premix could be stored upto 180 days at ambient condition without any adverse change.

From the results of the storage studies, it can be inferred that doughnut mix having initial moisture content of 9.0%, and packed in double PP and MPP packs will have a storage life of 50 and 30 days, respectively, at accelerated storage condition of 90%

RH and 38°C. However, the shelf-life can be increased to 110 and 85 days in these packs, if the initial moisture content of the premix is reduced to 7.7%. The shelf-life in both packs will be more than 150 days at 65% RH and 27°C.

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Biotyping of *Bacillus cereus* Isolates

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Forty natural isolates and 2 standard strains of *Bacillus cereus* were studied for the fermentation of 18 different sugars. These were variable with respect to fermentation of xylose, salicin and cellobiose. This character was utilised to develop a biotyping scheme. These strains were found to be distributed in seven out of nine theoretically possible biotypes. Isolates from ingredients of varieties of sausages, when clustered in distinct biotypes, suggested the usefulness of this biotyping scheme in epidemiological studies.

Keywords : *Bacillus cereus*, Biotyping, Sugar fermentation.

Isolation of *Bacillus cereus* from incriminated foods is not enough to assign aetiological association in an event of food poisoning. *Bacillus cereus* and its toxin are known to occur in foods, and have been isolated and characterized from a variety of foods (Sharma and Dogra 1983; Bachhil and Jaiswal 1988; Vijayalakshmi et al. 1981; Varadaraj et al. 1992). Therefore, a detailed characterization of isolates from incriminated food and victims' fomites is essential, and biological tests to demonstrate enterotoxigenicity of the isolates are required to support the aetiological association. The latter being difficult and time consuming, attempts have been made by many workers to correlate some biochemical characters with enterotoxigenicity. For example, isolates from diarrhoeal type of outbreaks have been found to hydrolyze starch, whereas those from the emetic type of outbreaks have been found to be starch non-hydrolyzing (Shinagawa 1990). Serotyping with a battery of H-antigen is not absolute, as Ueda and Kuwabara (1990) reported that only 7/122 isolates could be serotyped. Further, all 26 isolates of *B. cereus* were serologically untyped (Personal communication, Public Health Laboratory Services, Colindale, London). In this background, an attempt has been made in the present study, to evolve a biotyping system on the basis of the ability of isolates to ferment various sugars.

Test strains included 42 isolates of *B. cereus*, isolated from salami, trekker, frankfurter, ingredients used in the manufacture of these sausage varieties (Deva 1989) and environment (Mondal 1990), and standard strains 'NCTC 11143' and 'NCTC 11145' were tried for biotyping.

The ammonium salt-sugar (ASS) medium described by Cowan (1974) was used to study acid production by the isolates from 18 different sugars tested individually. These include D-glucose, D-fructose, D-mannose, rhamnose, dulcitol, inositol, mannitol, sorbitol, lactose, arabinose, galactose, inulin, saccharose, sucrose, D-xylose, salicin, cellobiose and mellibiose. The ASS medium containing test sugars at 1% level was inoculated with 20 h-old test cultures, grown in nutrient broth, mixed well and incubated at 37°C. Inoculated tubes were observed upto a period of 7 days for acid production. The scale used by Public Health Laboratory Services, Colindale, London, to declare isolates as positive, variable or negative (viz., 80, 20-80 and < 20% positive) for a particular reaction, was used.

Xylose, salicin and cellobiose were fermented, respectively, by 35.7, 46.62 and 26.19% of the isolates tested. All test isolates were positive for Voges Proskauer's, nitrate reduction, citrate utilization, urease, starch hydrolysis, and sucrose utilization. Therefore, the earlier described biotyping system (Shinagawa 1990) could not be applied. It is interesting to observe that all, except for one salicin positive natural isolates, were xylose negative and *vice versa*. According to Gilbert and Taylor (1976), the emetic enterotoxin products fermented salicin.

On the basis of variability with respect to fermentation of xylose, salicin and cellobiose, the 42 test isolates were grouped into different

TABLE 1. BIOTYPES OF *BACILLUS CEREUS*

Biotypes	Xylose	Salicin	Cellobiose
1	-	-	-
2	+	-	+
3	-	+	-
4	+	-	-
5	+	+	+
6	-	-	+
7	-	+	+

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TABLE 2. DISTRIBUTION OF BIOTYPES ON THE BASIS OF SOURCE OF ISOLATES

	Biotypes							Total isolates
	1	2	3	4	5	6	7	
Frankfurter	-	2	6	5	-	-	-	13
Ready-to-cook frankfurter			1		1	1	1	4
Flour	2	1	5	2	-	-	2	12
Spices			1	-	-			1
Heat-treated spices	1	-	2					3
Salami and trekker		2		1				3
Ready-to-eat sausage	2	1						3
Pig vomitus			1					1
Diarrhoeal food poisoning cases	1							1
Emetic type food poisoning cases	1							1
Typability, %	16.56	14.28	38.09	19.04	2.38	2.38	7.14	

fermentative groups (Table 1), considered as biotypes. These were found to be distributed in seven out of nine theoretically possible biotypes. The % typability of different biotypes, and their distribution on the basis of source of isolates are shown in Table 2. Nearly 87.5% (35 natural isolates) of the isolates fell in biotypes 1, 2, 3 and 4. Besides, the 2 standard strains, 'NCTC 11143' and 'NCTC 11145', fell in biotype 1. None of a cluster of 26 natural isolates, serologically untypeable by Public Health Laboratory Services, Colindale, London, fell in this biotype. It appears that biotypes 5 and 6 comprised isolates from ready-to-cook frankfurter only.

Biotyping may be useful for epidemiological investigation, as it helps in locating the source of an isolate. Flour is used for the preparation of frankfurter, salami and trekker. Isolates falling in biotypes 3 and 4 originated from these. Therefore, it appears that flour was the main source of these *B. cereus* isolates.

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Effect of Hot and Cold Deboning on the Yield and Quality of Quail Meat

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Meat yields, physico-chemical and sensory characteristics of hot and cold deboned meat from 6-weeks old Japanese quails were evaluated at 15 min, 2,4,6 and 24 h of post-mortem aging. Data showed that the deboning method had no significant influence on carcass components or total meat yields, which averaged 64.6% of eviscerated weight. The pH of meat declined rapidly within first 2 h and levelled off after 4 h aging in both leg (6.5-6.6), and breast muscles (5.8-5.9). The lowest water holding capacity, extractable myofibrillar proteins and tenderness score, as against highest cooking loss, and shear values of the meat were recorded at 2 h post-mortem, regardless of deboning methods. Subsequent aging for 4 h brought about an improvement in these quality attributes which, in general, did not differ significantly from those recorded for longer holding periods, thereby suggesting that a minimum of 4 h post-mortem aging prior to hot or cold deboning would be necessary for obtaining satisfactory meat quality.

Keywords : Hot/cold deboning, Japanese quail, Meat yields, Aging, Meat quality.

Hot deboning of poultry carcasses, over the conventional chilled deboning, has been receiving attention in recent years, as it saved energy, and reduced processing time as well as labour costs (Hamm 1981). However, hot deboned poultry meat has been reported to be generally less tender than chilled, and aged deboned meat (Stewart et al. 1984; Sams and Janky 1986). This has led to emphasis on holding the carcasses for a minimum period of 2 to 6 h, prior to deboning, to alleviate the toughness of hot deboned meat (Legare et al. 1985; Jones 1986). Some information on the chilling, refrigeration and shelf life and microbial quality of quail meat is available (Prabhakar Reddy 1991; Singh et al. 1989; Renukumari and Begum 1993). The relationship between post-mortem biochemical changes, and tenderness of chicken meat has been examined (Kijowski et al. 1982), but such a study on Japanese quail is lacking. The present study was, therefore, undertaken to assess the effects of hot and cold deboning on the meat yields, and of post-mortem aging on the physico-chemical and sensory quality of quail meat.

Forty, male Japanese quails (*Coturnix coturnix japonica*), of 6 weeks old, reared under identical feeding, and managemental conditions, were procured from the experimental quail farm of this institute, kept off the feed for 14 h, and slaughtered conventionally (Arafa et al. 1978). Half of the hot (unchilled) eviscerated carcasses were manually deboned within 2 h of exsanguination, while the remaining half were deboned after chilling in slush-ice (1:2, w/v) for 1 h, to an internal carcass temperature of 2.8°C. The yields of eviscerated carcass, and giblets were expressed as % live weight. Immersion chilled carcasses were weighed before and after chilling to determine water uptake (Benoff et al. 1984). Weights of deboned meat from breast, legs, and residual meat from cooked (1 kg/ cm^2, 10 min) carcass frame, and skin were recorded, and expressed as % eviscerated carcass weight. Meat and skin were considered as total edible components.

In order to evaluate the effect of post-mortem aging on physico-chemical and sensory quality of meat, a second lot of quails was processed as described above, and hot eviscerated carcasses were held at ambient temperature for 6 h followed by 18 h of refrigeration storage (4±1°C). Chilled carcasses were stored in the refrigerator for 24 h. Both leg and breast meats were excised at 15 min, 2,4,6 and 24 h post-mortem, freed from visible fatty and connective tissues, minced, and analyzed separately in triplicate for pH by inserting glass electrodes directly into the minced meat. Water holding capacity (Wardlaw et al. 1973), and myofibrillar proteins (Kang and Rice 1970) were determined. Cooking loss was estimated by placing 20 g minced breast meat samples, in triplicate, in polyethylene pouches, and heating in water bath at 80°C for 20 min (Kondaiah et al. 1988). Shear force was measured, in triplicate, on half-an-inch diam cores of cooked breast muscle, using Warner-Bratzler shear press. A seven-member trained sensory panel evaluated the breast meat for tenderness on a 7-point Hedonic scale (7 : extremely tender and 1:extremely tough). Data were subjected to analysis of variance, and means were compared for significant difference by Duncan's multiple

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range test (Snedecor and Cochran 1967).

The data showed that deboning method had no significant influence on the carcass components, viz., breast, legs and residual (frame) meat yields or total meat yield, despite 3.7% extraneous water during chilling pick-up by cold deboned carcasses (Table 1). Benoff et al (1984) and Kondaiah and Panda (1987) also found no appreciable effect of deboning methods on meat yields in broiler and spent hen, respectively. As expected, chilling treatment resulted in a significant increase in the dressed yield of carcass. The meat-to-bone (M:B) ratio is almost similar to that reported for 8-weeks old male broiler chicken (Hayse and Marion 1973). The total edible components also did not differ significantly between deboning treatments.

The pH of both dark (leg) and light (breast) meat (post-mortem) decreased rapidly during first 2 h, regardless of deboning method. The pH decline between 2 and 24 h was, however, non-significant, except for cold-deboned light meat, which showed a significant decrease in pH up to 4 h of aging. The rate of post-mortem glycolysis was much faster in light, than in dark meat, possibly due to low contents of adenosine triphosphate, and glycogen in the latter (Suzuki et al. 1985; Sams and Janky 1991). The pH reached a plateau within 4 h of aging. This is, in agreement with the observation of Stewart et al (1984) in broiler chicken. The ultimate pH of light meat was close to the literature values for chicken light meat (Kijowski et al. 1982; Jones and Grey 1989). However, the characteristic higher ultimate pH of dark meat was observed in the present study, as compared to chicken meat (Lyon et al. 1983; Jones and Grey 1989). This might be attributed to the species variation in post-mortem glycolytic and buffering capacity of meat.

The higher water holding capacity (WHC) of pre-rigor (15 min) meat was probably associated with its higher pH values. However, a significant reduction was observed in WHC after 2 h post-mortem, thereby indicating the rigor state of muscle, as reported by Kijowski et al (1982) in chicken meat. Subsequent aging brought about a significant increase in WHC, though the differences among 4,6 and 24 h periods were non-significant, regardless of the types of meat, and deboning method (Table 2). The higher pH of dark meat may be responsible for its consistently higher WHC than that of light meat, even though the latter had more myofibrillar proteins. The % myo-fibrillar proteins, which was maximum in pre-rigor meat due to the existence of free actin and myosin (Kijowski et al. 1982), got reduced significantly in meat deboned 2 h post-mortem. This could be attributed to the rigor-induced aggregation of actomyosin, which tended to dissociate during subsequent aging, as evident from an increase in their extractability with increasing holding time prior to excision.

Irrespective of deboning methods, aging period exhibited significant influence on the cooking loss and meat tenderness. Meat deboned, as early as 15 min after slaughter, gave significantly ($P<0.01$) lower cooking loss, required less force to shear, and had higher tenderness score, than that deboned 2 h post-mortem. However, with the increase in aging time, the cooking loss tended to decrease with concomitant improvement in meat tenderness, as revealed from lower shear values, and higher tenderness scores (Table 2). The reduction in muscle toughening of hot-deboned (Stewart et al. 1984), and cold-deboned (Lyon et al. 1985) light meat, with increase in deboning time intervals, has also been reported in broilers. The relatively higher cooking losses found in cold, than that in hot deboned meat, might be partly due to loss of water picked up during chilling. Even though meat deboned after 24 h of aging had the least shear values and highest tenderness scores, these attributes did not differ significantly from 4 or 6 h groups, except in case of shear values of hot deboned meat, which were significantly lower.

These results indicate that hot deboned quail meat had quality attributes comparable to those of cold deboned meat. However, a minimum of 4 h post-mortem aging would be desirable to overcome

TABLE 1. CARCASS AND MEAT YIELDS OF HOT AND COLD DEBONED QUAIL

Traits	Hot deboned (n = 20)	Cold deboned (n = 20)
Live wight, g	122.1 ± 3.0	124.5 ± 2.0
Dressing, %	69.7 ± 0.9[a]	73.1 ± 0.9[b]
Chilling gain, %	NA	3.7 ± 0.2
Giblets weight, %	5.1 ± 0.1	5.2 ± 0.1
Component yields (% Eviscerated weight)		
Breast meat	30.9 ± 0.4	31.7 ± 0.4
Legs meat	20.5 ± 0.3	20.7 ± 0.3
Residual (frame) meat	12.8 ± 0.3	12.4 ± 0.2
Total meat	64.3 ± 0.8	64.8 ± 0.6
Bone	26.3 ± 0.4	25.7 ± 0.3
Skin	9.3 ± 0.4	9.4 ± 0.3
Total edible components	73.7 ± 1.1	74.2 ± 0.7
Meat-to-bone ratio	2.4 ± 0.1	2.5 ± 0.1

Figures within a row bearing same or no superscript did not differ significantly ($p<0.05$). NA : Not applicable.

