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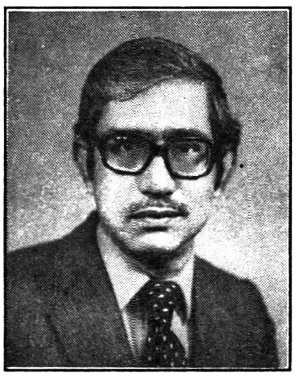

Dr. Susanta K. Roy, Principal Scientist/Project Coordinator, (Post - harvest Technology), Indian Agricultural Research Institute, New Delhi-110 012, India, has been elected by the members of the Association, with a thumping majority, for the Honorary Post of the President of Association of Food Scientists and Technologists (India), Mysore, for the period 1993-94. Accordingly, he has taken the helms of the Association into his hands from September 12, 1993 at the grand function held at the end of IFCON-93. On behalf of the Association and as a member of earlier and present Central Executive Council of the Association, I have the privilege to briefly introduce him to the readers of the Journal of Food Science and Technology.

Dr. Susanta Roy, born on October 16, 1939 in Calcutta, had his Bachelor's Degree in Agriculture from Calcutta University, Calcutta, in 1968, and Master's Degree in Food Technology (Assoc. CFTRI, Mysore) in 1963. He worked on "Storage & Processing of *Bael* Fruit" for his Ph.D degree, while at IARI and submitted his thesis to Bidhan Chandra Krishi Viswavidyalaya, Kalyani, in 1975. Subsequently, he underwent advanced training on Post-harvest Technology of Fruits, Vegetables and Root Crops at the Tropical Products Institute, London, in 1980. He was at the United States Department of Agriculture, Washington, D.C., in 1990 to receive training in the Management of Agricultural Research.

He has a distinguished record of serving in organizations of high repute. He has worked in the Research Department of the Metal Box Co., Calcutta, and then he moved to the Institute of Catering Technology and Applied Nutrition, Calcutta, for teaching and research assignment of the duration of about 3 1/2 years. For the past 25 years, he has been working at IARI, New Delhi, in various capacities, viz., Assistant Professor, Senior Scientist, Principal Scientist/ Project Coordinator and Head of PHT Unit. Thus, he has over 30 years of research and teaching experience in the field of Food Science and Technology in general, and Post-harvest Technology of Fruits and Vegetables, in particular. During this period, devoted to Science and Technology of Food, he has published over 120 papers in National and International journals of high repute. He has already guided 12 Ph.D. and two M.Sc. students, and is currently guiding three Ph.D. students.

Dr. Susanta Roy has widely travelled abroad; U.K. in 1980 and USA in 1986 and 1990. The first visit to USA, as a Member of a Management Team, was useful in interacting with various Universities and Institutions in the U.S.A. In September 1989, he was invited to attend the Third International Mango Symposium at Darwin, Australia. In October 1989, he visited Singapore to take stock of the recent developments in Post-harvest Technology of Fruits, Vegetables and Flowers.

Dr. Susanta Roy has been the recipient of three prestigious awards : a) Kejriwal Award given by AIFPA, b) N.N. Mohan Memorial Award given by AIFPA and c) Laljee Godhoo Smarak Nidhi Award given by the Association of Food Scientists and Technologists (India). Also, he is a member of the Scientific Panel of PHT of ICAR, Sub-committee of BIS and Editorial Boards of 'Journal of Food Science and Technology' as well as 'Indian Food Packer'. He has served as an External Examiner of Ph.D. of many Universities and as a Selection Committee member of many Universities and Research Institutions as well as the Union Public Service Commission.

On behalf of AFST (I), it is my honour to welcome Dr. Susanta Roy and the newly elected Central Executive Council Members (as specified on the earlier page). I wish all success to this talented and dedicated team in taking AFST (I) to greater heights.

B.K. Lonsane
Editor,
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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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Methods for Determining Bacterial Populations in Foods and Food Products : A Critical Evaluation

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Bacterial population in food may cause foodborne infections on consumption or spoilage of food, if stored under adverse environmental conditions. Therefore, it is of paramount importance to estimate their population in foods. There are several methods for determining the bacterial population in foods, each one having its own advantages and disadvantages. Rapid method to estimate microbial load in food and food products assumes critical importance from the view point of requirement of food industry and the consumers. This review critically analyses the methods for estimating bacterial populations in foods and discusses the rapid methods for food quality. The advantages and limitations of the methods are also delineated.

Keywords : Bacteriological population, Rapid methods, Estimation of microbes, Food quality, Shelf-life, Advantages and limitations of the methods.

Introduction

Safe microbiological quality of foods refers to the state of food and food products, which are free from pathogenic bacteria and have lower threshold number of those bacteria which are unlikely to cause spoilage of food at a given storage temperature (Elliott and Michener 1961). While the detection of pathogenic bacteria in food helps in controlling foodborne infections, the estimation of level of bacterial contamination in food allows assessing the shelf-life of food and mode of storage. The former is important from health point of view and the latter from the economic point of view, because the spoilage of food in a food processing factory results in great economic loss (Veeraraju and Rangarao 1990; Hays and Reister 1952). Many factors contribute to the bacterial load of food and food products (Jay 1974, 1987). Bacteriological quality of raw materials, processing environments, packaging conditions and subsequent storage environment are known to significantly influence the bacteriological quality of finished products (Sabbir and Sankaran 1990).

The internal parts of the healthy plants and animals are sterile (Jay 1987) and the bacteria in the processed food, therefore, are not inherent to the source of food but to the external environment that contributes microorganisms to the foods. It becomes imperative to somewhat compromise with the external environment, especially on economic considerations. Moreover, it is virtually impossible to process the foods to sterile products without altering the organoleptic changes in many cases. A nearly sterile food often gets contaminated during post-processing steps, such as transportation, storage and handling. It is, therefore, for these reasons that a certain number of microorganisms are permissible in the foods (McCoy 1961; Ingram 1961; Tompkin 1983), as they are unlikely to cause health hazard and spoilage. The total number of viable bacteria in the food is also an adequate index of the bacteriological quality of raw material used, cleanliness in the food processing area in the plant and the storage conditions (Silliker 1963). The food processing industries have to keep the number of microorganisms at or below the permissible levels, under most economic process of production.

A large number of methods for estimation of microorganisms in foods have been reported (Pishawikar et al. 1992; Daeschel and Eid 1992), but have not been evaluated for their comparative reliability. Methods for detection and enumeration of foodborne bacterial pathogens have been extensively reviewed recently, along with evaluation of their reliability, advantages and limitations (Varadaraj 1993). Such a critical evaluation has not been made of the methods for estimating bacterial populations in foods. The present review, therefore, attempts to evaluate available methods to assess the bacteriological criteria for foods.

Sample preparation

For a reliable determination of the total number of bacteria in the given food sample, it is necessary to homogenize the sample and this step is called as sample preparation (Jay and Margitic 1979). Earlier workers used Waring blender for homogenization of food samples (Emswiler et al. 1977). This procedure is adequate for distributing

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the bacteria throughout the sample, but the heat generated during the blending operation is detrimental to the injured and viable bacterial cells (Jay and Margitic 1979). To alleviate this problem, Sharpe and Jackson (1972) have devised less destructive homogenization system, which is called as stomacher. This system involves the pounding of the sample by two peddles in the bag. The food samples in the bag get sheared with mechanical pressure, thereby uniformly distributing the bacteria throughout the sample. It can handle the sample size of 40 to 400 ml. It has several advantages over the Waring blender. For example, the disposable sterile plastic pouches ensure contamination-free sample preparation. Stomacher does not transmit heat to the sample and it has a very low noise level. Further, the prepared sample can be stored in the refrigerator. Another significant advantage of sample preparation by stomacher is the minimum release of reducing agents from meat samples. Blending procedure releases a large amount of reducing substances and such a sample gives false dye reduction test due to non-microbial reducing agents (Holley et al. 1977). Jay and Margitic (1979) have shown that the sample prepared by blending and in stomacher showed the same number of bacteria in the sample.

Revival of injured bacteria

During the processing of food, the bacteria present are subjected to environmental stresses, such as heat during thermal processing and cold during freezing. Some of the cells do survive even after these stresses, but get metabolically injured (Tomlins et al. 1971). These bacteria may not multiply in the selective media, though the normal cells can (Hartsell 1951). Consequently, the presence of metabolically injured cells and their recovery has great significance in estimating the pathogenic as well as spoilage bacteria in the food samples.