TABLE 2. EFFECT OF DEBONING METHOD AND HOLDING TIME ON THE PHYSICO-CHEMICAL AND SENSORY QUALITIES OF QUAIL MEAT

Characteristics	Post-mortem holding period								
	15 min	2 h		4 h		6 h		24 h	
	HB	HB	CB	HB	CB	HB	CB	HB	CB
pH (DM)	6.8^{a} ±0.1	6.6^{b} ±0.0	6.7^{a} ±0.1	6.5^{b} ±0.0	6.7^{a} ±0.1	6.6^{b} ±0.0	6.6^{a} ±0.0	6.5^{b} ±0.0	6.5^{a} ±0.0
pH (LM)	6.2^{a} ±0.0	5.9^{b} ±0.1	6.0^{a*} ±0.0	5.8^{b} ±0.1	5.9^{b} ±0.0	5.8^{b} ±0.1	5.8^{b} ±0.0	5.9^{b} ±0.0	5.8^{b} ±0.1
Water holding capacity (DM), ml/100g	46.7^{a} ±0.8	31.6^{b} ±0.9	33.3^{a} ±1.7	40.2^{c} ±1.3	42.2^{b} ±1.7	42.7^{ac} ±1.1	40.0^{b} ±0.6	44.1^{ac} ±0.5	41.1^{b} ±1.1
Water holding capacity (LM), ml/100 g	38.3^{a} ±0.7	27.5^{b} ±1.8	28.3^{a} ±1.0	32.3^{c} ±0.4	34.2^{b} ±1.0	32.7^{c} ±0.7	33.1^{ab} ±1.4	34.2^{ac} ±1.1	36.4^{b} ±0.6
Myofibrillar proteins (DM), %	8.7^{a} ±0.5	6.5^{b} ±0.5	7.0^{a*} ±0.5	7.9^{ab} ±0.3	8.4^{b} ±0.3	8.0^{ab} ±0.5	8.4^{b} ±0.2	9.3^{a} ±0.1	9.0^{b} ±0.4
Myofibrillar proteins (LM), %	10.8^{a} ±0.3	8.6^{b} ±0.4	9.0^{a} ±0.3	9.1^{ab} ±0.2	8.9^{a} ±0.3	8.7^{b} ±0.6	9.4^{a} ±0.5	10.5^{a} ±0.3	10.4^{a} ±0.2
Cooking loss (LM), %	23.0^{a} ±0.9	28.6^{b} ±0.6	29.1^{a} ±0.8	24.5^{a} ±0.9	25.8^{b} ±0.5	24.9^{a} ±0.3	26.3^{ab} ±0.9	22.5^{a} ±0.4	24.6^{b} ±0.6
Shear force (LM), lb	4.7^{bc} ±0.2	8.6^{a} ±0.3	8.2^{a} ±0.7	5.9^{b} ±0.1	5.6^{ab} ±0.4	5.5^{b} ±0.6	6.0^{b} ±0.7	3.7^{c} ±0.3	4.1^{b} ±0.4
Tenderness score* (LM)	6.4^{a} ±0.3	5.1^{b} ±0.3	5.0^{a*} ±0.5	6.0^{ab} ±0.3	6.1^{b} ±0.3	6.3^{a} ±0.3	6.1^{b} ±0.4	6.7^{a} ±0.2	6.6^{b} ±0.3

Means in a row bearing similar superscripts within each deboning method did not differ significantly ($P<0.01$; $^{*}P<0.05$). HB : Hot deboned, CB : Cold deboned, DM : Dark meat, LM : Light meat, *7 : Extremely tender, 1: Extremely tough.

the problem of toughening and reduced functionality, irrespective of deboning methods.

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Compositional and Biochemical Changes in Cheese Manufactured from Cow Milk : Soy Milk Blends using Calf and Microbial Rennets

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Compositional and biochemical changes in cheddar cheese made from cow milk, and cow:soy milk blends (95:5, 90:10, 85:15, 80:20 and 75:25), using calf and microbial rennets during ripening (at 8±1°C and 75-80 % ERH) were studied at monthly intervals for 240 days. The retention of moisture in cheese was greater with increased proportions of soy solids in various blends. Moisture in cheese reduced with progress of ripening, the reduction being inversely related to soy solids in the blends. Proteins, fat and salt contents were greater in soy cheese, with no change during ripening. The titratable acidity, soluble proteins and free fatty acids showed an increasing trend with increase in the proportion of soy milk in the blends. During ripening, the titratable acidity showed a variable trend, while the soluble proteins, and fat contents showed little change.

Keywords : Cheddar cheese, Soy milk, Cow milk, Calf rennet, Microbial rennet, Ripening changes.

Technology for manufacturing cheese has been generally confined to cow milk, due to its compositional superiority over milks of other animals, bovines and ovines (Ganguli 1979). Buffalo milk is also widely used, but the flavour development is considerably slower than cow milk cheese, as it takes a longer time of 8-12 months (Kanwajia and Singh 1992; Singh and Kanwajia 1988). Besides, buffalo milk is also used for making mozzarella cheese (Ravi Sundar and Upadhyay 1991). Soybean, with its 38-42% proteins (Schroder et al. 1973), makes it an excellent and economic source of proteins. Soy proteins are unique among plant proteins by virtue of their relatively high biological value, and essential amino acid contents (Schroder et al. 1973). Hence, soy milk has the potential for replacing cow milk, at least partially, in production of cheddar type cheese (Del Valle et al. 1984).

Religious sentiments of a majority of Indian population, and the government regulations prohibit the use of calf rennet in cheese making. Some attempts have been made to replace calf rennet with microbial enzyme (Kumar and Angelo 1983), while microbial rennets are also used for cheese making (Krishnaswamy et al. 1976). Present investigations were conducted to study the compositional and biochemical changes due to substitution of cow milk with soy milk, using microbial rennet in cheddar cheese preparation and ripening.

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Materials : Cow milk and mature soybeans, (Variety 'PK-262'), were obtained from the Livestock Research Centre and University Farm, respectively. Pure culture of *Streptococcus lactis* was from the National Dairy Research Institute, Karnal. Bulk culture was prepared by using skim milk (Scott 1981). Modilase, double strength microbial rennet, produced from *Mucor miehei* was supplied gratis by Chr. Hansens Laboratory Inc. Horsholm, Denmark. Calf rennet was from Chr. Hansens Laboratory Pvt. Ltd., Adelaide, Australia.

Preparation of soy milk and cheese : Soy milk was prepared as per method described by Nelson et al (1976). Standardized cow milk (casein/fat = 0.7) was pasteurized to 145°F for 30 min, and soy milk, heated to 100°C for 5 min, was added to it in different proportions (95:5, 90:10, 85:15, 80:20 and 75:25). Bulk starter culture (1.5%, v/v) was added to milk at 30°C, and incubated for 30 min. Calf and microbial rennets were added to different blends of cow milk and soy milk at the rate of 0.0015% and 0.001%, respectively, at 30°C. The resultant curd was cut with cheese knives and scalded for 50-60 min at 1°C rise/min upto 37-39°C. After scalding, whey was removed, and curd was subjected for cheddaring/texturing (Davis 1965), until acidity reached 0.55%. The curd was milled, and salted at 2% level (w/w), and pressed. The cheese samples were paraffined, and ripened at 8±1°C (equilibrium relative humidity 75-80%) for 240 days.

Analytical method : Moisture was determined gravimetrically (MIF 1959), while protein and salt were estimated by standard methods (BIS 1961,

TABLE 1. CHEMICAL AND BIOCHEMICAL CHANGES[1] DURING RIPENING (AT 8±1°C) OF CHEESE MADE FROM DIFFERENT BLENDS OF COW:SOY MILK USING MODILASE

Blend	Ripening period, months				
	0	2	4	6	8
Cow milk : Soy milk			**Moisture, %**		
*100 : 0	38.3	36.3	35.9	35.6	35.4
**100 : 0	37.9	36.8	36.3	35.7	35.2
95 : 5	40.6	40.0	39.6	39.2	38.9
85 : 15	41.6	41.1	40.8	40.4	40.0
75 : 25	49.1	48.8	48.2	48.1	47.8
			Proteins,%		
*100 : 0	38.8	37.7	37.6	37.5	37.4
**100 : 0	40.2	39.7	39.5	39.1	39.0
95 : 5	39.7	39.5	39.3	39.9	39.0
85 : 15	37.0	35.9	36.8	36.6	36.4
75 : 25	31.2	30.8	30.5	30.3	30.1
			Fat,%		
*100 : 0	51.1	50.1	51.1	52.0	52.0
**100 : 0	50.1	50.0	50.7	51.2	51.4
95 : 5	50.1	50.4	50.7	50.7	50.7
85 : 15	48.9	49.5	50.0	50.3	50.2
75 : 25	44.3	44.0	44.4	44.6	44.9
			Salt,%		
*100 : 0	2.3	2.3	2.3	2.3	2.3
**100 : 0	2.3	2.3	2.3	2.3	2.3
95 : 5	2.4	2.5	2.5	2.5	2.5
85 : 15	2.7	2.8	2.8	2.8	2.8
75 : 25	3.5	3.4	3.4	3.4	3.4
			Titratable acidity,%		
*100 : 0	0.9	1.3	1.6	1.6	1.8
**100 : 0	0.9	1.3	1.7	1.7	1.9
95 : 5	1.0	1.4	2.0	2.0	2.0
85 : 15	1.1	1.4	1.9	2.0	2.2
75 : 25	1.2	1.5	1.9	1.8	2.3
			pH		
*100 : 0	5.3	5.2	4.9	5.5	5.5
**100 : 0	5.3	5.2	5.1	5.4	5.4
95 : 5	5.2	5.1	5.1	5.4	5.3
85 : 15	4.9	4.7	4.8	5.3	5.0
75 : 25	4.8	4.5	4.7	5.3	5.0
			Soluble proteins,%		
*100 : 0	1.1	1.4	1.8	2.1	2.3
**100 : 0	1.2	1.4	1.7	2.3	2.7
95 : 5	1.2	1.8	1.8	2.4	2.7
85 : 15	1.3	1.7	2.3	3.2	3.9
75 : 25	1.4	2.3	3.0	3.7	4.5
			Free fatty acids,μ mole/g fat		
*100 : 0	10.5	15.3	25.0	35.3	39.0
**100 : 0	10.0	17.3	28.7	35.7	36.3
95 : 5	11.0	33.3	36.7	37.0	39.3
85 : 15	13.3	36.6	40.1	42.3	44.1
75 : 25	16.3	42.8	44.0	46.7	49.3

[1]Average of triplicate experiments, *Calf rennet was used for clotting cow milk, ** Modilase alone was used for clotting cow milk and cow-soy-milk blends.

1965). Fat, titratable acidity (AOAC 1975) and pH (BIS 1961) were determined. Soluble proteins and free fatty acids (FFA, μ mole/g fat) were determined by the methods of Kosikowski (1966), and Rama Murthy and Narayanan (1974), respectively.

Chemical changes : The moisture content increased with the increase in concentration of soy proteins (Table 1), due to hydrophilic nature of soy proteins (Aworh et al. 1987). Fat, proteins and salt in green soy cheese ranged from 44.3 to 50.1, 31.2 to 39.7 and 2.4 to 3.5% on dry weight basis, the values being highest and lowest in green soy cheese made from cow milk to soy milk ratio of 95:5 and 75:25, respectively. Decrease in protein and fat contents in cheese with increase in the proportion of soy milk in the blends has also been reported along with increase in salt (Hwang et al. 1987; Metwalli et al. 1982). The proteins, fat and salt contents (on dry weight basis) did not show any change during ripening. As there were not much changes during ripening of cheese made from various blends, data for each month have not been reported.

Glycolysis : The titratable acidity in all cheese samples increased up to 120 days of ripening, as in control sample (Table 1). Total increase in titratable acidity was maximum (2.3%), and minimum (2.0%) in soy cheese samples, containing 25 and 5% soy milk, respectively. This could be attributed to a higher percentage of moisture in soy cheese, containing 25% soy milk, which resulted in optimum conditions for the growth of lactic acid bacteria (Aworh et al. 1987). All the soy cheese samples as well as the control followed a similar trend in pH changes during the ripening period of 240 days (Table 1). The control cheese had the highest pH values at the end of ripening, whereas soy cheese containing 25% soy milk had the lowest value.

Proteolysis : Initial soluble proteins were lowest and highest in soy cheese containing 5 and 25% soy milk, respectively (Table 1). The control and all soy cheeses showed increasing trend in soluble proteins, throughout the ripening period. The values of soluble proteins at the end of ripening period ranged from 2.7 to 4.5%, lowest and highest values being in cheese samples, containing 5 and 25% soy milk, respectively. The results confirm the findings of Metwalli et al (1982); Hwang et al (1987); and Gooda et al (1988). During ripening, the degradation of proteins showed an increasing trend, as evidenced by the increase in soluble nitrogen content.

Lipolysis : FFA levels at the beginning were lowest and highest in soy cheese containing 5 and 25% soy milk, respectively. These increased sharply up to 60 days in cheese, containing 25% soy milk, as compared to other cheeses, and control samples. During the later period of ripening, increase in FFA was more in cheese samples containing soy milk. The amount of FFA in cheese increased, as proportion of soy milk increased. However, cheese having 25% soy milk showed poor flavour development in spite of high FFA value. FFA content in cheese made from cow milk using modilase was 36.3 μ mole/g fat, whereas cheese made from cow milk:soy milk blend (75:25) contained 49.3 μ mole/g fat at the end of ripening period (Table 1). The results confirm the findings of El-Safty et al (1978) and Marsile (1986), who demonstrated that addition of soy milk to buffalo milk increased the lipase activity in Ras cheese.

It can be concluded that the changes in the cheese made from cow milk using calf rennet and modilase are negligible. Increases in the titratable acidity, soluble proteins, and FFA occur with increase in the soy milk proportion in the blend.