Recovery of *Staphylococcus aureus* from pasteurized milk on a medium containing 7% NaCl will be less than that on an enriched medium like trypticase-soy-agar (Hurst et al. 1973). This is mainly due to the inability of injured bacteria to withstand high salt concentrations and multiply (Flowers et al. 1977). Other treatments such as irradiation, freeze-drying, salting, presence of metal ions as well as antibiotics also lead to the metabolic injury of bacteria (Tomlins et al. 1971). Hartsell (1951) has stressed that the use of selective media for recovery of bacteria from the food products should be done with caution, due to the presence of injured bacteria in the sample. Highly nutritive media could recover more salmonellae from frozen food than the selective media like MacConkey and violet red-bile-agar (Hartsell 1951). Metabolically injured bacteria have prolonged lag phase of growth and are sensitive to a number of selective agents used in the media (McDonald et al. 1983). Pyruvate is known to increase the recovery rate of metabolically injured *Staphylococcus aureus* and *E. coli* from food products (Hurst et al. 1976; McDonald et al. 1983).

Sublethally injured *Staphylococcus aureus* are known to lose their NaCl tolerance and cannot grow on media with higher concentrations of NaCl, but can regain NaCl tolerance after restoring of the membrane functions (Flowers et al. 1977). The latter can be achieved by growing such cells in a more nutritious media (Hurst et al. 1976). Milk imparts maximum protection from heat and freeze injury and the components in the milk which provide such adequate shield are phosphate, lactose and casein (Janssen and Busta 1973). Recovery of injured bacteria can also be done by employing tryptose-soy- broth (Indolo and Ordal 1966). Baird-Parker medium is found to be the best for the recovery of *Staphylococcus aureus* from food products (AOAC 1984). Catalase is also known to increase the recovery of injured organisms such as *E. coli, Salmonella, Pseudomonas and Staphylococcus aureus* (Martin et al. 1976).

Heat injured *E. coli* can be recovered by employing 3, 3-thio-di-propionic acid (McDonald et al. 1983). Radiation injury reduces the recovery of microorganisms from foods, so also the injury caused by hypochlorite (Tomlin et al. 1971). Special plating techniques are known to enhance the recovery of microorganisms (Speck et al. 1975). For example, a bottom layer of tryptose-soy-agar is surface-plated with sample and incubated for 2 h at 25°C for recovery of the stressed microorganisms. Over such agar surface, a layer of violet red-bile -agar is then poured (Ordal et al. 1976). In this method, the injured coliforms get recovered easily. The technique can be adopted to other microorganisms employing different selective media (Speck et al. 1975).

Peroxides get accumulated in the metabolically injured cells as the cells are unable to degrade the toxic peroxides (McDonald et al. 1983). Catalase and pyruvate help in the degradation of toxic peroxides and speed up the recovery process (Martin et al. 1976). Use of calcium pectate gel,

in place of agar, also helps in the recovery of injured bacteria from food samples (Fung and Chain 1991). Methoxyl pectin is sensitive to metal ions and gets gellified at room temperature upon exposure to metal ions. Calcium chloride is used as a hardening agent for methoxyl pectin due to the formation of calcium pectate. By this process, the heat shock due to molten agar can be eliminated (Fung and Chain 1991). Further, this procedure can be used in field conditions with minimum laboratory facilities by employing sterile ingredients.

Estimation of anaerobes in foods

Anaerobes vary in oxygen sensitivity and include those, which can grow at low levels of oxygen as well as the methane bacteria, which do not grow above the redox potential of Eh - 330 mV. (Hungate 1969). For strict anaerobes, the cultivation should be carried out under the continuous flow of CO_2, which is passed through a preheated copper filings column to remove the traces of oxygen (Pierson et al. 1974). For intermediate group of organisms, the anaerobic jar, with 90% hydrogen and 10% CO_2 together with cold catalyst, can be used (Barach et al. 1975). Traces of oxygen present in the jar react with H_2 to produce water (Cruickshank 1965). Alternately, the liquid media containing reducing agents and sealed with mineral oil can be used (Hungate 1969). Before inoculation, dissolved oxygen in the broth is removed by keeping the broth in the boiling water for 20 min (Barach et al. 1975).

The food articles likely to support the growth of clostridia are the tinned meat products, which have low redox potential. Raw meat has Eh of -130 mV and the presence of sulphydril groups further help to maintain the low Eh (Lawrie 1966). Some of the canned products with Eh in the range of -18 to 438 mV (Monville and Conway 1982) support the growth of many anaerobes, but the pasteurized milk with redox potential of +230 to +290 mV does not permit the growth of anaerobes. The sensitivity of clostridia to oxygen varies to a large extent and the faulty processing and storage of food may permit rapid multiplication of clostridia without spore formation (Barnes and Ingram 1956). For the isolation of *Clostridia*, the use of reinforced clostridial medium is most ideal and the growth should be observed only after two days of incubation for spores (Bernes 1985). Moreover, the viability of the spores has to be reconfirmed after heat treatment by subculture on a suitable media under anaerobic conditions (Bernes1985).

Spore-forming anaerobes are common in low and medium acid canned foods, which may cause spoilage. *Clostridium thermosaccharolyticum* is the typical organism having strong saccharolytic activity, thereby leading to gaseous swell due to CO_2 and H_2 (Jay 1987). In tropical countries, these organisms are the real threat in food processing units. *Cl. nigrificans*, another thermophilic H_2S producing clostridia, leads to 'sulphur stinker' spoilage of canned foods with or without can bulge (Schmitt 1966). The blackening of the product takes place due to reaction of H_2S with iron salts. Mesophilic toxin producing *Clostridium botulinum* is often encountered in canned foods (Smith 1977). Destruction of the spore of this organism is taken as an index of successful processing of low and medium acid foods (Thatcher et al. 1962). Other putrefactive organisms in canned food are *C. putrificum, C. bifermentans, and C. sporogenes.* Facultative anaerobes such as *Bacillus thermoacidurans and B. polymyxa* also cause spoilage of acid foods (Hays and Reister 1952). Some of these organisms may survive heating in tomato juice at a pH 4.5 for 20 min or more (Fields 1970).

Conventional methods of estimation of bacteria in food

A number of conventional methods for estimating the total number of bacteria are available and these include (1) direct count by microscopy, (2) total viable counts, (3) statistical estimations of most probable number of specific bacteria and (4) dye reduction test based on the ability of viable cells to reduce the dye. The results of these procedures provide an extent of bacterial contamination in the food sample.

Microscopic count: This method has a significant place in dairy industry to assess the microbial load of raw milk (Marth 1978). A known quantity of the sample is spread over a predetermined area on a microscopic slide, fixed, defatted and stained. Under oil immersion objective, the number of bacteria are enumerated (Jay 1987). Direct visual counting of microorganisms in the sample under microscope gives the total number of bacteria, both live and dead (Pettipher 1983).

The method is rapid, and economical (Marth 1978). But the technique is cumbersome, gives fatigue to eyes, and it is not accurate, if the bacteria are present in clumps (Pettipher 1983). The direct count estimates are also higher due to the counting of dead bacteria. The problem of fatigue, due to continuous microscopic observation, has been

overcome by employing image analyser (Jaeggi et al. 1989). A good correlation between the manual and image analyser counts has been reported, as the analysis of variance showed equal results. Thus, the use of image analyser would greatly increase the sensitivity and reproducibility.

Pettipher (1983) and Pettipher et al (1985) have developed and evaluated a rapid direct epifluorescent filtration technique, which is most ideal for liquid foods, like milk and beverages. In this method, the sample is filtered through polycarbonate membrane and the bacteria trapped on the surface of the membrane are stained with fluorescent dye. The membrane is observed under epifluorescent microscope and the fluorescent bacteria as well as the clumps are counted. The sensitivity of the method can be improved by treating the sample with proteolytic enzymes and non-ionic detergents like Triton X-100 (Pettipher 1982). The improved method gives good agreement with viable count technique, if the microbial load in the sample falls in the range of 10^4-10^7 /ml (Pettipher 1983).

In spite of inability to differentiate between the live and dead bacteria, and difficulty in counting, when bacteria are in clumps, the rapidity of the method is the main advantage. Among all the methods, direct microscopic counting is the most rapid technique to estimate the number of bacteria in the food sample (Pettipher et al. 1985).

Total viable counts : The number of viable bacteria in the food can be estimated by pour plate method (Marth 1978). In this method, a known amount of homogenized and serially diluted food sample is mixed with molten agar, at 45°C in a petri dish. Care should be taken to ensure that the injured bacteria are not killed in molten agar, by not using the medium at higher than 45°C. After setting, the plate is incubated at 37°C and the colonies formed are counted. The dilution which gives 200 to 300 colonies per plate is the most ideal for counting and gives accurate result (Marth 1978). In another method, the known quantity of diluted food sample is surface -plated to avoid the heat shock of molten agar and the colonies formed are counted after incubation (Jay 1987). The colonies in the surface-plating method are relatively larger in size and higher in numbers, than those in the pour plate method (Cruickshank 1965), due to differences in oxygen availability in these culturing methods. In pour plate method, the bacteria embedded inside the agar do not develop well due to limited availability of oxygen, as compared to surface colonies which are exposed to higher oxygen concentration (Angeloti and Foster 1958). Further, the pour plate method is not ideal for frozen samples containing psychrotrophs, which are sensitive to heat, and the molten agar may kill significant percentage of bacteria. For such food samples, pour plate method of Fung and Chain (1991) is the ideal method to recover maximum number of bacteria.

Statistical estimation of most probable number of bacteria : The technique is most often used for microbiological analysis of water (WHO 1961). It is a statistical method (Prescott et al. 1946), which is not very precise. This has been discussed extensively by Dutka (1973) to indicate that coliform count of 10/100 ml water actually means that the true value lies between 2 and 23. In another study (Silliker et al. 1979), the estimated value of 10 was found to be 34, with the upper limit of 60. The method involves the dilution of the sample and inoculating it into 15 to 11 tube systems. The number of organisms in the original sample is calculated on the basis of fermented tubes, employing McCrady's table (WHO 1961). It is simple, reproducibile, and the method is of choice for faecal coliform estimation. Specific organisms can be isolated from the fermented tubes. Its major disadvantages are the lack of precision, requirement of large amounts of media and glasswares. In spite of these draw backs, the method is most commonly used in water bacteriology (Manja et al. 1982; WHO 1961).

Dye reduction test : Microorganisms have the ability to reduce the dye and this property is made use of in estimating the bacteria in milk (Garvie and Rowlands 1952). The time required to reduce the dye is inversely proportional to the number of microorganisms in the sample (Holley et al. 1977). The most commonly used dyes are methylene blue and resazurin (Garvie and Rowlands 1952). Supernatant of the properly prepared food sample is added to methylene blue solution and the time taken to decolourize the dye is taken as an index of contamination (Saffle et al. 1961). In case of resazurin, the colour turns from slate blue to pink or white (Garvie and Rowlands 1952). Good correlation between the time taken to reduce the dyes and the number of bacteria present in the sample has been reported (Garvie and Rowlands 1952; Saffle et al. 1961).

Endogenous reducing substances in the raw meat are known to accelerate the dye reduction,

thereby giving the increased estimate of microbes in the sample (Holley et al. 1977). The use of stomacher for sample preparation greatly reduces this problem, since this process releases less of reducing substances (Sharpe and Jackson 1972). Using dye reduction test, Dodsworth and Kempton (1977) could detect 10^7 bacteria/g raw meat within 2 h. To assess the microbiological quality of milk, dye reduction test is routinely used in dairy industry (Garvie and Rowlands 1952). The test is simple, inexpensive, rapid and only the viable cells are capable of reducing the dye (Saffle et al. 1961). However, the ability of the microbes to reduce the dye varies considerably (Jay 1987). The test may give false positive result, if the food sample contains non-microbial reducing agents (Sharpe and Jackson 1972). In spite of these drawbacks, the dye reduction test has its own significance in the rapid analysis of food samples.

Rapid methods for determining bacteriological quality of foods

The conventional methods for microbiological analysis of foods take long time to arrive at the results and require services of a skilled technician as well as a large quantity of media and reagents. Several alternative and indirect techniques to this method are available for estimating microbial load of the food samples, based on biophysical and biochemical principles. Some of these methods detect metabolic products of microorganisms (Manja and Rao 1983), while others rely on small increase in temperature of media during multiplication of bacteria (Shabbir and Sankaran 1991) or an increase in electrical impedance (Eden and Eden 1984). However, for many of the statutory purposes, the conventional viable count continues to be the reference method.

Adenosine triphosphate (ATP) detection method : ATP is unique to living system and its detection indirectly indicates the presence of living organism. The test is based on the principle that the ATP is necessary to oxidize leuciferin with the help of an enzyme called leuciferase from fire fly (Kennedy Jr and Oblinger 1985; Patterson et al. 1970) Oxidized leuciferin emits light and this luminiscence is detected by luminometer. The intensity of luminiscence is proportional to the amount of ATP present in the sample (Kimmich et al. 1975). The quantum of ATP in the sample is an indication of microbial load (Sharpe et al. 1970). The system can be automated and the computer interfaced luminometer consists of a software, printer and an automatic sampler to analyse samples with sensitivity of one organism in 200 ml, as the light produced from the reaction permits the estimation of ATP upto 10^{-12}g. Theoretically, it is possible to detect as low as 200 cells.

The presence of nonmicrobial ATP poses a problem in food samples. This problem can be overcome by degrading nonmicrobial ATP by ATPase, before processing (Thore et al. 1975). Alternately, the microbes can be separated from the food sample and the ATP is estimated (Stannard and Wood 1983). But the success of this method depends on the separation of microorganisms from food, presence of nonmicrobial ATP and the transfer of extracted ATP into the vial of the luminometer (Stannard and Wood 1983). The presence of ATP inherent to food is the critical factor in this procedure. Therefore, the success of ATP detection for the estimation of bacterial load depends strongly on the efficiency of the procedure to separate the bacteria from food or to destroy its intrinsic ATP, before the assay.

The methodology is as follows: The sample is vaccum-filtered, and the filter membrane entrapped with bacteria is placed into a cuvette. The cuvette, once placed inside the luminometer, the ATP releasing agent is automatically added, followed by leuciferin and leuciferase system. The light emitted is measured by photo-multiplier tube and converted by a computer software system into a microbial load for printing on a chart paper (Thore et al. 1975). The Lumae biocounter is routinely being used in the wine, dairy and meat industries.

Radiometric estimation of microorganisms : Microorganisms can utilise ^{14}C labelled substrates and produce $^{14}CO_2$, which can be detected radiometrically. The time taken to detect $^{14}CO_2$ is inversely proportional to the number of organisms present in the sample (Lampi et al. 1974). Head-space $^{14}CO_2$ can be regularly monitored and the positive results can be obtained on the same day, if the sample is heavily contaminated (Rowly et al. 1978). This technique has been used in clinical microbiology and has great potential in food microbiology (Bacharch and Bacharch 1974). In an extensive study (Rowly et al. 1978) on food samples, it was observed that 75% of them were classified as acceptable or unacceptable within 6 h and the false positive result was observed in less than 1% samples. Stewart et al (1980) have modified this technique for the detection of radio-labelled dulcitol. The production of $^{14}CO_2$ could be suppressed by

employing salmonella polyvalent 'H' antiserum. The specific suppression of $^{14}CO_2$ production by antisera is an indication of *Salmonella* in the sample. Use of radio-labelled substrates for screening the food samples for microorganisms is unlikely to cause any radio-active contamination of food products in the production area (Prentice et al. 1989).

Gas chromatographic method : Gas chromatography (GC) has great potential in the detection of microorganisms (Brooks 1983; Brooks et al. 1983), since it is rapid and extremely small amount of material is required for analysis. Bassette and Claydon (1967) have successfully used GC for detection of microorganisms in foods. A rapid gas chromatographic method of detection of *Salmonella* has recently been reported (Manja and Kaul 1987). The test is based on the detection of ethanol as metabolic product from the fermentation of rhamnose and specific suppression of ethanol production by preservative-free *Salmonella* polyvalent H antiserum in parallel cultures. Dulcitol fermentation has been used (Rowely et al. 1978) for the detection of *Salmonella* in foods, but many strains of *Salmonella typhi* do not ferment it. Hence, rhamnose is considered superior to dulcitol. The use of antiserum in this GC technique is a critical step, because the commercial antisera contain cresol or merthiolate as preservatives, which are nonspecific inhibitors (Manja and Kaul 1987). The GC method has also been extended for the detection of a variety of other bacteria (Brooks et al. 1980; Manja 1988; Manja and Rao 1983; Manja and Kaul 1987). Looking at the sensitivity of the equipment and rapidity of the procedure, the gas chromatography method appears to have a promising and prominent role in food microbiology (Manja and Kaul 1987).