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Detection and Determination of Ester Gum (Substitute for Brominated Vegetable Oil) in Ready-to-Serve Beverages and Their Concentrates

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A paper chromatographic method has been developed to detect and determine ester gum present in ready-to-serve beverages and their concentrates, utilizing Halphan-Hicks reaction, where ester gum reacts with bromine and phenol, giving a violet spot on the chromatostrip. Ester gum has been estimated by measuring the absorbance at 245 nm. Other substitutes of brominated vegetable oils (i.e., glycerol mono stearate and polysaccharide gums) have also been detected.

Keywords : Detection and determination of ester gum, Abietic acid, Paper chromatography, Glycerol mono stearate, Polysaccharide gums.

For citrus-based carbonated beverages, there is a need for a weighting agent to raise the specific gravity of the flavour oil to enable its uniform distribution (Branen et al. 1990). So far, soft drink industries were using brominated vegetable oils (BVO) for this purpose (Branen et al. 1990). But on the recommendation of World Health Organisation, BVO has been banned, by many countries including India, as it was found to be carcinogenic in nature (Murno et al. 1971). Hence, substitutes for BVO- like rosin ester (ester gum), sucrose ester (SAIB), polyolbenzoates, protein clouds and starch-based gum have been reported (Anon 1990 a,b). Among these substitutes, the government has allowed the use of ester gum in RTS beverages to the maximum of 100 ppm in place of BVO (MHFW 1991). Since no method is available, a method has been developed to detect, and determine ester gum in RTS beverages and their concentrates, using Halphan-Hick reaction (Thorpe and Whiteley 1946).

Ester gum, a glycerol ester of wood rosin, is a pale yellow colour solid, having the following characteristics, as envisaged by FAO/WHO Expert Committee (WHO 1988) : acid value 3 to 9; density 1.06 to 1.1 g/cm^{-3}; drop softening point 88° to 96°C; hydroxyl No. 15 to 45; arsenic (as As) $\geq$ 3 ppm; heavy metals (as Pb) $\geq$ 40 ppm. Ester gum consists of approximately 90% rosin acids, and 10% neutrals (WHO 1988). In addition to isomers of abietic acid, ester gum also contains neoabietic, pimeric, isopimeric, and palustric acids (MHFW 1991). Ester gum is insoluble in water, but soluble in acetone, benzene and chloroform. Purity of ester gum to be incorporated in the concentrate is of utmost importance (MHFW 1991). For this purpose, a Gazette Notification was issued by Government of India (MHFW 1991), where standard and quality requirements for ester gum are laid down.

Chloroform, methanol, carbon tetrachloride, diethyl ether, petroleum ether, phenol, bromine, potassium hydroxide, sodium sulphate, and hydrochloric acid, used in the present studies, were of analytical reagent grade. Ethyl alcohol was distilled over sodium hydroxide. The commercial samples of RTS beverages were purchased from the local market, whereas concentrates were procured from the local bottling industries. Phenol (1:1, v/v) and bromine (1:4, v/v) were prepared in carbon tetrachloride. Fehling's solution 'A' and 'B' were prepared as per standard procedures (AOAC 1990).

Extraction of ester gum from RTS beverages : RTS beverage (200 ml) was extracted thrice with 25 ml chloroform each, using 500 ml separating funnel. The chloroform extract was washed twice with 25 ml water, and dried over anhydrous sodium sulphate. Flavour concentrate (25 g) was dissolved in 100 ml distilled water, before extraction with chloroform. The chloroform extracts were filtered, dried, and residue preserved for tests.

Paper chromatographic method for the detection of ester gum : The above residue and a standard ester gum sample were dissolved in 0.5 ml chloroform separately, and spotted on a chromatostrip (3x18 cms, Whatman No. 3 paper). The chromatostrip was developed in pure methanol to a distance of ten cm. After development, the chromatostrip was air-dried, and soaked in phenol solution. In wet condition, the chromatostrip was

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TABLE 1. DETECTION AND DETERMINATION OF ESTER GUM, GMS AND POLYSACCHARIDE GUMS IN RTS BEVERAGES AND CONCENTRATES

RTS beverage

Market sample	No. of samples	Ester gum	GMS	Poly-saccharide gum
Orange flavoured				
A	6	-	-	+
B	6	-	+	+
B + ester gum, 100 ppm	4	+ 95 ppm	+	+
Lemon flavoured				
C	6	-	-	+
D	6	-	-	+
E	6	-	-	+
C + ester gum, 100 ppm	4	+ 96 ppm	-	+
D + ester gum, 100 ppm	4	+ 95 ppm	-	+
E + ester gum, 100 ppm	4	+ 95 ppm	-	+

Concentrates

Samples	No. of samples	Ester gum	GMS	Poly-saccharide gum
Orange flavoured compound				
1. Batch No. 0896	2	-	-	+
2. Batch No. 1088	2	-	-	+
3. Batch No. 64	2	-	-	+
4. Batch No. W003	2	-	+	+
5. Batch No. A 035-B	2	-	+	+
Lemon flavoured compound				
1. Batch No. 0905	2	-	-	+
2. Batch No. 2592	2	-	-	+
3. Batch No. 101001	2	-	-	+
4. Batch No. 33-791	2	-	+	+
5. Batch No. 204166	2	-	+	+

suspended in a stoppered conical flask, containing bromine solution by pressing upper end of chromatostrip between stopper, and neck of the flask in such a way that the lower end of the strip hangs just above bromine solution. Development of a violet spot at the application point within 5 min for sample extract and standard ester gum, confirmed the presence of ester gum. Orange and lemon oils did not interfere. Sometimes, a blue spot at R_f 0.4-0.5 may be observed due to triterpenic acids, but it will be different from the violet spot at the application point. This method can detect ester gum up to the minimum of 400 µg.

UV-spectral determination of ester gum : Residue dissolved in 0.5 ml chloroform was quantitatively applied on a chromatostrip, and developed as

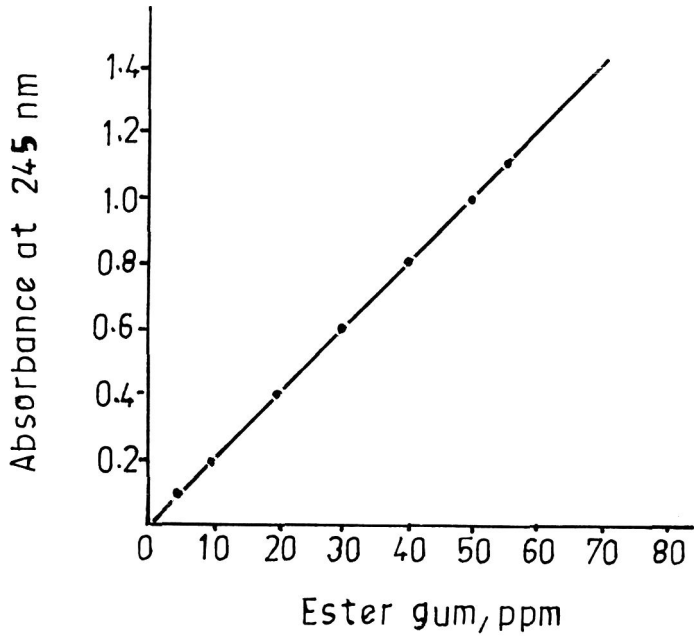

Fig. 1. Absorbance of ester gum solution

before. The chromatostrip at the base line was cut into small bits, extracted thrice with 10 ml chloroform, filtered, made up to 100 ml and absorbance measured at 245 nm. Using standard calibration curve, the concentration of ester gum in the solution and in turn, in the RTS beverage/ concentrate was calculated.

Detection of GMS in RTS beverages and concentrates : RTS beverages (200 ml) or 10 g of flavour concentrate, dissolved in 100 ml water, was extracted thrice with 50 ml of chloroform and methanol (2:1 v/v) mixture, using 500 ml separating funnel. The aqueous layer was preserved for detection of polysaccharide gums, whereas chloroform methanol extract was washed thrice with 25 ml water and dried over anhydrous sodium sulphate. The extract was filtered, and evaporated to dryness for determining the melting point. In the presence of GMS, the residue will melt at 63°C. To confirm, the residue was saponified and unsaponifiable matter was removed using diethyl ether. The aqueous soap layer was acidified with aqueous hydrochloric acid (2:1 v/v), and fatty acids were extracted thrice with 25 ml diethyl ether, which was dried over anhydrous sodium sulphate and evaporated. The fatty acid residue melted between 68-70°C, thereby confirming the presence of stearic acid and in turn, GMS. A Co-TLC (using petroleum ether and diethyl ether(60:40 v/v) moving phase of GMS, and the residue obtained after extraction had further confirmed the presence of GMS.

Detection of polysaccharide gums in RTS

beverages and concentrates : The aqueous layer, which was preserved during the extraction of GMS, was taken in a glass bowl and evaporated to dryness. The residue obtained, thereafter, was washed 15 times with 20 ml of 80% aqueous alcohol to remove sugars. The whitish residue was dissolved in 10 ml distilled water, treated with 1 ml concentrated hydrochloric acid, and left overnight. The hydrolyzed solution was neutralized with 50% aqueous sodium hydroxide in the presence of phenolphthalein. This neutralized solution was subjected for Fehling's test. Deposition of brick-red coloured precipitate of cuprous oxide at the bottom of the tube confirmed the presence of reducing sugar and in turn, the presence of polysaccharide gums.

The results on detection and determination of ester gum, GMS and polysaccharide gums in RTS beverages, and concentrates are shown in Table 1. All these samples were found to be devoid of BVO and ester gum, but some of them showed the presence of GMS, and polysaccharide gums. Recovery (95 to 96%) was achieved in case of admixture with ester gum in orange and lemon flavoured RTS beverages, following the presently developed procedure. Calibration curve (Fig. 1) followed the Beer-Lambert's law (Denney and Sinclair 1991) up to 60 ppm ester gum.

During screening programme of citrus flavour based RTS beverages and concentrates, it was found that most of the samples were lacking BVO as well as ester gum. The absence of both the unpermitted and permitted weighting agents prompted to look for any other permitted emulsifier or clouding agent being used. Hence, attempts have been made to isolate these undeclared additives, and identify them. It has resulted in the formulation of methods for detection of glycerol monosterate (GMS), and polysaccharide gums in RTS beverages and concentrates. Use of polysaccharide gums in beverages and other related food products have been well documented (Branen et al. 1990). Glucose, which is a part of the most of the water-soluble polysaccharide gums, has been utilized as a component to detect the presence of polysaccharide gums (substitute for BVO) in RTS beverages. Table 1 shows that in all the RTS beverages and concentrate samples, polysaccharide gum was present and ester gum was absent, whereas GMS was found to be present only in certain samples. Whenever ester gum was added, it responded to the test, and showed the recovery up to 95-96%, by the presently developed method.

The methodology developed for detection of ester gum depends upon Helphan Hick's reaction (Thorpe and Whiteley 1946), where abietic acid forms a violet colour complex with phenol and bromine. Abietic acid and their isomers contain a conjugated double bond system, which is responsible for UV absorption of ester gum at 245 nm. This conjugated system reacts with phenol and bromine, which are added at 1 and 4 position to yield phenoxy-bromo-derivative of abietic acid, which is violet in colour.

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Effect of Different Concentrations of Sodium Chloride on the Growth of *Yersinia enterocolitica*

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Studies on the effect of 0.5 to 10.5% sodium chloride on the growth of *Yersinia enterocolitica* on MacConkey agar at room temperature, revealed decrease in the viable cells with increase in sodium chloride concentration. Sodium chloride at 10.5% level was able to completely inhibit the growth of the organism at 23 days. Data indicate the potential of 10.5% or higher concentration of sodium chloride, as effective curing agent for killing *Yersinia enterocolitica* in the environment existing in Edo State, Nigeria.

Keywords : *Yersinia enterocolitica*, Sodium chloride, Curing agent, Killing effect.

Yersinia enterocolitica, a Gram-negative rod, is an enteropathogen of the family Enterobacteriacea (Agbonlahor et al. 1983). It is an established agent of gastro enteritis (Agbonlahor et al. 1983; Bercovier et al. 1980), and has also been incriminated in a variety of human and animal infections, including septicaemia, bacteraemia, mesenteric lymphadenitis, terminal ileitis, and pseudoappendicitis (Agbonlahor et al. 1983; Winbald 1973; Mayer and Greenstein 1976). Its role in cases of diarrhoea has received adequate attention (Agbonlahor et al. 1983; Bercovier et al. 1980; Greenwood and Hooper 1985; Anjorin et al. 1979). The organism has also been isolated the world over from healthy humans and animals (Lambin et al. 1985; Igumbor 1991; Nwosuh and Adesiyun 1987; Kwaga et al. 1984).

The effect of salt on the growth of bacteria has received extensive attention (Greenwood and Hooper 1985; Raccah and Henningson 1984). *Staphylococcus aureus*, for example, has been reported to tolerate high salt concentrations (Brackett 1986), while *Y. enterocolitica*, on the other hand, has been shown to be more sensitive to curing agents, than other enteropathogens (Raccah and Henningson 1984). Sodium chloride (3%) has been reported to abrogate the growth of *Y. enterocolitica* (Raccah and Henningson 1984).

In spite of the established pathogenic role of *Y. enterocolitica* in cases of yersiniosis (Agbonlahor et al. 1983; Winbald 1973), and the increasing awareness among the population of Nigeria, negligible efforts are put up to control the bacterium. In fact, no report on the effect of salt on the growth of *Y. enteroolitica*, in Nigerian environment is available. The present communication represents the first attempt to document sodium chloride, as a curing agent against Nigerian isolates of *Y. enterocolitica*.

Bacteria : Yersinia enterocolitica, serotype 0:3, was isolated from domestic and wild animals. The isolate was resuscitated, and purity test performed, following standard microbiological procedures (Agbonlahor et al. 1990).