Electrical impedance measurement : Microorganisms break the large molecules into small ions and change the resistance of the medium to electrical current (Silverman and Munoz 1979). The time required to detect the measurable change in the resistance is proportional to the number of organisms present. Hence, the change in the electric resistance can be correlated to the microbial concentration in the sample (Eden and Eden 1984). The time required to reach the detectable impedance is the function of initial concentration of microorganisms. This corresponds to the microbial concentration of $10^7 - 10^8$/ml. Based on the methods of Easter and Gibson (1985), an impedance method for the detection of *Salmonella* was compared with the conventional method for milk powder samples (Prentice et al. 1989). In this method, initial growth is obtained on an enriched media, which is followed by the growth on enrichment medium with selenite and trimethylamine oxide. Impedance measurement is made on enrichment medium and the detection of *Salmonella* could be made in 46 h.

The method can be automated and 120 samples can be simultaneously analysed (Eden and Eden 1984). During the period of incubation, the computer regularly screens the samples for change in impedance and the time to reach the detection threshold for impedance change, which is shown on display unit. Ability of the instrument to analyse samples in large numbers simultaneously, continuous monitoring of data, and minimum sample preparation, makes the technique unique (Gnan and Leudecke 1982). Impedimetric methods have been employed in food microbiology for the rapid estimation of total viable organisms (Bulte and Reuter 1984). Hardy et al (1977), while estimating the microorganisms in frozen vegetables, reported that 10^5 microorganisms/g vegetables could be detected in 4.5 h. Shorter detection time indicates that the sample has more than 10^5 organisms. Main drawback of the technique is the construction of calibration curve for each type of product (Firstenbug-Eden and Zindulis 1984). Optimization of growth condition is also critical in this method. Moreover, the more the dilution of the sample, the more will be the dilution of toxic substances. The technique can also be used in food microbiology for the rapid estimation of microorganisms in starter cultures for fermentation and also for the assay of vitamins and antibiotics (Firstenberg-Eden and Zindulis 1984).

Microcolorimetric estimation : During growth of microorganisms, a small amount of heat that is produced can be measured by using a highly sensitive microcolorimeter (Lampi et al. 1974). The technique is based on the correlation of the resulting thermogram with the absolute number of microorganisms (Forrest 1972). After establishing the reference thermogram, it is possible to extrapolate the data with the unknown sample. Microorganisms in milk, meat, molasses and canned foods were quantified by using microcolorimetry (Gram and Soggard 1985). Using thermal activity monitor (LKB Instruments), Shabbir and Sankaran (1991) have estimated *Staphylococcus* growth at different concentrations of sorbic acid. The instrument consists of four sample measuring cylinders, immersed in a constant water bath. Heat generated during microbial growth is detected by

the thermopiles and converted into electrical signals, which are amplified and recorded on a potentiometer recorder or fed to a computer (Lampi et al. 1974).

Immunological methods : With high specificity and sensitivity of antigen-antibody reaction, immunochemical techniques represent highly useful tool for the routine microbiological analysis of food. The promising immunological techniques for bacterial detection in food are enzyme-linked immuno-sorbent assay (ELISA), radio-immuno-assay (RIA) and reversed passive agglutination assay (Rogemond et al. 1991; Manja and Kaul 1990; Van Vunakis 1980). Reverse passive agglutination test involves the use of polystyrene latex or red blood cells, as inert carrier of antibody. If the antigens of the bacteria are present in the food sample, the coated particles get agglutinated (Finkelstein and Young 1983). This method is rapid, inexpensive and does not need any special skill (Hadfield et al. 1987; Manja and Kaul 1990). Limitation of this method is that only one antigen can be coated at a time (Silverman et al. 1968). With the improvement in the technology, it is now possible to use coloured latex particles and different antigens can be coated on different coloured latex (Ibrahim and Fleet 1985). Depending on the colour of the agglutination, it is possible to specify the antigen present in the sample. Mixed colour agglutination indicates the presence of more than one antigen (Ibrahim and Fleet 1985).

Radio-immuno assay (RIA) is based on the principle of competitive binding, in which the isotopically labelled antigen competes for receptor binding sites on the antibody with unlabelled antigen present in the sample (Johnson et al. 1971). Johnson et al (1973) used RIA for the detection of staphylococcal enterotoxins. RIA is used more often for the detection of fungal toxins, since the quantity of toxin present in the sample is very small (El-Nakib et al. 1981) and the method is very sensitive.

ELISA is an enzyme assay in which antigen or antibody is coated on a polystyrene plate and allowed to react with enzyme-tagged antibody or antigen for assaying the bound enzyme, using a chromogenic substrate (Clark and Engvall 1980; Manja 1990). It is being extensively used for the detection of *Salmonella* in foods (Rowly et al. 1978). The commercial kits for the detection of *Salmonella* are also being accepted for statutory purpose (Flowers et al. 1986). Immunological assays can be the powerful and useful tools for the detection of microorganisms and their byproducts. The disadvantage of the method is that it does not differentiate between the viable and non-viable cells. But the technique is rapid, accurate and needs less reagents and facilities (Clark and Engvall 1980). The sensitivity and specificity of the immunological methods can be further improved by the use of monoclonal antibodies and it is possible to complete the analysis within a period of 1 h (Meyer et al. 1984). Latex agglutination tests are extremely simple and have great potential in food microbiology (Finkelstein and Young 1983).

Obvious advantage of immunological assay is that it can be used for large scale screening of food samples (Freed et al. 1982). Further, it is easier and faster to detect slow growing organisms in food by immunological method than by the conventional methods, specially in the cold-stored food samples having psychrotrophs (Manafi and Sommer 1992). When compared to conventional methods, immunological methods offer similar detection limits and confidence levels (Meyer et al. 1984). Further, the sample preparation is simpler, requiring no elaborate extraction procedures for metabolites or isolation of organisms (Stiffer and Fey 1978). However, it becomes necessary to analyse a large number of food samples to find out the presence of cross-reacting antigens that may interfere with the reaction.

Limulus amoebocyte assay : The endotoxins of Gram negative bacteria have the ability to gellify the lysates of amoebocytes of horseshoe crab (Jorgenson and Smith 1973). In this test, endotoxins are extracted from the bacteria present in the foods by mixing the sample with pyrogen-free distilled water. Serial dilutions of the extracts are mixed with known quantities of amoebocyte lysate and incubated at 37°C for 1 h. Formation of clot is the end point of endotoxin presence and the titre is recorded as the reciprocal of the highest dilution forming a clear gel (Jay 1974). The technique has also been used to estimate the shelf-life of pasteurized milk (Byrne and Bishop 1990), based on the microbial load present in the milk. In this study, the limulus amoebocyte assay was compared with direct epifluorescent filter technique for psychrotrophic bacteria count. Each milk sample was evaluated before and after the incubations, while milk alone and milk with benzalkolium chloride were employed. The technique could be useful as a rapid method of shelf-life estimation.

Polymerase chain reaction : The technique involves the rapid amplification of target DNA *in vitro* and identification of the amplified DNA, using

suitable DNA probe (Wernars et al. 1991). DNA polymerase of *Thermus aquaticus* can be used to amplify DNA fragments to millions in 3 to 4 h by heating and cooling cycles in quick succession. The technique has been used extensively for detection of variety of bacteria in foods (Wernars et al. 1991; Brooks et al. 1992). Recently, new user friendly DNA probe assays have been developed, which eliminate the use of radioactive probes (Groody 1991). Non-radioactive DNA probes have also emerged for the safer use of DNA fragments (Manja 1990). The DNA of unknown bacteria present in the food is denatured at 94°C and hybridized with the primers at 37°C, in the presence of thermostable DNA polymerase and deoxyribonucleotide phosphate. Then at 75°C, polymerase synthesizes the new complementary strand of DNA. In the repeated cycles of heating and cooling, double the amount of DNA is synthesized (Brooks et al. 1992). The technique is much faster than the natural multiplication of bacteria, hence the advantage of the method for rapid detection of bacteria (Toranzos and Alvarez 1992). One great disadvantage of this method, in using it for foods, is the presence of large amount of non-microbial DNA, which might cross-react (Fits 1985). Since it uses the amplification principle, even a small contamination may amplify. Further, the test only detects the DNA and not necessarily from the living cells (Wernars et al. 1991). Now, the newly synthesized oligonucleotide probes, coding for heat labile and heat stable enterotoxins of *E. coli*, have been successfully employed for the detection (Wachmuth et al. 1991). Polymerase chain reaction and DNA probes revolutionise the detection methods for enteric pathogens.

Conclusion

In recent years, a large number of rapid methods for microbial detection have emerged and each one is having its advantages and disadvantages. These methods have not yet taken over the conventional colony count method completely. The statutory food microbiologists and also the conventional microbiologists have not yet realised the significance of these rapid methods. This has been critically put forth by Sharpe (1979). He questioned the obsession of food microbiologists for total counts, which has retarded the development of more rational and realistic technologies for the changing needs of the consumer. In the total count methods, one often determines the cluster of bacteria and it is not a true viable count, but cluster count of bacteria. It is now vitally essential to use the newer and rapid methods of microbial estimation in food microbiology and follow the changing trends in science. These methods should ultimately replace the present day labour and reagent intensive microbial estimations.

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Studies on the Changes in Peroxidase Levels in Cluster Bean (*Cyamopsis tetragonoloba*) and Cowpea (*Vigna unguiculata L.*) During Development

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Peroxidase activity and protein content in the crude as well as acetone precipitate extracts, from cluster bean and cowpea, during eight stages of development were studied, along with average weight and moisture content. Peroxidase activity increased upto the stage of maturity, followed by a large decline till the stage of senescence. The soluble proteins first declined, then started increasing and reached the peak at the senescent stage. The isoenzyme patterns of peroxidase, during development of both the vegetables, showed varying intensities. Cluster bean peroxidase possessed two isoenzyme bands throughout the development and the cowpea showed five bands initially and a sixth band appeared at maturity stage.

Keywords: Peroxidase activity, Isoenzyme patterns, Soluble protein content, Cluster bean, Cowpea, Development.

Many chemical and physical changes, occurring during the ripening of fruits, are attributed to enzyme action (Haard 1977). There is a rapid increase in the activity of several enzymes during maturity and ripening of several fruits, such as mango, apple, peach, pear and banana (Mattoo et al. 1969; Kunive 1969; Flurkey and Jen 1978; Ranadive and Haard 1972; Haard 1973). Further, isoenzyme patterns of some of the enzymes are reported to change during fruit maturation, while some isoenzymes disappear and some others appear at various stages of ripening, thereby suggesting a role of isoenzymes in the ripening process (Bonisolli and Gorin 1977; Gorin and Heidema 1976). Changes in the isoenzyme patterns of peroxidase enzymes have been reported to occur with the climacteric rise in pear (Ranadive and Haard 1972) and tomato (Frenkel 1972). Bonisolli and Gorin (1977) have determined the isoenzyme patterns of peroxidase and correlated the maximum activities with appearance of new isoenzymes. Gorin and Heidema (1976), while studying the apple stored under controlled atmosphere, have shown two peaks in the isoenzyme patterns, one corresponding to climacteric and the other to the start of senescence; one of the enzyme fractions representing the phase of ripening only.

Although studies on the peroxidase enzyme activity, protein content and the role of isoenzyme patterns during ripening in fruits are reported, not much information is available on these factors in vegetables during development. It was, therefore, considered worthwhile to study these aspects in two vegetables, cluster bean and cowpea, which are known to possess high concentrations of peroxidase (Ramanuja et al. 1988).

Materials and Methods

Cluster bean (*Cyamopsis tetragonoloba*) and cowpea (*Vigna unguiculata* L.) seeds were procured from local horticulture department. Solvents and chemicals used were of analytical grade. The seeds were sown in the premises of the laboratory. The first picking of the beans was done after 50 and 70 days for cluster bean and cowpea, respectively. They were harvested periodically, starting from the pod setting (initial) stage upto the senescent stage, which reached after 130 and 140 days in cluster bean and cowpea, respectively. Both the seed samples and crude as well as acetone precipitated extract were analyzed at eight stages of development-pod setting (initial), pod setting (early), pod setting (late), pod filling (early), pod filling (late), mature, overmature and senescence.

The average weight of ten representative samples from each vegetable was recorded after every harvest. Moisture was determined by the AOAC (1960) method. Protein concentration in the extract was measured by the method of Lowry et al (1951), using crystalline bovine serum albumin as standard. Extraction of peroxidase, assay of enzyme activity and partial purification of crude enzyme extract were performed as reported earlier (Ramanuja et al. 1988).

Polyacrylamide gel electrophoresis : It was carried out by the method of Davis (1964), using glass tube of 5 mm inner diameter, which was filled to 8 cm with 7.5% polyacrylamide in 0.375 M Tris-

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HCl buffer (pH 8.9) as the resolving gel (Gabriel 1971). Tris-glycine buffer (10 mM), at pH 8.3, was used as the electrode buffer. The electrophoresis was run for 3 h by applying a current of 4 mA per tube. The gels were stained for peroxidase activity by equilibrating with 0.5% benzidine dihydrochloride in 10 ml glacial acetic acid in test tubes for 30 min and then immersing in a solution of 0.14% hydrogen peroxide for 10-30 min. The colour of the stained bands was fixed and the clarity of bands was increased by treating the gels subsequently with 10% acetic acid for 10 min, followed by storing in 30% aqueous ethanol.

Results and Discussion

Changes in physico-chemical parameters : Average weight in both cluster bean and cowpea increased with the advancement of the stage of maturity (Table 1). However, a small decline occurred, when these reached the senescent stage. The moisture content showed a steady decrease, setting (initial) stage upto the stage of maturity. This was followed by a large decline, till the stage of senescence. The increase was found to be about 3 times in both the vegetables at the mature stage, as compared to the pod setting (initial) stage. The protein content of the crude as well as acetone precipitate extract from cluster bean and cowpea first declined, then started increasing, and reached the peak at the senescent stage. It was found to be about one and a half times more at the mature stage as compared to the pod setting (initial) stage. The peaking of both peroxidase activity and total solids content at the mature stage indicated the most active growing period. Ku et al (1970) have shown that total activity of peroxidase enzyme in tomato increased from 1.1 to 8.2 units from pre- to post-climacteric, whereas in the present study, the activity of peroxidase in crude extract increased from 42 to 133 units/g in cluster bean and from 16 to 48 units/g in cowpea, from pod setting (initial) to mature stage.

TABLE 1. CHANGES IN PEROXIDASE ACTIVITY AND OTHER PARAMETERS IN CLUSTER BEAN AND COWPEA DURING DEVELOPMENT.

		Cluster bean						Cowpea					
				Crude extract		Acetone ppt.				Crude extract		Acetone ppt.	
Day of picking	Stage of development	Moisture, %	Average wt. g	Peroxidase U/g	Protein, mg/ml	Peroxidase U/g	Protein, mg/ml	Moisture, %	Average wt. g	Peroxidase U/g	Protein, mg/ml	Peroxidase U/g	Protein, mg/ml
10	Pod setting (initial)	85.5	0.6	42.0	7.4	67.0	3.0	84.8	1.3	16.0	3.5	28.0	1.6
20	Pod setting (early)	84.2	0.9	45.0	6.4	67.0	2.9	84.1	2.8	21.0	2.5	30.0	1.0
30	Pod setting (late)	83.2	1.3	60.0	5.9	83.0	2.6	78.1	3.3	28.0	2.5	38.0	0.7
40	Pod filling (early)	82.5	1.8	64.0	5.4	94.0	4.3	79.0	3.3	28.0	4.0	47.0	1.5
50	Pod filling (late)	78.0	2.0	93.0	9.1	152.0	7.4	76.1	4.0	38.0	5.3	76.0	1.7
60	Mature	76.2	2.0	133.0	9.9	213.0	7.4	64.0	5.0	48.0	5.8	80.0	1.9
70	Over-mature	74.0	1.8	100.0	10.0	150.0	8.0	62.0	4.5	37.0	6.2	62.0	2.1
80	Senescence	74.0	1.5	60.0	10.6	82.0	8.5	62.0	4.0	25.0	6.2	47.0	2.5

as the beans developed from the pod setting (initial) stage to the mature stage, and remained at a constant level of 74% in cluster bean and 62% in cowpea. The total solid content, consequently, was in the range of 14.5-26% in cluster bean and 15.2-38% in cowpea.