Bacterial inoculation : This was done as per the scheme adopted earlier (Igumbor 1991). Bacterial culture from the stock (1 ml) was inoculated into peptone water (Igumbor 1991), and incubated at 37°C overnight. The culture grown in peptone water (1 ml) was then used to inoculate 10 ml each of the sodium chloride solutions of different concentrations (0, 0.5, 0.6, 0.8, 1.0, 2.0, 5.0, 7.5 and 10.5%.). After incubation at room temperature for 0,6 and 12 h and between 2 to 23 days, serial ten-fold dilutions were done ranging from 10^{-3}–10^{-8}, and 1 ml of the 10^{-3} dilution was plated on to MacConkey agar plates. After incubation at 37°C for 24-48 h, the colony forming units (cfu) were counted. Also, 1 ml of the solution without any dilution was plated out. Distilled water was used as a positive control.

Results obtained revealed that the cfu decreased, as the concentration of sodium chloride as well as the time of incubation increased (Table 1). Growth was totally abrogated at 23 days in case of 10.5% sodium chloride concentration, while 0.7×10^2 cfu/ml were present at 6 h.

Salt has been used for controlling bacterial growth (Raccah and Henningson 1984). The present study revealed that the effect of salt on the growth of *Y. enterocolitica* depended largely on the salt concentration, and the period of incubation. This finding is in agreement with an earlier report (Raccah and Henningson 1984). It has been reported that *Y. enterocolitica* did not survive in 3% sodium

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TABLE 1. EFFECT OF DIFFERENT CONCENTRATIONS OF SODIUM CHLORIDE ON THE GROWTH OF *YERSINIA ENTEROCOLITICA*.

Sodium chloride concentration, %	Colony forming units, cfu/ml x10^2 at time								
	0 h	6 h	12 h	2 d	7 d	10 d	14 d	18 d	23 d
0	6.0	6.2	6.4	6.6	6.9	7.0	7.2	7.6	7.8
0.5	4.5	3.9	3.6	3.4	3.1	2.8	2.6	2.4	2.1
0.6	4.4	3.6	3.4	3.2	2.9	2.7	2.4	2.2	1.9
0.8	3.3	2.0	1.8	1.6	1.5	1.3	1.1	1.0	0.8
1.0	2.2	1.8	1.6	1.4	1.2	1.2	0.9	0.7	0.3
2.0	1.0	1.5	1.3	1.2	1.1	1.0	0.8	0.5	0.9
5.0	0.9	1.4	1.1	0.9	0.8	0.9	0.6	0.3	0.02
7.5	0.6	0.9	0.6	0.5	0.4	0.3	0.1	0.06	0
10.5	0.4	0.7	0.4	0.3	0.2	0.1	0.09	0.03	0

chloride solution (Raccah and Henningson 1984). In contrast, a concentration of 10.5% was found to totally inhibit the growth of the organism in the present case. The bactericidal effect of sodium chloride could be due to the different osmotic pressures between the bacterium cells, and the salt solution. Consequently, the bacterium loses water, which eventually leads to rupturing of the plasma membrane.This could be related to the different geographical locations in which the studies were carried out, as microorganisms have been reported to vary with time and geographical locations (Eko and Utsalo 1990).

Chareonpong and Chen (1980) also observed that canned gizzards treated with 7.7, 15.3 and 23% sodium chloride, when stored for a week and three months, did not show any microbial growth. Salt is an effective curing agent in our environment. It has been used to inhibit the growth of *Y. enterocolitica* in meat, snail, fish and vegetable stew (Igumbor 1991; Igumbor and Owhe-Ureghe 1992). It has also been used to prolong shelf life in poultry (Chatterjee et al. 1971), and also in fermented product (Gangopadhyay et al. 1971). Similarly, a combination of lactic acid and sodium chloride was found to have inhibitory effects on different species of bacteria responsible for microbial spoilage of meat, and meat products as well as those causing meat-borne infections and intoxications (Ziauddin et al. 1993).

The use of sodium chloride at concentrations equal to or greater than 10.5% is, therefore, suggested for control of *Y. enterocolitica* in the environment of Edo State of Nigeria.

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Composition and Functional Properties of Washed Ground Buffalo Meat During Refrigerated Storage

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Effects of washing ground buffalo meat with water and 2% ethylene diamine tetra acetic acid (EDTA) solution (pH 4.5) on the composition, and functional properties during refrigerated storage were studied at intervals of 0,5,10,15 and 20 days. Washing of meat reduced the moisture content, total pigments, myoglobin and water extractable proteins (WEP) significantly ($P<0.01$), while crude protein contents and salt extractable proteins (SEP) were significantly higher than control. During storage, the SEP content in all the meat samples increased significantly ($P<0.01$). Total lipid contents were not influenced by washing and storage. Water-washed meat recorded higher pH, followed by raw and EDTA-washed meat. pH did not change significantly during storage. EDTA-washed samples recorded significantly higher emulsifying capacity, as compared to raw and water-washed samples.

Keywords : Washing, Buffalo meat, Extractable protein, Functional properties, Refrigerated storage.

Washing of meat with chilled water, usually employed in case of whole fish and pork cuts, offers certain technical advantages, such as improved microbial quality, deflavouring, improved functional properties, and removal of substances promoting protein denaturation as well as lipid oxidation, during storage at low temperature (Lee and Toledo 1970; Colmenero and Matamoros 1981). However, the composition and functional properties of washed pork or buffalo meat during refrigerated storage have not been reported. Similarly, little work has been done on the effect of EDTA salt, in spite of its antioxidant and antibacterial properties (Igene et al. 1979). The microbial counts in EDTA-washed samples were lower than those for raw and water-washed meat samples (Kulkarni et al. 1993). Hence, the present study was undertaken to observe the effect of water and EDTA washing treatment in ground buffalo meat.

Four adult healthy entire male buffaloes (*Murrah* type) of uniform conformation were slaughtered by *Halal* method at the local slaughter house. Medial round cut (*Mukkadam*, a common wholesale cut in India) comprising semi-membranosus, semi-tendinosus and gluteal muscles, was brought to the laboratory in chilled condition; trimmed off the fat, facia and excess of connective tissue; and cut into approximately 2.5 cm cubes. These were minced through 8 mm, followed by 4 mm plates. Minced meat was divided into 3 groups, one served as control, and the other two were subjected to washing. For washing, minced meat samples were soaked separately in chilled distilled water or 2% EDTA (disodium salt) solution (pH 4.5) in a ratio of 1:2 (w/v) in sterilized stainless steel vessels with frequent stirring for 10 min. The meat was squeezed in a double-layered sterilized muslin cloth by applying hand pressure to the extent that no continuous drip occurred from the meat. In all, two washings were given to each group of meat samples. About 100 g samples were packed separately in 150 gauge polyethylene bags, and stored in a refrigerator at 4±1°C for 20 days. Samples were drawn at 5 day intervals upto 20 days of storage for analysis.

Moisture, pH and total proteins were estimated by the method of AOAC (1980). Water-extractable proteins (WEP), and salt-extractable proteins (SEP) were recovered by the method of Kang and Rice (1970), and was determined according to Lowry et al (1951). Total pigments and myoglobin were determined by the procedure of Araganosa and Henrickson (1969). Iron content was estimated by the method of Ponder (1942). The method of Folch et al (1957) was used for extracting total lipids for gravimetric determination. Water holding capacity (WHC) was determined by the procedure of Whiting and Jenkins (1981) with slight modifications. The meat flake was weighed immediately after pressing, and the filter papers were weighed after drying at room temperature. The weight due to meat proteins was added to the weight of meat flake, and WHC was expressed as % weight loss. Emulsifying capacity (EC) was estimated by the procedure of Pearce and Kinsella (1978), and expressed as ml of oil emulsified/mg protein. Statistical analysis of the data was done by three way analysis of variance

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(ANOVA) technique (Snedecor and Cochran 1967).

Analysis of variance revealed insignificant effect of treatment on total lipids, iron and storage period on moisture, total proteins, total lipids, pigments and pH.

Moisture content was significantly ($P<0.05$) reduced during washing. Similar results were obtained by Holmquist et al (1984) in water-washed fish flesh, though Colmenero and Matamoros (1981) observed higher moisture content in water-washed mechanically deboned pork. This could be due to squeezing adopted in the present study, as against centrifugation adopted by them. Insignificant changes in moisture contents during refrigerated storage were in accordance with the reports of Agnihotri (1988), and Kesava Rao (1988) in raw buffalo meat (Table 1).

Crude protein contents were significantly affected by treatments, and were lower in raw samples. Lower moisture content (higher dry matter) in the washed meat samples exhibited higher total protein contents in these samples, although significant amounts of WEP were removed during washing. SEP contents of the water-washed samples were significantly higher as compared to other samples due to higher pH. Lower extractability of SEP in EDTA-washed meat may be due to comparatively low pH, nearing the isoelectric pH (Table 1). Similar observations were reported by Hamm (1960) in beef. WEP content increased significantly during storage. A significant ($P<0.05$) increase in SEP content of all samples during storage was in general agreement with the increased extractability of myofibrillar proteins, as observed by Anjaneyulu (1988) in buffalo meat. This increase was attributed to the activity of natural enzymes or microbial growth, causing release of myofibrillar proteins (Chen et al. 1981). In support of this, a significant correlation was observed between SEP content and length of storage period in raw ($r=0.46$), and water-washed ($r=0.58$) samples. However, insignificant correlation in EDTA-washed samples suggested the possibility of different microflora, low pH and/or unknown effect of EDTA salt itself on meat proteins.

Washing of meat significantly increased pH in water-washed samples, as compared to raw and EDTA-washed meat samples. Similar rise in pH was observed by Colmenero and Matamoros (1981) in water-washed pork, which was attributed to the loss of soluble acid substance during washing. The lowest pH in EDTA-washed samples might be due to low pH of EDTA solution.

Water holding capacity (WHC), expressed as % weight loss of loose water from the meat tissue, was reduced due to washing of meat. Colmenero

TABLE 1. TREATMENT AND STORAGE EFFECT ON BUFFALO MEAT

	Treatment effect			
	Raw	Water	EDTA	Critical difference ($p<0.05$)
Moisture, %	77.82^{b}	75.18^{a}	74.82^{a}	0.49
Total proteins, %	20.15^{a}	22.24^{b}	21.73^{b}	0.68
WEP, %	3.02^{c}	1.65^{b}	1.23^{a}	0.39
SEP, %	4.22^{b}	7.29^{c}	3.07^{a}	0.85
TP, mg/g	4.49^{b}	1.06^{a}	0.81^{a}	0.50
MB, mg/g	1.94^{b}	0.42^{a}	0.27^{a}	0.18
pH	5.37^{b}	5.77^{b}	5.17^{a}	0.15
WHC, % wt loss	58.48^{a}	63.61^{b}	62.04^{b}	2.65
EC, ml/mg protein	0.52^{b}	0.44^{a}	0.70^{c}	0.07

	Storage effect, days					
	0	5	10	15	20	Critical difference
WEP, %	1.38^{a}	1.82^{ab}	1.73^{a}	2.24^{bc}	2.65^{bc}	0.50
SEP, %	3.06^{a}	4.24^{b}	4.86^{b}	6.21^{c}	5.94^{c}	1.01
Iron, mg/g	0.22^{ab}	0.19^{a}	0.23^{ab}	0.24^{b}	0.24^{b}	0.04
WHC, % wt loss	64.54^{a}	61.77^{ab}	60.25^{ab}	59.92^{a}	60.41^{ab}	3.42
EC, ml/mg protein	0.65^{b}	0.53^{a}	0.52^{a}	0.52^{a}	0.56^{a}	0.08

Means with same superscript in rows do not differ significantly ($p<0.05$).

and Matamoros (1981) also observed similar decrease in WHC of water-washed pork. This could be due to loss of water-soluble substances, mainly WEP and protein insolubilization. Changes in the moisture content observed in the present study as well as in the findings of Colmenero and Matamoros (1981) showed a little effect on WHC of washed meat samples. Increase in WHC during refrigerated storage in all the samples was in general agreement with the report of Anjaneyulu (1988) in raw buffalo meat.

Emulsifying capacity (EC) of the raw buffalo meat was comparable to the values reported by Swift et al (1961). EDTA-washed samples recorded significantly higher EC than raw and water-washed ones. This might be due to lower protein concentrations in the extracts of these samples, because of lower extractability of proteins (Swift 1965). The efficiency of salt soluble proteins tended to vary inversely with respect to both the amount of proteins removed from the solution, and the original protein content (Swift et al. 1961). During storage, the EC decreased significantly. This might be correlated to higher SEP, as reported by Swift (1965). A significant ($P<0.05$) correlation between EC and pH of raw ($r=0.34$)/water-washed samples ($r=0.47$) was observed.

Effect of washing on compositional and functional properties was more pronounced in EDTA-washed, as compared to water-washed samples. Reduced moisture and pigment content can offer certain advantages by decreasing water activity and rate of oxidative changes, which may off-set the observed losses.

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Radiation Disinfestation of Spices and Spice Products I. Radiation Sensitivity of Developmental Stages of *Lasioderma serricorne* and *Stegobium paniceum*

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Gamma radiation treatment to eggs and larvae of *Lasioderma serricorne* and *Stegobium paniceum* showed an age and stage dependent response. A dose of 25 Gy prevented eggs from developing to adult stage. A dose of 50 Gy was required to prevent older larvae from developing. Larvae of *S. paniceum* were comparatively more sensitive than those of *L. serricorne*. In case of adults, doses above 30 Gy appear to produce a sterilizing effect in both the species.

Keywords : Disinfestation of spice and spice products, Radiation sensitivity, Developmental stages, *Lasioderma serricorne*, *Stegobium paniceum*.

Among the insect pests, which infest spice commodities, *L. serricorne* (the cigarette beetle), *S. paniceum* (the drug store beetle), and *Tribolium castaneum* are of major importance (Padwal-Desai et al. 1987). Considerable work has been directed towards determination of effects of gamma radiation on various stored product insects (Golumbic and Davis 1966;Tilton and Brower 1973), particularly *Tribolium* (Benham 1962). Earlier work on exposure of whole and ground spices in packaged conditions to gamma radiation doses ranging between 7.5 and 100 kGy doses indicated efficacy for microbial decontamination (Sharma et al. 1984; Munasiri et al. 1987; Padwal-Desai et al. 1987) However, comparatively little information is available on the stage/age dependent sensitivity of *S. paniceum*, though some data are available in respect of *L. serricorne* (Tilton et al. 1966). Moreover, it is difficult to make full use of available data reported by other workers, because of variations in techniques, local conditions, methods of assessing sensitivity and other factors (Golumbic and Davis 1966; Tilton et al. 1966; Tilton and Brower 1973). Hence, it is necessary to work out optimum radiation dose for commercial application. As a first step towards optimization of radiation dose, studies were undertaken to determine radiation sensitivity of different metamorphic stages of *L. serricorne* and *S. paniceum*. Present communication describes the results of these investigations.

Insects for experiments were drawn from cultures of *L. serricorne* and *S. paniceum* maintained at 28±1°C on wheat flour + yeast diet (Tilton et al. 1966). For obtaining eggs, large number of adults were released on flour, which was obtained after passing through 80 mesh sieve. Eggs were collected after 24h, by passing the flour through a 60 mesh sieve (Fletcher and Long 1971). Eggs were counted in groups of 25, and incubated for varying periods. Normally, the eggs of *L. serricorne* hatch on fifth day at 28±1°C. Therefore, eggs incubated for 1,2,3 and 4 days were evaluated for radiation sensitivity, by irradiating at appropriate time with gamma radiation doses between 5 and 30 Gy. These were then placed on wheat flour diet (Tilton et al. 1966), (25 g), and left undisturbed till adult emergence. Number of adults emerged at each dose treatment was recorded.

For determining radiation sensitivity of larval stages, freshly eclosed larvae of *L. serricorne* and *S. paniceum* were placed in 25 g food medium (Tilton et al. 1966), and allowed to develop. Larvae were exposed to radiation doses ranging from 10 to 75 Gy after 1,5,10,15,20 and 25 days aging. Adult emergence was scored.

In order to determine the sterilizing dose for adults, freshly emerged adults of *L. serricorne* and *S. paniceum* were irradiated with doses ranging between 10 and 80 Gy. Ten pairs of irradiated adults were released on appropriate quantity of food medium. These adults were removed from the medium, when dead, without disturbing the medium. Number of adults of F_1 progeny was recorded in all the dose treatments to assess reproductive performance of irradiated adults.

All irradiations were carried out in Gamma-Cell 220 (Atomic Energy of Canada Ltd., Ottawa, Canada) with dose rate of 30 Gy/min under ambient conditions (28±1°C, 60-70% RH). After irradiation, the medium was left undisturbed. Ability of the irradiated immature stages to reach adult stage, and capacity of irradiated adults to

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TABLE 1. EFFECT OF GAMMA RADIATION ON POST-EMBRYONIC DEVELOPMENT OF EGGS OF *LASIODERMA SERRICORNE*

Radiation dose (Gy)	Eggs (%) developing to adult stage, when irradiated at indicated age, days*			
	1	2	3	4
0 (control)	84	87	76	76
5	25	68	81	84
10	0	29	72	74
15	0	0	54	68
20	0	0	33	20

The values were zero on all days in case of 25 and 30 Gy doses.

* 25 eggs/replicate, 4 Replicates/treatment (dose)

produce F_1 progeny, were considered as the main criteria for radiation sensitivity.

When eggs of *L. serricorne* of different ages were exposed to gamma radiation, the response was both age and stage dependent. For example, 1 day old eggs were sensitive even to a dose of 5Gy, and none of the eggs could develop after gamma irradiation with 10 Gy dose (Table 1). As the age of the eggs advanced, tolerance to radiation treatment increased. The 3 and 4 days old eggs could withstand 15 Gy dose and develop to a considerable extent, but 1 and 2 day old eggs could not develop. The older eggs (3 or 4 days) required about 25 Gy for complete inhibition of development, whereas 2 days old eggs required 15 Gy dose for similar effect. In the case of unirradiated control groups, % eggs successfully completing development ranged from 76 to 87.

Response of aging larvae to gamma radiation doses showed a similar pattern, i.e., age and dose related response. At 10 Gy dose, 5 days old larvae were most sensitive, while 25 days old larvae were the least sensitive (Table 2). Similarly, none of the 5 or 10 days old larvae could reach adult stage at 25 Gy. Percentage of larvae reaching adult stage increased with advancing age of larvae in case of irradiated samples. But, it never reached the level of unirradiated controls. The larvae reaching adult stage in control groups ranged between 81-99%. The dose of 50 Gy and above completely inhibited the development of irradiated larvae (data not given). These dose levels were higher than those required for preventing eggs from developing to adult stage.

In case of larvae of *S. paniceum*, sensitivity appeared to be more pronounced than that of *L. serricorne* larvae. None of the larvae, irrespective of the ages tested, could reach adult stage at 25 Gy dose (Table 2). Sudha Rao et al (1984) have

TABLE 2. RADIATION SENSITIVITY OF LARVAL STAGES OF *LASIODERMA SERRICORNE* AND *STEGOBIUM PANICEUM*

Radiation dose, Gy	Larvae % reaching adult stage, when irradiated at indicated age, days*					
	1	5	10	15	20	25
	Lasioderma serricorne					
0	-	92	90	81	99	99
10	-	28	54	-	92	87
25	-	0	0	15	32	36
	Stegobium paniceum					
0	91	91	90	97	97	-
10	48	66	76	58	44	-

The values were zero on all the days tested in case of *L.serricorne* with the use of 50 and 75 Gy and at 25,50 and 75 Gy doses in case of *S.paniceum*

* 4 Replicates/dose, 25 Larvae/replicate, -: Not done

shown the same effect on *rawa*, where the predominant infesting species was *Tribolium castaneum*. The response at 10 Gy dose was much varied. However, the larvae successfully completing development at this dose was much lower, as compared to control larvae.

Reproductive performance of the adults, when exposed to varying doses of radiation soon after emergence, was severely affected even at doses of 30 Gy, and above for both the species tested. In case of *L. serricorne*, the average number of F_1 progeny produced/female in control was 20. But, it was only one fourth of the control at 10 Gy dose treatment. In case of *S. paniceum*, the F_1 progeny production in control was at a low level of only 5/female. At 10 Gy dose, the number of F_1 progeny adults produced/female was only one tenth of the control. Gamma radiation doses of 30 Gy and above (data not given) induced sterility in the irradiated adults, since no F_1 progeny is produced.

Although *L. serricorne* is one of the test insects employed during the earlier period for radiation effects (Runner 1916), comparatively scanty information is available on this species. Same is the case with respect to stage dependent radiation sensitivity of *S. paniceum*. Tilton et al (1966) studied radiation sensitivity of *L. serricorne* along with three other stored grain pests. However, the dose employed by these workers ranged between 13.2 and 100 Kr. They observed the hatching of some of the irradiated cigarette beetle eggs, but none of eclosed larvae transformed into pupae. These workers had not specified the age of eggs at irradiation. From response pattern, advanced eggs appear to be the stage tested. Similarly, the insects irradiated as larvae transformed into pupae, but could not eclose as adults.

Ruangpos (1966) studied radiosensitivity of eggs of *L. serricorne*, and reported that the doses of 8 and 40 Gy caused 14% and 100% mortality, respectively, when irradiation treatment was given to 1 day old eggs. Such observations were reported for the disinfestation of wheat at 0.3 to 0.5 kGy (Bongirwar et al. 1981). These observations are somewhat similar to those observed in the present studies. On the other hand, Lapis et al (1977) reported the killing effect on 1 day old eggs by doses ranging between 0.1 and 1 kGy, which are considerably higher than those studied earlier. Further, these workers observed that the doses upto 0.4 kGy did not prevent hatching of 4 days old eggs, but the ensuing larvae failed to pupate in spite of the survival. Lethal doses for pupae and adults were in the range of 0.4 to 1 kGy. Ravetti (1973) observed that doses ranging between 0.1 and 0.5 kGy killed all stages of *L. serricorne*. Els et al (1978) reported that the doses of 0.75 and 2.5 kGy caused 100% mortality in immature stages and adults, respectively. It is thus evident that all these workers have used comparatively higher doses for studying radiosensitivity of *L. serricorne*. Moreover, they have used mortality as one of the criteria. However, the present studies involved the assessment of the ability of irradiated stages to develop into adult in case of egg as well as larval stages, and reproductive capacity in case of adults.

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Effect of Different Nitrogen Sources on Ethanolic Fermentation of Glucose by *Zymomonas mobilis*

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Among several organic and inorganic nitrogen sources tried, urea (0.5 g/l) was found to be better especially than ammonium sulphate, which is generally used in defined medium for ethanol production by *Zymomonas mobilis*. Calcium pantothenate has been found to be an essential vitamin for ethanol production. Addition of yeast extract as a source of both nitrogen and vitamins, rather than peptone, was effective for obtaining enhanced yield of ethanol, particularly in high glucose (200-300 g/l) fermentation. A final pH of 4.1 to 4.4 in case of urea, as compared to that of 3.3 to 3.9 with the use of ammonium sulphate, appears to contribute to enhanced ethanol production, as the bacterium is intolerant to lower pH.

Keywords : *Zymomonas mobilis*, Ethanol, Nitrogen sources, Glucose, Vitamins, Medium pH.

In all the earlier studies with *Z. mobilis*, many workers (Baratti et al. 1986; Swings and De Ley 1977; Rogers et al. 1982) have used ammonium sulphate as the supplementary source of nitrogen to yeast extract. To enhance the ethanol production, different levels of the nutrients (Rogers et al. 1982) were studied. and Nath (1987) used *Zymomonas mobilis* for ethanol production, and studied the effect of high substrate concentration on the kinetic parameters. The information available on exploring the potential of other nitrogen sources is meagre for this organism (Rogers et al. 1982; Baratti et al. 1986). In the present study, both organic and inorganic nitrogen sources were evaluated in a defined medium for ethanol production along with efficacy of urea in replacing generally used $(NH_4)_2 SO_4$ in presence of yeast extract or peptone, in both defined and fermentation media.

Zymomonas mobilis ZM4, obtained from laboratorie de Chimie Bacterienne, Centre National de la Recherche Scientifique (CNRS), Marseille, France, and its thermotolerant mutant (ZMI2) (Sreekumar and Basappa 1991) were used. These cultures were maintained on glucose (2%), yeast extract (1.0%), agar (2%) medium, at 4°C by sub-culturing every two weeks.

All chemicals used were of analytical grade. The protein isolates (95% pure) of oilseeds were obtained from Protein Technology Department of this Institute. Yeast extract and casein hydrolysate were from Difco, USA. Defined medium (Baratti et al. 1986) used included (g/l) glucose 100 to 300, $MgSO_4$ $7H_2O$ 0.5, KH_2 PO_4 2.0, and $(NH4)_2$ SO_4 1.0, in addition to calcium pantothenate at 5 mg/l level. Ammonium sulphate was replaced in the medium by other nitrogen sources on equimolar nitrogen basis. A nitrogen percent of 15-16 was considered in undefined nitrogen sources based on purity. In fermentation medium, calcium pantothenate was replaced by yeast extract or peptone (5 g/l). The media were sterilized at 121°C for 15 min. Calcium pantothenate and urea solutions were filter-sterilized by using membrane filters (Type HV, Pore size 0.45µ; Millipore, Bedford, USA), and added to media just before inoculation. All the experiments were performed in triplicate, and the average values are reported. The experiments were repeated at least once to confirm the results in some cases.

Whole of the growth from two-days old slant culture was inoculated into 100 ml sterile glucose (2.0%), yeast extract (1.0%), broth (pH 5.5) in 250 ml conical flask, and incubated at 30°C under stationary conditions for 24h for inoculum preparation. Ten ml of this inoculum was transferred into 100 ml of sterile fermentation medium (pH 5.5) in 250 ml conical flask, and fermented at 30°C under stationary conditions for 72 h. The inoculum for the defined medium was prepared by centrifuging the above inoculum at 10,000 **g** for 10 min, washing the cells with sterile distilled water, suspending in sterile defined medium and used as inoculum. The fermentation was carried out as above.

Biomass was estimated by measuring the optical density (OD) of known volume of culture broth at 600 nm in Spectronic 21 (Bausch and Lomb, New York, USA), and comparing with standard curve, drawn for known volumes of culture broth against their dry weights. The latter was determined by filtering the medium through pre-weighed Millipore membrane (Type HV, Pore size 0.45µ; Millipore, Bedford, USA) filters and drying the biomass at 105°C for 6h. Glucose was estimated

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by the dinitrosalicylic acid method (Miller 1959). Ethanol was assayed by gas chromatographic (FID, 16 A, Shimadzu, Kyoto, Japan) method (Anthony 1984), which was simpler, more rapid, more sensitive and less expensive than the other methods (Sreekumar et al. 1990). Ethanol yield (Yp/s, g/g) was calculated as the ratio between g ethanol produced and g sugar consumed. (Sreekumar and Basappa 1991). The % theoretical ethanol yield was calculated by the formula: Ethanol yield/0.511 x 100 (Sreekumar and Basappa 1991).

Both the strains utilized sodium nitrate and sodium nitrite very poorly, whereas the use of urea as the sole nitrogen source gave better ethanol production, when compared to ammonium sulphate in the defined medium, containing 100 g glucose/l. Ethanol production in media containing tryptone or protein isolates was equal to that in urea containing medium. This suggests the production of proteinase and urease by cultures. The occurrence of these enzymes in *Z. mobilis* has been reported by Swings and De Ley (1977), and Baratti and Bu'lock (1986).

Since, urea was found to be the best and cheapest nitrogen source in the defined medium, different levels of urea were tested in the presence and absence of calcium pantothenate, an essential vitamin (Baratti et al. 1986;Swings and Deley 1977; Rogers et al. 1982; Toran-Diaz et al. 1983). The increase in concentration of urea upto 1.0 g/l, though improved the sugar utilization and pH maintenance, it did not enhance ethanol production (Table 1). However, the production of ethanol was always better in the presence of calcium pantothenate than in its absence.

TABLE 1. EFFECT OF DIFFERENT NITROGEN SOURCES (ON EQUIMOLAR NITROGEN BASIS) ON ETHANOL PRODUCTION BY *Z. MOBILIS* ZM4 AND ZMI2 IN DEFINED MEDIUM (GLUCOSE 100 g/l WITHOUT NITROGEN SOURCE) AT 30°C.