The peroxidase activity, in both crude extract as well as acetone precipitate, increased from pod

Bailey and McHargue (1943) found that peroxidase activity in tomato fruits and leaves increased with increasing maturity and decreased with the onset of senescence. Same pattern was also observed in the present study. Prabha and Patwardhan (1986) have reported that the peroxidase activity may serve as an index of ripening in fruits. Haard (1977) has reported a rise in the activity of

peroxidase during climacteric in ripening flesh of apples, banana, grapes and pear. The observations in the present investigation on the peroxidase activity, reaching a peak at the time of maturity in these vegetables, are in agreement with the above trend and support the above views.

Isoenzyme pattern : Figs. 1 and 2 show the isoenzyme patterns during development in cowpea and cluster bean, respectively. Cowpea peroxidase separated into a maximum of 6 isoenzyme bands (Fig. 1). In cowpea, the fastest moving band F of the isoenzyme pattern decreased in intensity at the pod filling stage and thereafter, the intensity was consistent throughout the development (Fig.1). Though the bands B and C got smudged and diffused in the pod setting (initial) stage, all the other bands were generally sharp and clear, during the development and senescent stages. Bands A to C persisted with almost same intensity from the pod setting (initial) stage to the senescent stage. The band D showed higher intensity after pod filling and its intensity remained same thereafter. One more band of low intensity, i.e. band E, appeared at the mature stage and remained upto the senescent stage.

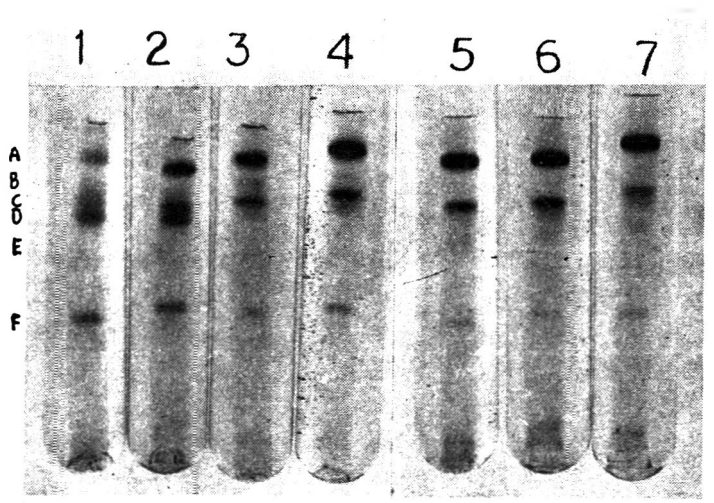

Fig. 1. PAGE pattern of crude cowpea peroxidase during development
1 - Pod setting (initial) 2 - Pod setting (early), 3 - Pod setting (late), 4 - Pod filling (early), 5 - Pod filling (late), 6 - Mature, 7 - Senescence.

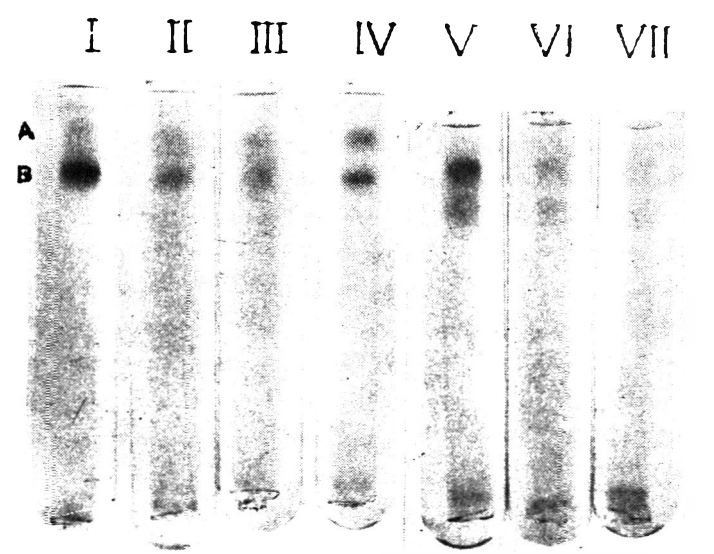

Fig. 2. PAGE pattern of crude cluster bean peroxidase during development
1 - Pod setting (initial) 2 - Pod setting (early), 3 - Pod setting (late), 4 - Pod filling (early), 5 - Pod filling (late) 6 - Mature, 7 - Senescence.

In cluster bean, the bands A and B were seen throughout consistently (Fig. 2). It possessed a maximum of two bands, right from the pod setting (initial) stage to the senescent stage. But, the intensities of the two bands varied as the vegetable developed. The high intensity band reduced in intensity gradually, while the low intensity band increased in intensity, as the vegetable developed. The intensities of the bands, however, reduced after maturity and remained constant, until the senescent stage.

According to Prabha and Patwardhan (1986), the enhanced peroxidase activity, observed at the climacteric stage in peel and pulp of all the fruits studied, could be associated with the appearance of new isoenzyme bands as well as intensification of the already existing ones. Similar results have been reported by Frenkel (1972) in case of pear, tomato and blue berry fruits during ripening. In the present study, one additional band was observed in cowpea at the stage of maturity, thereby suggesting a role of isoenzymes in the maturity process. In cluster bean, decrease in peroxidase activity after maturity coincided with reduced intensity of the isoenzyme bands. Similar results have been reported by Bonisolli and Gorin (1977) and Gorin and Heidema (1976) in apples.

In cowpea peroxidase, initially, there were five bands and the sixth band appeared at the mature stage, whereas in cluster bean peroxidase, there were only two bands and no additional band appeared during development, although the intensities of the bands varied at different stages of development.

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Effect of Common Salt Substitution on the Dough Characteristics and Bread Quality

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Effect of substituting common salt (sodium chloride) with *sendha* (rock salt) or low sodium containing salt mixtures on the quality of wheat flour dough and bread was studied. In general, the common salt substitutes reduced water absorption capacity, but increased dough development time, stability, extensibility, resistance to extension and stiffness of the dough. The extent of changes with different common salt substitutes was almost similar and comparable to the dough made with common salt. Incorporation of common salt substitutes improved the loaf volume, crust colour, and crumb characteristics of the bread to the same extent as the common salt. However, only *sendha* yielded bread which had comparable taste and overall quality to that made with common salt.

Keywords : Common salt substitutes, Low sodium salts, Dough characteristics, Bread quality, *Sendha* (rock salt)

Common salt (NaCl) is an essential ingredient in bread and is used as a flavour enhancer and dough stabilizer (Salovaara 1982). Consumption of sodium chloride in excess has been associated with hypertension in certain sensitive individuals (Gillette 1985) and low sodium intake has been recommended for such individuals (Ndabikunze and Lahtinen 1989). Studies have been reported on the possibilities of replacing part of common salt with potassium or magnesium chloride in bread (Salovaara 1982; Guy 1986). Though no serious adverse effect was found on the dough characteristics or loaf quality, a slight astringent mouthfeel was, however, felt when 50% of common salt was replaced with potassium or magnesium chloride (Salovaara 1982; Stroh et al. 1985).

Several salt substitutes, such as *sendha* (rock salt) or the blends of different salts, containing low amounts of sodium, are now commercially available in India. This work was carried out to study the effect of such common salt substitutes on the rheological and bread making qualities of commercial flour, made from wheat grown in India.

Materials and Methods

A commercially available refined wheat flour (*maida*) was obtained from the local market. Reagent grade sodium chloride, potassium chloride, and magnesium chloride were obtained from S. D. Fine Chemicals Ltd., Boisar. Commercially available low sodium containing salt mixture, common salt and *sendha* were procured from the local market. One g of commercial low sodium salt mixture contained 135 mg sodium and 340 mg each of potassium and magnesium carbonates. Rock salt had 315 mg sodium and 187 mg potassium per g. Common salt contained 400 mg sodium/g.