Nitrogen source	Sugar utilised, g/l		Biomass, g/l		Ethanol, g/l	
	ZM4	ZMI2	ZM4	ZMI2	ZM4	ZMI2
Sodium nitrate	1.0	2.0	0.01	0.01	0.0	0.0
Sodium nitrite	2.0	8.0	0.01	0.04	0.0	0.0
Ammonium sulphate	78.0	74.0	1.78	1.40	33.5	30.0
Urea 0.5g/l	96.0	95.0	1.50	1.70	39.2	40.0
Urea 0.5 g/l*	60.0	66.0	1.20	1.20	25.0	30.0
Urea 1.0 g/l	94.0	95.0	1.40	1.40	33.0	35.0
Urea 1.0 g/l*	94.0	93.0	1.40	1.20	31.2	32.0
Tryptone	96.0	97.0	1.40	1.50	39.0	40.1
Casein hydrolysate	22.0	22.0	0.50	0.60	7.9	8.7
Mustard protein	98.0	98.0	1.80	1.50	38.4	36.8
Soya protein	98.0	98.0	1.70	1.60	40.8	38.8
Groundnut protein	97.0	98.0	1.80	1.40	40.0	36.8

* Without calcium pantothenate. Initial inoculum: 0.01g/l

The level of calcium pantothenate in yeast extract is about 20-100 µg/g (Bridson and Brecker 1970) and it supplies about 0.5 mg/l of calcium pantothenate in the medium at 5 g/l level. Though the effect of calcium pantothenate is pronounced on ethanol production in media containing lower concentrations of glucose (100 g/l) and urea (0.5 g/l), its effect is not so discernible at higher glucose (200 g/l) and urea (1.0 g/l) concentrations (Data not presented). Hence, yeast extract, which supplies not only calcium pantothenate, but also other vitamins, was used in place of pure calcium pantothenate for fermenting higher sugar concentrations in order to obtain higher yields of ethanol. The experiment with yeast extract (Table 2) indicates that vitamin (s) other than calcium pantothenate is/are also required for better growth, and higher ethanol production by *Z. mobilis*.

Table 2 also shows a comparison of the effect of equimolar nitrogen of $(NH4)_2SO_4$ (1.1 g/l) and urea (0.5 g/l) in a fermentation medium (with yeast extract), containing varying concentrations of glucose ranging from 100-300 g/l. In high glucose concentration medium, urea served as a better nitrogen source. Biomass, ethanol and sugar utilization were more in urea containing medium, and pH was maintained around 4.1 to 4.4 as compared to 3.3-3.9 in $(NH4)_2SO_4$ containing medium. Formation of a strong inorganic acid (H_2SO_4) in $(NH4)_2SO_4$ medium is perhaps the reason for more acidic pH condition than that in urea medium. It is known that *Z. mobilis* is low pH intolerant (Rogers et al. 1982). Experiment with pH stat at 5.0 to 5.5 yielded maximum concentration of ethanol (Sreekumar and Basappa 1992).

When peptone (5 g/l) was used in defined medium (glucose 200 g/l) along with urea, it yielded higher ethanol level (76 g/l) than when ammonium sulphate was used (71 g/l). Twice the concentration of urea (1 g/l) alone, though equated the nitrogen level to the above media, yielded only 42 g/l ethanol (data not presented). This indicates that complex organic nitrogen is better than inorganic nitrogen for ethanol production.

It may be concluded that urea (0.5 g/l) could be used successfully to replace $(NH4)_2SO_4$ in both defined and fermentation media. Its role in maintaining a suitable pH is a significant feature, especially in high sugar fermentation. Though

TABLE 2. EFFECT OF EQUIMOLAR NITROGEN OF AMMONIUM SULPHATE AND UREA ON ETHANOL PRODUCTION BY *Z. MOBILIS* ZM4 AND ZM12 IN A FERMENTATION MEDIUM** WITH DIFFERENT CONCENTRATIONS OF GLUCOSE.

Glucose, g/l	Final pH		Sugar utilised, g/l		Biomass, g/l		Ethanol, g/l		Ethanol yield, g/g		Theoretical yield, %	
	ZM4	ZMI2	ZM4	ZMI2	ZM4	ZMI2	ZM4	ZMI2	ZM4	ZMI2	ZM4	ZMI2
100#	3.6	3.7	97.5	98.0	1.9	2.0	43.0	44.0	0.44	0.45	86.5	88.0
100•	4.3	4.4	97.0	97.5	1.7	2.0	42.5	43.1	0.44	0.44	85.9	86.7
200#	3.6	3.8	180.0	166.0	2.2	1.6	76.0	75.0	0.42	0.45	82.8	88.6
200•	4.4	4.1	194.0	186.0	2.4	2.1	87.0	82.0	0.45	0.44	87.9	86.4
250#	3.8	3.9	150.0	155.0	2.5	2.0	66.0	68.0	0.44	0.44	86.3	86.0
250•	4.3	4.2	217.5	225.0	2.6	2.2	80.4	82.0	0.37	0.36	72.5	71.5
300#	3.5	3.7	150.0	155.0	1.5	1.6	62.0	66.0	0.41	0.42	81.0	83.5
300•	4.4	4.1	189.0	186.0	2.2	1.8	79.0	82.0	0.42	0.44	82.0	86.4

With ammonium sulphate 1.1 g/l, • With urea 0.5 g/l, •• With yeast extract 5.0 g/l

calcium pantothenate is an essential vitamin for growth and ethanol production, addition of extra vitamin (s), and organic nitrogen through yeast extract is required in order to obtain better yields of ethanol, especially at higher glucose concentrations.

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Separation Technology for Infested Wheat in Bulk Storage

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Studies on separation of infested grains from a bulk of wheat showed unsuitability of a common vibratory type air screen cleaner at 6.8 m/s air velocity and poor performance of specific gravity separator. An entoleter was, therefore, developed to break the comparatively weaker infested grains. At 1470-1780 rpm, if the infestation level in wheat is limited to 20%, the entoleter separated 95% of the infested grains.

Keywords : Infested wheat, Separation technology, Air screen grain cleaner, Specific gravity separator, Separation efficiency, Entoleter.

The annual production of foodgrains in India is around 180 million tonnes (Anon 1994). Food grains are stored at farmer's level for their own use and by Government agencies, traders and millers. A loss of 10% of foodgrains during post production phase has been reported (Sawant 1985), and a major portion of this loss occurs during storage (Pandya et al. 1980) due to physico-chemical changes (Leelavathy et al. 1984), leading to reduction in the nutritive value and functional property (Emefu et al. 1992; Indudhara Swamy et al. 1993). It was reported that common man could detect infestation in wheat at 6% level (Dakshinamurthy 1990). Datta and Nagarkar (1977) have reported a centrifugal device for separation of lighter materials, like chaff and straw from the grains. Bilanski et al (1962) have investigated the aerodynamic properties of grains. Clarke (1985) studied the separation of round and healthy grains/seeds by fluidization. However, separation of insect-infested grains from uninfested grains has not been attempted so far. Data on investigations on different methods for separating infested grains are reported in the present communication.

For this study, wheat was stored in gunny bags for 4-5 months in hot and humid conditions (25 to 35°C., R.H. between 50 and 90%). It resulted in 40% infestation and 20-30% weight loss. The samples were prepared by mixing 50% by weight of infested grains (with visible damage) with 50% uninfested grains stored similarly, but in insect-proof containers. Other ratios of uninfested and infested wheat at 60:40, 70:30, 80:20 and 90:10 were also tried.

The physical properties (Mohesenin 1970), viz., dimensions, equivalent diam, specific gravity and hardness of infested and unifested wheat were measured. The equivalent diam of wheat was 4.2 mm. The specific gravities of uninfested and infested wheat were 1.41 and 1.31, respectively, whereas the hardness values of uninfested and infested wheat were 9.0 kg and 2.7 kg, respectively. Based on the equivalent diam, the screen sizes for cleaning were identified. Tests were conducted on the separation of infested wheat using three different devices.

Power operated vibratory air screen grain cleaner: The unit developed in the Institute (Kachru and Sahay 1990), was used. The wheat from hopper was dropped to top scalping sieve by gravity, and controlled by a feeding mechanism (Fig. 1). With the help of a blower, air was blown across the falling wheat, whereby lighter materials are blown away. Separation with two sieves was achieved by difference in size. Larger impurities were retained over the top screen, and the under size was separated by the bottom grading sieve. The cleaned

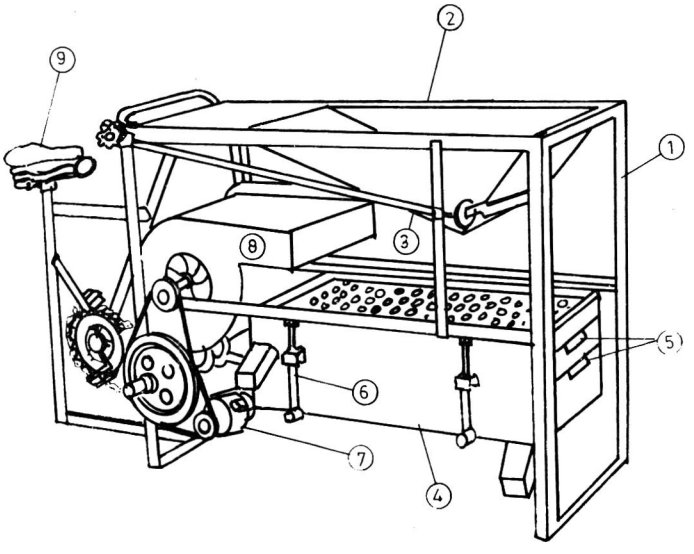

Fig. 1. Pedal-cum-power operated air screen grain cleaner
1. Main frame 2. Hopper 3. Feeding mechanism
4. Sieve box 5. Scalping and grading sieves 6. Shoes
7. Electric motor 8. Blower 9. Standard bicycle parts

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and sound wheat was retained over the bottom sieve, and delivered through a spout. Bulk of wheat with 50% infested grains were run thrice to separate the infested grains, and observations were noted.

Laboratory model specific gravity separator : A commercial unit (Westrup, Denmark), which can separate grains from other impurities using the difference in specific gravity was used (Fig. 2). The wheat from hopper was dropped to the vibrating deck. The feeding was controlled by a magnetic vibrator. Air was blown across the deck from beneath. The end and side slopes of the gravity tables were adjustable. Wheat, when dropped to the deck, was stratified by blowing air. At gentle side slopes, the grains were collected in all the 4 spouts, along the triangular deck. At increased side slopes, the grains were collected in first two spouts. The

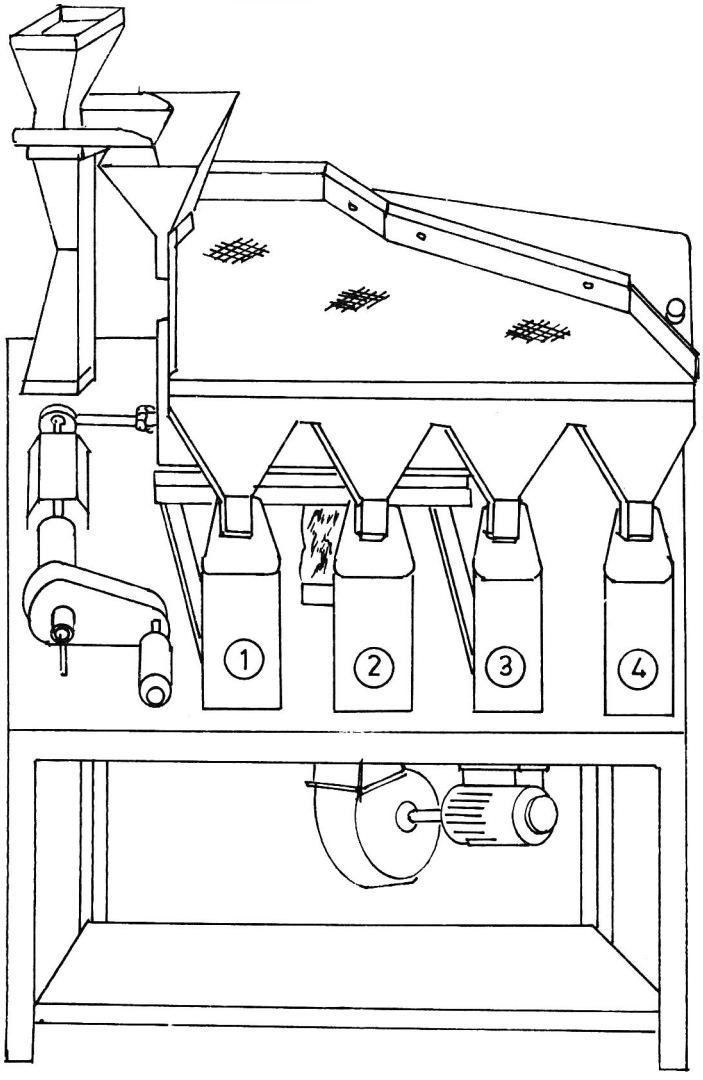

Fig. 2. Specific Gravity Separator

specific gravity separator was run twice to observe its utility towards removal of infested grains.

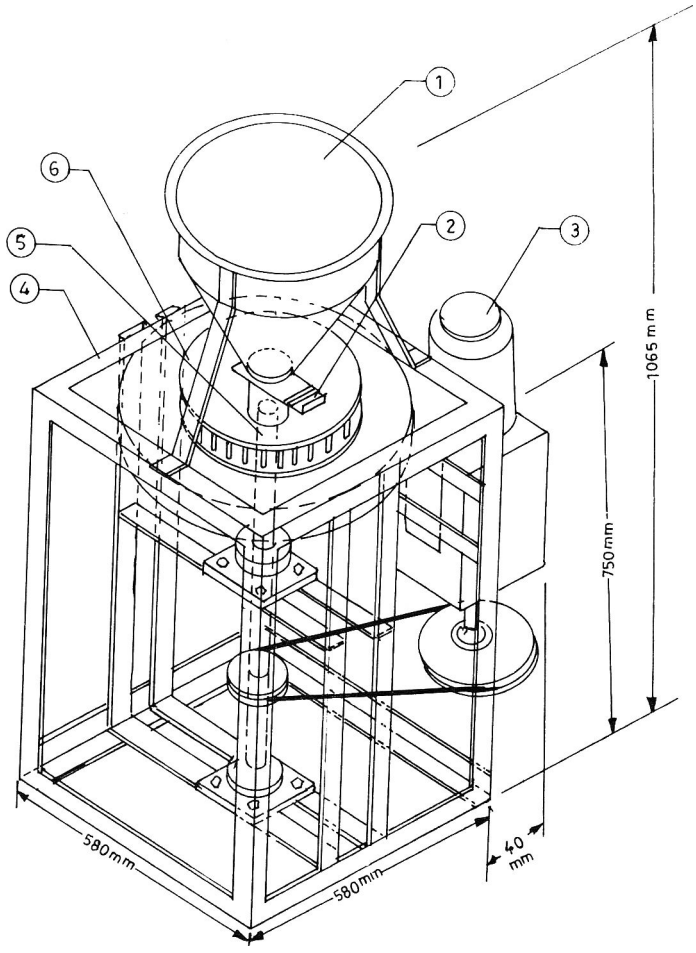

Fig. 3. Entoleter

1. Hopper 2. Feeding gate 3. Motor
4. Main frame 5. Pinned rotor 6. Metal liner

Random samples of different weights from the spouts and the uninfested, infested wheat, and debris were separated by hand picking. The percentages of infested, uninfested wheat, and debris collected in each spout were determined.

Entoleter : The unit developed to break the weaker infested grains is shown in Fig. 3. The entoleter has a pinned rotor rotating inside a metal casing. Wheat fed in at top of the mill, on reaching the rotating pined rotor is struck by the row of 12 mm pins, and thrown against the metal casing or liner. The weaker grains are broken by these impacts.

To test the entoleter, wheat with various proportions of uninfested and infested grains was fed into the machine. The entoleter was run at different speeds (rpm). Three replications of observations of grain breakage for various infestation levels, and at different speeds were recorded by taking samples of 100 g and counting the infested and uninfested grains (by number and on weight basis). The entoleted wheat was first separated

TABLE 1. TEST RESULTS OF THE SPECIFIC GRAVITY SEPARATOR FOR SEPARATION OF 50% INFESTED WHEAT

End slope, degree	Side slope, degree	Constituents	Amounts received at different spouts, %			
			1	2	3	4
3.5	1.5	Uninfested wheat	10.5	46.7	71.5	87.0
		Infested wheat	77.1	51.6	26.0	11.6
		Debris	12.2	1.5	2.5	1.3
3.5	2.9	Uninfested wheat	46.8	59.7	72.9	82.7
		Infested wheat	50.9	38.6	26.3	17.2
		Debris	2.16	1.6	0.7	0.0
3.5	3.6	Uninfested wheat	58.2	65.1	73.3	86.3
		Infested wheat	40.0	34.0	26.2	13.0
		Debris	1.6	0.8	0.3	0.6
3.5	4.4	Uninfested wheat	65.5	76.4	45.2	9.8
		Infested wheat	33.3	22.7	17.3	11.4
		Debris	2.4	0.7	37.4	78.7
3.5	5.1	Uninfested wheat	70.7	86.9	-	-
		Infested wheat	28.7	12.2	-	-
		Debris	0.5	0.7	100	100
4.3	1.5	Uninfested wheat	4.5	49.0	75.2	88.8
		Infested wheat	90.9	50.2	24.5	10.6
		Debris	4.5	0.7	0.2	0.5
5.2	1.5	Uninfested wheat	-	0.6	29.7	27.2
		Infested wheat	86.9	96.2	69.0	72.0
		Debris	13.0	3.1	1.2	0.7

Details of spout No. 1,2,3,4 are indicated in Fig. 2.

using air screen grain cleaner with a top sieve of 5 mm round holes and a bottom sieve of 2x20 mm, rectangular holes. Further separation was necessary, and an indented cylinder separator with a rotating drum dent size of 5.2 mm was used to separate broken grains.

Screen grain cleaner : It was observed that the 6.8 m/s winnowing air velocity did not separate the lighter infested wheat completely. Also being equal in size, the infested wheat was retained over the bottom grading sieve, and came out along with the clean grains. When wheat containing 50% infested grains was fed into the device, the cleaned wheat contained 30% infested grains. In contrast, the efficiency (grain purity) of the cleaner for freshly harvested, and threshed wheat was reported to be 99.8% (Kachru and Sahay 1990).

Specific gravity separator : When wheat occupied the entire deck surface for 3.5-4.3 and 1.5 degree end and side slopes, respectively, the machine was able to separate 87-89% of the uninfested wheat in the spout number 4. However, in the spout number 3, 71-75% of the uninfested wheat was separated out. On the other hand, the above deck angles could efficiently eliminate the infested wheat to the extent of 77-91% in the spout number 1 which is normally the inferior quality grains and debris (Table 1).

TABLE 2. TEST RESULTS FOR THE ENTOLETER AT 1470 TO 1780 RPM

Uninfested : Infested grain ratio			Unbroken infested grains, %
50	:	50	16
60	:	40	13
70	:	30	9
80	:	20	5
90	:	10	2

Entoleter : At entoleter speeds in the range 1470-1780 rpm, the infested grains left unbroken was maximum, when the ratio of uninfested and infested grains was 50:50 (Table 2). However, when this proportion was 80:20 and 90:10, the percentage of unbroken infested grains was reduced to 5 and 2%, respectively. At higher speeds, even the healthy grains were broken. Thus, the performance of the entoleter was best for wheat of less than 20% infestation.

Due to the similar size and small difference in specific gravity of infested and uninfested grains, separation of infested wheat by the air screen vibratory sieve cleaner or by the specific gravity separator was not very successful. An entoleter, developed to break the comparatively weaker infested grains, separated 95% of infested grains, when the infestation level in wheat was limited to 20%.

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***Toxicological Evaluation of Certain Veterinary Drug Residues in Food - WHO Food Additives Series 33.* Prepared by 42nd meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) Published by WHO, Geneva, 1994 and IPCS-International Programme on Chemical Safety, WHO, 1211, Geneva 27, pp 152, Price Swfr 40/= or US$ 36/= in developing countries Swfr 28/=**

This monograph is the culmination of the untiring efforts put in by all the 32 members of the joint FAO/WHO expert committee on food additives, who met in Rome from 1-10 Feb, 1994. Special appreciation has to be accorded to the eight contributors, who have done the onerous task of gathering all the vital information on some of the very important veterinary drugs, and presenting them as working papers in a highly intelligible manner. This volume has been prepared with the kind support of International Programme on Chemical Safety (IPCS), which is a joint venture of the United Nations Environment Programme, International Labour Organization and World Health Organisation. The ICPS devotes itself mainly to evaluation of the toxicological effects of chemicals on human health, and has been performing a laudable task of disseminating the available information.

The monograph contains a treasure of valuable information on the following drugs. Anthelminthic-Levamisole, antimicrobial agents-chloramphenicol, Flumequine, Olaquindox, Spectinomycin, Sulfadimidine; Glucocorticosteroid-Dexamethasone; Trypanocide-Diminazene.

The chapters begin with a concise introduction on each drug, its application in the veterinary medicine, the problems associated with their use, and possible adverse reactions in humans. This is followed by the description of biological data, which include biochemical aspects and biotransformation studies. Adequate information covering different toxic effects, namely hematological, immunological and reproductive toxicity following drug exposure has been presented. The information contained in the monograph is based on studies conducted in experimental animals such as mice, rats, dogs, pigs, guineapigs and rabbits. Observations in humans were also reported, wherever the information was available. Each of the eight papers focusses attention on special toxicological studies, that were specifically conducted for each drug.

Ronidazcle-the antiprotozoal drug was evaluated by the 34th joint FAO/WHO expert committee on Food Additives, and reported in WHO Technical report series No 788, 1989. A temporary ADI of 0-0.025 mg/kg body weight was fixed for this drug by the then committee. Since no new data were available to this committee, the above temporary ADI was not extended.

The reports compiled in the monograph include tables summarising the results, and highlighting the important observations.

There are useful annexures at the end of the monograph. Annexure 1 lists 112 important references available on food additives/drugs. Annexure 2 gives the abbreviations used in the monograph. Annexure 4 is a comprehensive presentation of the outcome of the meeting in a tabular form. A glance at this table would provide quick information on the ADI levels and toxicological recommendations.

This monograph is a valuable guide for workers in veterinary institutions, food regulatory officers, scientists in industries and toxicological laboratories. With the joint efforts of WHO/IPCS, one more highly informative and technically useful book has been added to the WHO food additives series.

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***Physico-chemical Constituents and Engineering Properties of Food Crops* by R.P. Kachru, R.K. Gupta and A. Alam; Published by Scientific Publishers, Jodhpur, 1994, pp 188, Price Rs. 450/-.**

For want of basic data, researchers, crop processing machinery designers, food engineers, those involved in manufacture of harvest and post-harvest machinery, and related professionals used to face a variety of problems in process development and machinery design. Many attempts lead to faulty design, owing to lack of facilities for determination of properties and related data generation. The usual approach of researchers has been to generate the data/information needed for the purpose and proceed. Data, thus, generated either remained with researchers or published in various journals, to which everyone does not have ready access. The engineer working in the field is, thus, deprived of the information, and left with the choice of making assumptions arbitrarily. The attempt made by the

authors in bringing out this book, giving data on engineering properties, physico-chemical constituents for food crops, fruits and vegetables grown in India, is worth complimenting. This book is first of its kind in Indian context, and is likely to satisfy many users.

This book provides the normal reader the understanding of procedures and state-of-the-art data on the subject, and vividly describes some procedures and basic concepts for each property. It is mentioned that the data presented in the book were more particularly, the work carried out at various centres of All India Co-ordinated Research Project on Post-harvest Technology of ICAR. Some related data published in different journals has been included. However, at least equal quantity of published data may have been left out. In spite of this, the data presented in the book clearly depict that the information on the subject is scanty and a lot remains to be done to get desired quantum of data on properties and constituents of fruits and vegetables, spices, oilseeds, and food grains. The book, written by authors closely involved in research on the subject under ICAR, thus draws an attention of the researchers for taking up the systematic and targeted work on generation of much needed basic data on engineering properties, and constituents of food raw materials, food products, by-products, plant residues etc. The work also needs to be initiated on properties, not covered in the book e.g., dielectric properties, surface area etc.

The book has been divided into eight chapters covering valuable information on physico-chemical constituents, and physical properties such as dimension, size, sphericity, shape and engineering properties viz., gravimetric, frictional, aerodynamic, rheological, thermal and hygroscopic properties of food crops, and their products/by-products.

Chapter I on physico-chemical properties, the richest data source among the chapters, gives exhaustive and valuable information on various aspects like straw percentage, height of the plant, proteins, mineral contents etc. However, use of different terminology like water content, moisture content and dry matter or terms like grain to straw ratio, grain to stalk ratio, grain to stubble ratio, and grain to crop ratio may be inconvenient to the reader in the absence of glossary of terms or definition of each. The non-uniform nomenclature and variation in data presentation pattern deprives the reader of similar information for other category product, and may affect the significance of information reported. For example, in the absence of subject index, it may be near impossible to imagine the availability of the data on *Chapati* under the head chemical constituents of cereals (p. 13). In chapter on dimensions (Ch. II), useful data have been reported systematically. However, use of terms like weight with unit of mass 'g' is unexpected. In chapter on gravimetric properties (Ch. III) rarely available data useful to design engineers have been presented. In the age of 'SI' units, terms like 1000 grain mass would have suited most over 1000 grain weight (pp 65-66). Presentation of linear regression equation for any property (p 77) without mention of validity range for independent variable appears superfluous. In chapter IV, procedural description does not include static and dynamic frictional pro-perties, as against the data on 'coefficient of static friction' (p 92). Near blank data tables (pp 93-97), indicate non-availability of sufficient data, and leave enough scope for redressal. Combination of chapter on aerodynamic and rheological properties could have eliminated 4 or 6 page chapters. Absence of procedures for determination of rheological properties make the chapter less attractive. Unit of hardness or crushing load may have been 'Newtons' instead of kg. Chapter on thermal properties presents useful data. In chapter on hygroscopic properties, the Henderson Equation of the form: $1-Rh=e-CTMe^{n}$ has been printed as : $1-RH=e^{CT}Me^{n}$ (p 137 & 145) and value of 'C' reported 10^{5} times in Table 8.5 (p136). Existence of such is likely to magnify errors in estimations, particularly by non-expert professionals. More abbreviations for same journal reflect on citation of references. Uniformity in presentation could have added lucidity, and lusture to the book. Appendix-I giving botanical names and Hindi names is informative and highly useful, but for listing of botanical names to food products. Few more such examples can be seen. But for some of these shortcomings, the content of the book is eminently valuable, informative, and useful.

Compilations of this type are bound to receive the data with different systems of units from different sources. Conversion of such data preferably to 'SI' units could improve the suitability of the book for ASIAN countries, where more or less similar crops are grown. Expression of views by the authors, at the end of each chapter, in the form of discussion on indicative priorities and research gaps may have drawn the attention of researchers for future attempts, and paved the way for future edition with more data. It is believed that we would

get a detailed future edition from the authors, elaborating all the needed information to match international standards, and complete requirements of professionals.

All in all, this book is a welcome addition to the current spate of publications on crop process/ food engineering and noteworthy contribution in the area of engineering properties and chemical constituents of food crops. Despite its relatively high price, it being the first systematic data source, the reviewer earnestly hopes that it would be widely referred. The book is likely to find a place among process and design engineers, researchers, and crop processing industry people.

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***Food Additive Toxicology:* Edited by Joseph A. Maga and Anthony T. Tu; Published by Marcel Dekker, Inc. N.Y., 1994, pp 552, Price US $175.00/=**

With urbanization and changed life style, food habits are changing and processed food is finding its place in daily meals, even in developing countries like India. Food additives used intentionally or unintentionally have become intricate part of our food supply. The intentional food additives are used to enhance and preserve food qualities, especially nutritive value, sensory qualities, and some may be incorporated as processing aids. The unintentional food additives include compounds used for agricultural efficiency, which may be insecticides, herbicides, plant hormones etc. Different packaging materials may also result in unintentional presence of plasticizers, printing inks, pigments in particularly high fat foods.