Chemical characteristics : Estimations of moisture, gluten, total ash, Zeleny sedimentation value, and Kent-Jones colour grade value of the flour were carried out according to standard AACC (1969) methods. Dough raising capacity of flour, containing different salts, was measured by ISI (1972) method.

Rheological characteristics : Farinograph and extensograph characteristics of dough, containing different salts, were assessed according to standard methods (AACC 1969; Venkateswara Rao and Haridas Rao 1993). Texture profile of the dough was assessed using General Foods Texturometer (Tanaka 1975) under the following conditions : voltage 1 V, clearance 2 mm. Amylograph characteristics of flour were determined as per the AACC (1969) procedure, using 80 g flour.

Baking characteristics : Breads were prepared by standard 'Remix' baking test (Irvine and McMullan 1960). Fat (1%) was also included in the formulations. Breads were assessed for loaf volume by rapeseed displacement method (Malloock and Cook 1930). Evaluation of crust and crumb characteristics was carried out by a panel of six trained judges. All the tests were carried out in triplicate and average values are reported.

Statistical evaluation : The data were subjected to statistical analysis by Duncan's new multiple range test (Duncan 1957).

Results and Discussion

The flour used in the present study had the

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following quality characteristics (expressed on 14% moisture basis) : dry gluten 10.86%, sedimentation value 22 ml, total ash 0.47% and colour grade value 3.2. Reasonably high value of gluten as well as sedimentation value and low ash content in flour, indicated its suitability for bread making.

Effect of salts on the dough raising capacity of flour : Effect of different salts on the dough raising capacity of flour is shown in Table 1. At the end of 60 min, common salt had a severe effect on the dough raising capacity of flour, as it decreased from 96.6% for control to 75.9%. KCl and the commercial salt mixture had the minimal effects on the dough raising capacity. Effect of *sendha* was slightly more than that of the above two compounds. The decrease in the dough raising capacity with the addition of salts was probably due to their toughening effect on the dough (Hylnka 1962), which, in turn, affected the expansion of the dough.

TABLE 1. EFFECT OF SALTS ON DOUGH RAISING CAPACITY

Time (min)	Control (without salt)	Salt used				
		Common salt, 1%	KCl, 1%	0.5 NaCl +0.5 KCl	Commercial salt mixture, 1%	*Sendha* 1%
		Dough volume, ml				
0	145	145	145	145	145	145
15	170	160	165	165	170	170
30	215	195	200	200	210	205
45	270	235	250	250	260	260
60	285	255	275	265	275	270
% Dough raising capacity	96.6	75.9	89.7	82.8	89.7	86.2

Effect of salts on rheological characteristics of dough : The farinograph water absorption (FWA) of the flour decreased to different extents, with the addition of various salts (Table 2). Greater effect was, however, found with the use of common salt. At 2% level, common salt decreased the water absorption from 59 to 56%. When part of the common salt was replaced with 50 to 75% of either KCl or $MgCl_2$, only a marginal decrease in the FWA was observed. Similar results were earlier reported by several other workers (Galal et al. 1978; Salovaara 1982; Guy 1986). Galal et al. (1978) explained this effect of common salt, based on the increase in the amount of free water in the system, thereby altering the gluten structure in such a way that the salt occupied the sites which were once occupied by the bound water. Among the various other salts, *sendha* had the minimal effect on FWA, which decreased to 58.0 from 59.0%.

All the salts, at 2% level, increased the development time of the dough (Table 2). Minimal effect was, however, seen with the addition of $MgCl_2$. Addition of different salts, at 2% level, with the exception of $MgCl_2$, increased the dough stability by almost three-fold. Salovaara (1982) also reported similar effect on farinograph characteristics, with the addition of $MgCl_2$. Addition of salts made the dough more elastic. This was also indicated by an increase in the band width of the respective farinograms.

Effect of salts on the extensograph

TABLE 2. EFFECT OF DIFFERENT SALTS ON THE FARINOGRAPH AND EXTENSOGRAPH CHARACTERISTICS OF DOUGH

Salt type	Level	Water absorption, %	Farinograph				Extensograph		
			Elasticity, BU	Dough development time, min	Stability, min	MTI,* BU	Resistance to extension, R, BU	Extensibility, E mm	Ratio figure R/E
Control	0	59.0	115	3.0	5.5	60	150	140	1.07
Common salt	0.5	57.8	130	3.0	9.0	40	–	–	–
	1.0	57.0	135	4.0	11.0	20	325	165	1.97
	1.5	56.4	145	4.0	14.0	20	–	–	–
	2.0	56.0	150	5.0	15.0	20	450	170	2.65
Commerical salt mixture	0.5	58.0	125	4.0	7.0	50	–	–	–
	1.0	57.6	125	5.0	9.0	40	440	160	2.75
	1.5	57.2	130	5.0	12.0	30	–	–	–
	2.0	57.0	140	6.0	14.0	30	775	170	4.56
Sendha	1.0	58.4	145	3.5	9.5	20	310	180	1.70
	2.0	58.0	160	4.0	15.0	40	325	220	1.48
NaCl + KCl	0.50 + 0.50	58.4	145	4.0	13.0	20	300	155	1.94
NaCl + KCl	0.25 + 0.75	58.2	135	4.0	12.0	20	290	160	1.82
NaCl + $MgCl_2$	0.50 + 0.50	57.6	145	6.0	10.0	20	270	150	1.80
NaCl + $MgCl_2$	0.25 + 0.75	57.2	135	5.0	8.0	10	260	160	1.63

* Mixing tolerance index

characteristics is shown in Table 2. All the salts used had a greater effect on the resistance to extension of the dough, than its extensibility. Commercial salt mixture caused maximum resistance to extension, at both the levels added. *Sendha* had maximum effect on the dough extensibility, increasing it to 220 mm from 140 mm at 2% level. Bennett and Ewart (1965) and Tanaka et al (1967) have reported increased resistance as well as extensibility of dough, with increase in the concentration of the salts. Salovaara (1982) observed decreased resistance, and increased extensibility, when NaCl was substituted, at 20 and 40% levels, with chlorides of potassium, calcium and magnesium.

Effect of salts on the hardness, cohesiveness, and stickiness of dough, as measured by using General Foods Texturometer, is shown in Table 3. Hardness of the dough increased with the addition of various salts. These values ranged between 1.3 and 1.7 kg/v, as compared to 1.1 kg/v for the control dough made without any salt. Dough containing the commercial salt mixture had the maximum effect, probably as a result of the combined action of several salts present in the mixture. Cohesiveness of the dough increased wherever common salt was used. NaCl is reported to decrease the intermolecular and intramolecular repulsions by interacting with charged groups on protein molecules, thereby increasing the dough cohesiveness (Bennett and Ewart 1965).

All the salts reduced the stickiness of dough and common salt showed greater effect than the other salts. As the levels of salts increased, their effect on stickiness decreased, probably due to the presence of more free water in the dough system.

Effect of salts on amylograph characteristics of flour : All the salts, in general, lowered the gelatinisation temperature and increased the peak viscosity of flour paste. Gelatinisation temperature of the flour decreased marginally from 60°C to 59°C, when different salts were used at 2% level. Addition of common salt increased the peak viscosity from 2830 AU to 3010 AU, respectively, while the common salt substitutes caused greater increase. Maximum increase in peak viscosity (3330 AU) was

TABLE 3. EFFECT OF SALTS ON TEXTUROMETER CHARACTERISTICS OF DOUGH

Salt type	Level, %	Hardness, kg/V	Cohesiveness	Stickiness, cm
Control	0	1.10	0.80	1.75
Common salt	0.5	1.31	0.87	1.50
	1.0	1.40	0.96	1.20
	2.0	1.55	0.91	1.00
Commercial	0.5	1.51	0.85	1.60
salt mixture	1.0	1.65	0.87	1.50
	2.0	1.68	0.80	1.50
Sendha	1.0	1.45	0.87	1.50
	2.0	1.60	0.78	1.50
NaCl+KCl	0.50 + 0.50	1.34	0.90	1.40
NaCl+KCl	0.25 + 0.75	1.47	0.87	1.60
NaCl+$MgCl_2$	0.50 + 0.50	1.39	0.92	1.60
NaCl+$MgCl_2$	0.25 + 0.75	1.45	0.88	1.70