In the United States, nearly 2800 compounds are approved as food additives. Most of these compounds are generally recognized as safe (GRAS), and are not expected to show direct toxicity. Further, the amounts used are very small compared to their LD_{50} level. However, the intake of small quantities of numerous food additives over a long period may have some adverse effects on the consumer population. Toxicology and risk factor analysis for long term uses of food additives is not completely studied in human *per se* from a scientific stand point. However, this book has attempted to document the available information on animal models and for human systems for a variety of food additives.

FAO/WHO Expert Committee on Food additives has been giving recommendations on this subject for a number of years, but these reports are limited to only certain food additives. This book is unique, since it has almost covered the whole spectrum of food additives. The book includes classification, manufacture, application and toxicology of each class of food additive. It may be looked up as a compilation of different review articles on a variety of food additives with special reference to toxicology.

The book consists of 11 chapters, contributed by various food scientists, nutrition experts from industry and universities of the United States. Each chapter is devoted to a single class of food additives, such as acidulants, antioxidants, food colours, flouring agents, salts, antimicrobial agents etc. Out of 2800 compounds from the American List of Approved Food Additives, 1300 are flavouring agents. Thus, chapter 6 on flavouring agents is quite big. On the basis of data, perhaps, antioxidants and acidulants contribute significantly, and hence two important chapters (2 and 3) cover these two types of food additives.

To summarize, this book is quite informative, and it will be useful for students, researchers as well as professionals working in food industry. The title is slightly misleading, which gives an impression that toxicology is the main objective. In fact, the format of the book is analogous to an encyclopaedia where a successful attempt has been made to classify, and document major food additives with information on production, application, and special emphasis on toxicology.

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***Solid State Fermentation* : Edited by Ashok Pandey; Published by Wiley Eastern Ltd. New Age International Publishers, New Delhi, 1994, pp. 188, Price:not mentioned.**

The book under review is intended as both a text for fermentation technology, and as a reference for industry professionals. The book is compiled from a course of invited lectures from experts during the "National Level Specialists Group Meeting and Symposium on Solid State Fermentation", held on March 23-24, 1994, at Regional Research Laboratory, Trivandrum, India. The book contains a detailed information on several aspects of Solid State Fermentation (SSF), like present status of SSF, fermenter design, production of food, feed, fuel, enzymes etc., employing SSF, in addition to

the safety aspects of SSF technology.

This book reminds the first ever book on Solid State Fermentations (SSF) 1992, entitled as "Solid Substrate Cultivation" edited by H.W. Doelle, A. Mitchell and C.E. Carlos and E. Rolz, published by Elsevier Applied Science. The book under review may be the second book totally devoted to the theme of SSF. Through a number of contributed papers in this book by CFTRI scientists, their command on the theme of book and technical know-how developed in India can be realized. The credit goes to Dr. B.K. Lonsane, the internationally famous personality, especially for his expertise in SSF technology, and his colleagues, who have contributed 8 Chapters in this book, highlighting very important aspects of SSF technology.

During the last decade, the simple and cost-effective SSF technology has emerged and developed the world over, because the advantages of technology evidently outweigh the problems associated with the process. The technology is of value for the growth of microorganisms due to its familiarity to the growth conditions in nature. Usually, the reactor volume per unit substrate converted remains small, because only a little amount of water is to be added to the substrate, resulting in lower capital as well as operating costs and little space requirement, and less processing is required due to simplicity of reactor. In case of fungi, very low moisture content is adequate to get good yield, eliminating the chances of bacterial contamination. Hence, the process can even be operated under non-sterile environmental conditions. Product recovery is very easy, since the product is highly concentrated in the solid substrate, and there is no need of removal of water at the commencement of fermentation. In some areas, for instance, upgradation of feed value of crop-wastes, SSF offers the best hope for a direct applicability of the processed material.

No doubt, scanty literature is available on industrial biotechnology containing informations on SSF technology, and there are hardly a couple of books in the subject, specifically. Hence, since long, the need was felt to compile a literature on the strategies for technology of research and development on SSF. To meet that requirement, the present book on SSF has been compiled, and sub-divided into five sections like-general; fundamental aspects of SSF; production of food, feed and fuel; production of enzymes and other products by SSF; miscellaneous.

Section one describes the present status and scope of SSF technology for its commercial exploitation of microorganisms in an economic way. Section second comprises five chapters, which refer to the fundamental aspects of SSF in general. Out of these, one chapter describes the optimization of SSF parameters, and two chapters are dealing with design and process control during SSF, the other one provides recent informations on biomass estimation in the fermented products, especially in case of fungal SSF, and the last one reviews the methods involved in determining the water activity in the substrate, an important parameter which directly reveals the quantity of water available to the microorganisms for growth and metabolic purposes. Next section, the third one includes nine chapters on the production of food, feed and fuel using SSF. The first four chapters discuss the significance of SSF in production of foods, like fermented foods of Indian origin, fermented vegetables, mushroom cultivation and cocoa, coffee and vanilla fermentation. The next three chapters describe the upgradation of poor quality feed components, involving SSF techniques such as "Karnal Process", cassava fermentation, and the double stage SSF-ENLAC, a novel bioconversion technology. The remaining two chapters are on the production of fuel employing SSF, the one dealing with liquid i.e., ethanol from apple pomace and the other with gaseous fuel from willow dust. Section four of the book throws light on the production on enzyme and other products like organic acid, and mycotoxins. This section consists of 10 chapters, out of which, the first 8 describe the process optimization of different categories of enzymes like food enzymes, dehairing enzymes, alkaline proteases, cellulase, β-glucosidases, keratinase etc. Between the remaining two, the first one goes to citric acid production, and the last one explains the aflatoxin production using SSF. The fifth and last, the so called miscellaneous section comprising four chapters is mainly dealing with industrial aspects, for instance, the scope of industrial exploitation of cassava, a tuber crop, in India. Apart from this, the information on utilization of marine micro-life is of paramount importance, and the last chapter of this section and book itself describes the safety aspects of SSF.

Overall, the book is an ideal text and one of the few up-to-date volumes available in the SSF technologies. The book is clearly printed, well bound, handy and the figures and tables are extremely clear. Finally, the chapters are well

written by contributors, easy to read, thoroughly referred. The book may prove to be valuable to microbiologists, biochemists, biotechnologists, chemical engineers and industrialists.

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***Supercritical Fluid Processing of Food and Biomaterials:* Edited by S.S.H. Rizvi, Published by Blackie Academic and Professional of Glasgow, U.K., 1994 pp. 257. Price £ 79/-**

This is a publication compiled to assist in understanding the fundamentals of supercritical fluid processing, and their applications in food processing. The volume is based on the papers presented at the symposium on Supercritical fluid processing of biomaterials-basics of process design and applications, organized during the 8th World Congress of Food Science and Technology, held in Toronto during September-October 1991.

The book contains 19 invited papers, by different authors from developed and developing countries, each one discussing an important aspect of supercritical fluid food processing. The first article by SSH Rizvi et al., gives an overview of the fundamentals of supercritical processing. The paper is an introductory type, explaining fundamentals starting from phase equilibrium and solubility principles, and extending through different aspects of heat, and mass transfer upto salient features of scale up and cost analysis. This is a very well written primer to begin with.

There are a number of articles, which are case studies of different applications of the technology. Some applications discussed are, recovery of alpha-tocopherol and removal of water from fatty acid/ triglycride mixtures; extraction of oil from fried potato chips; production of natural antioxidants from rosemary to sage; fractionation of butter oil and removal of cholesterol; separation of volatiles from orange juice-effects on cloud and pectinesterase activity; selective recovery of volatile extractives from black pepper and ginger; evening primrose oil from the seed; separation of organics like ethanol, 2-propanol and certain antibiotics from water solutions; reduction of fat/cholesterol from high fat products such as chicken, cooked beef and dried lean meat; fractionation of beaf tallow, again to reduce cholesterol; extraction of oil from certain seaweeds; and production of low calorie peanuts.

The last mentioned paper on production of low calorie peanuts is a very interesting study. Generally, SCFE is believed to be feasible for high value products. However, the authors have established that it is both technically and economically viable to set up an SCF extraction plant for defatting peanuts for snack food and high grade edible oil purposes.

Two papers of direct interest to engineers are-studies on mass transfer phenomena during production of spice oils, and selection of pumps for supercritical fluid service. In the first article, the authors, using ginger as an unsteady state model, studied the effect of particle size on mass transfer coefficients under different extraction pressures and temperatures.

The paper on selection of pump for supercritical fluid services is of great interest to design engineers. This is one of the rare papers published on the hardware of supercritical extraction technology. The article discusses different types of pumps like diaphragm, packed plunger and piston types, their construction in brief and applications of these pumps under different conditions.

Overall, the book is a very useful reference material for scientists working in the field of supercritical fluid extraction. It helps to some extent to fulfil the gaps of information in this emerging technology.

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***"Basic Biotechnology"* by Rev. Fr. Dr. S. Ignacimuthu, S.J., Entomology Research Institute, Loyola College, Madras and Published by Tata McGraw - Hill Publishing Co. Ltd., New Delhi. 1975, pp 317, Price Rs. 120/-**

The author has included the recent and relevant information regarding various facets of biotechnology in a most lucid manner, and supplemented with beautiful illustrations, wherever necessary.

The book comprises the applications of biotechnology at a glance, in the introduction part followed by eleven chapters which are :

1) Overview, 2) Genetic engineering and gene cloning, 3) Gene transfer mechanism in bacteria, 4) Transfer of gene into plants and animal cells, 5) Plant cell and tissue culture, 6) Animal cell and tissue culture, 7) Agricultural biotechnology, 8) Industrial biotechnology, 9) Healthcare

biotechnology, 10) Environmental biotecnology and 11) Biotechnology and ethics.

The author starts the 1st chapter with all that goes to explain, what is Biotechnology? He traces the history of biotechnological processes to Babylonian period, obviously beginning with the production of alcoholic beverages. He has outlined the various processes of commercial applications with hosts of products and their current economics.

Chapter II is devoted to various steps in genetic engineering, starting from gene cloning, construction of recombinant DNA plasmid and finally transformation of plasmid and growth of cell, describing all the techniques, and processes involved.

All the aspects and methodology of gene transformation and conjugation have been highlighted in chapter III.

Chapters III and IV reveal the information on plant processes, plant tissue culture with necessary details and genetic manipulations in plants.

Various facets of animal tissue culture and DNA hybridization in animals have been aptly elucidated in chapter VI.

Data related to micropropagation for biomass production and development of disease-free plants go under the application of biotechnology in forestry, which forms the earlier half part of chapter VII. While, the later part is devoted to techniques useful in horticulture, specially with reference to development of economic and fruit crops with improved yield together with nutition.

The author has presented a summary of industrially useful products from microorganisms, plants and animals, starting from SCP to human growth harmone (Somatotropin) in the 8th chapter.

Important health care products, common diagnostic tests, metabolic and genetic diseases/ defects, and their correction by gene therapy, forms the subject matter of the 10th Chapter.

Waste treatment, biodegradation processes and bioconversions for fuel, and renewable source of energy have been outlined in the subsequent chapter.

The author has rightly raised the ethical question in the genetic engineering experiments and moral problems involved in the other biotechnological applications. Few words also have been spared for the code of conducts for the scientists associated with this subject.

One wonders to note the vast, introductory and basic information, that has gone into making of this book. It will serve as a guide for all those interested to study biotechnology, in general, and genetic manipulations, in particular.

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***Cell Adhesion Fundamentals and Biotechnological Application*, Edited by Marthin A. Hjortso and Joseph W.Roos; Published by Marcel Dekker Inc., 270, Madison Avenue New York, NY- 10016; 1994, pp 288, Price US $ 135/-**

The book is an excellent treatise on biochemical, biophysical and bioengineering aspects of the biological phenomena "Cell adhesion" in microbial, plant and animal cell line. Basic as well as applied sides of subject are presented with beautiful illustrations, latest experimental data or research evidences together with exhaustive bibliography. The present book is the outcome of concerted editing efforts of W.C. McGregor, as a part of bioprocess technology series, brought out by Marcel Dekker, Inc.

The first chapter includes the kinetics of ligand-receptor bond formation, such as rate constant for complex formation and ligand-receptor binding, diffusion coefficient and *in vivo* and *in vitro* methods of their estimation. Effect of equilibrium, density, membrane composition, forces on adhered cells have also been highlighted.

Mathematical modelling with regard to single cells, segregated cell population on the basis of kinetics of ligand receptor bond formation have been proposed. Several models have been suggested. Various events taking place during cell adhesion are illustrated, and fluid mechanics to as seen in the phenomena have been described in the second chapter.

First two chapters are contributed jointly by the editors of the book. Mechanism of cell adhesion its biological implications and physiological changes resulting from it, are well elucidated in the third chapter, using animal cell culture as a model. Tissue engineering of bone marrow culture forms the speciality of this chapter. Efforts of M.R. Koler and E.T. Papoutsakis as the contributors of this chapter needs special mention.

Jean Archambaut, who compiled the chapter IV has done a commendable job. The chapter is devoted to the commercial exploitation of plant cell culture. The scale up of plant cell culture, various

techniques of plant cell immobilization, details of SPIC culture engineering, and processes based on it have been explained well.

Cell aggregation and sedimentation phenomena have been appropriately stuffed in the preceding chapter by Robert Davis. An excellent account of various factors affecting cell aggregation, its measurement, sedimentation velocities, and applications in beer fermentation, recombinant bacterial fermentation, SCP production together with activated sludge process are covered in this chapter.

B.M. Peyton and W.G. Characklis have aptly summarised the various aspects of biofilms, their role in industrial, environmental processes and types of biofilm reactors with self explanatory photographs, figures and schematic diagrams in the 6th chapter.

The concluding chapter in the book with masterly writing by William Scouten encompasses a variety of matrices, and a number of methods of their, activation. It has also enlisted selected commercial sources of materials and examples of use of affinity chromatography for clinically important products.

The book is extremely useful to the graduate students of chemical engineering, biotechnology, P.G. students and researchers in the subjects like microbiology, biochemistry, horticulture, pharmaceutical sciences, biophysics, tissue culture, genetics, botany and zoology. It opens up new vistas for personnel in research and development laboratories and plants (works) in various industrial establishments.

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