TABLE 4. QUALITY OF BREAD AS AFFECTED BY DIFFERENT SALTS

Salt type	Level, %	Volume, cc	Specific volume, cc/g	Crust colour*	Cell structure and texture *	Taste*
Control	0	465	3.435^{A}	5.625^{A}	5.00^{A}	3.75^{A}
Common salt	0.5	475	3.590^{B}	7.375^{B}	7.50^{B}	5.38^{B}
	1.0	480	3.595^{B}	9.125^{C}	9.25^{C}	8.75^{C}
	1.5	465	3.405^{A}	7.625^{B}	7.25^{B}	8.38^{C}
	2.0	455	3.392^{A}	5.750^{A}	6.50^{D}	5.38^{B}
SEm	15 df		±0.018	±0.274	±0.18	±0.24
Commercial salt mixture	0.5	495	3.705^{B}	7.250^{B}	7.00^{A}	7.50^{B}
	1.0	490	3.678^{B}	8.875^{C}	8.75^{B}	7.83^{B}
	1.5	480	3.650^{B}	7.250^{B}	7.25^{B}	6.38^{D}
	2.0	460	3.420^{A}	6.125^{A}	6.25^{D}	4.75^{E}
SEm	15 df		±0.022	±0.161	±0.17	±0.20
NaCl + KCl	0.50 + 0.50	485	3.610^{B}	8.250^{B}	8.50^{B}	7.50^{B}
NaCl + KCl	0.25 + 0.75	485	3.565^{B}	8.250^{B}	8.12^{B}	8.25^{C}
NaCl + $MgCl_2$	0.50 + 0.50	500	3.690^{B}	8.375^{B}	8.38^{B}	6.50^{B}
NaCl + $MgCl_2$	0.25 + 0.75	500	3.638^{B}	8.250^{B}	8.25^{B}	7.25^{B}
Sendha	1	470	3.580^{B}	9.115^{C}	8.75^{C}	8.75^{C}
SEm	15 df		±0.020	±0.180	±0.19	±0.20

Names followed by different letters differ significantly ($p < 0.05$)
*Sensory evaluation was carried out on a 9 point Hedonic scale

observed, when $MgCl_2$ was added at 2% level. Increase in peak viscosity could be attributed to suppressed enzymatic activity in the presence of the salt (Galal et al. 1978).

Effect of salts on bread quality : Bread quality, as affected by the various salts, is presented in Table 4. Addition of 1.0% of any type of salt or salt mixtures significantly improved the loaf volume, crust colour, crumb grain, and texture of the bread. However, the use of higher levels of salts (2.0%) yielded bread, with harder texture and darker crust colour.

The extent of improvement in loaf volume was similar in all breads, made with different types of salts or salt mixtures. The crust colour of the bread, made with 1.0% commercial salt mixture or *sendha*, was comparable to that made using 1.0% common salt. However, only the bread, made with 1% *sendha* had comparable crumb characteristics as well as taste to those made with 1.0% common salt. Thus, comparable results were observed in spite of *sendha* containing less sodium (315 mg/g) than common salt (400 mg/g). Presence of higher levels of potassium chloride (more than 2.5%) in commercial salt mixture or the salt blend containing magnesium chloride, imparted undesirable after-taste to the bread.

Conclusion

Common salt is an essential ingredient in bread formulation, as it imparts desirable flavour to bread. In addition, it also strengthens the gluten proteins and controls yeast activity. Therefore, substitutes of NaCl need to have these attributes, without adversely affecting the bread quality. The data showed that *sendha* had these qualities and hence could be satisfactorily used in the preparation of low-sodium breads.

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Histochemical Changes in Mesocarp of Oil Palm (*Elaeis guineensis*) Fruit During Development

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Immature mesocarp cells of oil palm fruit (12 weeks after anthesis) were thin-walled and oval-shaped with intercellular spaces, while mature mesocarp contained densely packed thick-walled polygonal cells, filled with numerous oil bodies (oleosomes), 24 weeks after anthesis. Unlike oilseeds, the oil palm fruit mesocarp contains fat and fibres as the major storage and structural components. Structural components increased slowly during the early stages of fruit development (4 to 16 weeks), followed by a steep increase in the mature stages (20 to 24 weeks). Similarly, the lipid content in the fresh mesocarp increased steeply from 7% at 16th week to 48% by 24th week after anthesis. Proteins and sugar, though minor constituents, also showed a progressive increase.

Keywords : Oil palm fruit, *Elaeis guineensis*, Histochemical changes, Fruit development, Mesocarp constituents.

The oil palm fruit is a drupe with a fleshy fibrous mesocarp that encloses a hard nut. Of the three varieties of oil palm, the 'Tenera' variety has high mesocarp content of 60 to 95%, with a shell thickness of 0.5 to 4.0 mm. Oil palm fruit takes about 6 months for its full development. While palm oil is derived from the mesocarp, the endosperm is the source of palm kernel oil, the ratio of these two oils being approximately 9:1 in the mature fruit (Arumughan et al. 1989). It is also rich in carotene, which is retained well during traditional process of extraction, without damage (Jideani 1992). Studies, reported so far, on oil palm fruit development have been confined to lipid biosynthesis, fatty acid composition and lipid profiles, probably because the lipids are the most abundant constituent of the fruit (Oo et al. 1986; Bafor and Osagie 1988). Other constituents such as structural carbohydrates, proteins and other physiologically active enzymes have not been investigated, in spite of their role in biogenesis of lipids. Recently, histochemical and biochemical evidence for the lipid-lipase association in the developing mesocarp of oil palm fruit has been reported (Mohankumar et al. 1990). This paper reports the histological and quantitative changes in the structural and storage components of mesocarp of the developing oil palm fruit.

Materials and Methods

Inflorescence of 'Tenera' oil palms was randomly selected at anthesis and hand-pollinated in the Central Plantation Crops Research Institute Plantation at Palode. For each developing stage (4,8,12,16 and 24 weeks after anthesis), inflorescence of 5 palms was selected and the fruit samples were collected separately and pooled. The chemical analyses were carried out in duplicate for each stage and the average values were recorded.

Histochemical methods : The fruits were cleaned, surface-sterilised (using 0.5% sodium hypochlorite) and washed in distilled water (Abigor et al. 1985). Small pieces of mesocarp tissue were cut into thin sections of 10-15 μ thickness, using a freezing microtome. Sudan black staining method was adopted for the detection of lipids (Bayliss and Adams 1972). Coomassie brilliant blue and periodic acid-Schiff's reagent were used to detect protein and carbohydrates, respectively (Cawood et al. 1978; Hotchkiss 1948). Lignin was located by Maul's and acid phloro glucinol methods (Faulkner and Kimins 1975).

Analytical methods : Mesocarp moisture content, lipids, proteins, sugars, lignin, pectic substances, hemicellulose, and cellulose were estimated at various stages of fruit development. Moisture was estimated by the oven-drying method (Paquet and Hautfenne 1987), while fresh mesocarp weight was recorded by gravimetry. For lipid estimation, fresh mesocarp tissue was homogenized and extracted with chloroform-methanol, in the ratio of 2:1 (Folch et al. 1957). Protein was estimated from the dried and defatted mesocarp tissue following micro-Kjeldahl method. The fat-free dried mesocarp tissue was refluxed in 80% methanol and the methanol extract was used for the estimation of sugars. The sugars, structural carbohydrates and lignin were estimated by the methods described by Southgate

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(1976). Pectic substances, hemicellulose and cellulose were estimated by gravimetry, following the sequential extraction techniques (Southgate 1976).

For estimation of lignin, fat-free dry mesocarp powder was digested overnight in pepsin-HCl solution (0.5% in 0.1 N HCl). The mixture was filtered and the residue was collected. The residue was washed in hot water and then sequentially treated with various concentrations of H_2SO_4 (w/v) for 2 h (5% at boiling temperature, 72% at 20°C and 3% at boiling temperature). The acid mixture was filtered, using a weighed ashless filter paper and the residue was washed thrice with hot water, dried and weighed. The filter paper was ignited and the ash weighed. The loss in weight was taken as lignin.

Results and Discussion

The mesocarp of mature oil palm fruit (24 weeks after anthesis) contained 48% fat, 18% structural constituents (lignin, hemicellulose, cellulose and pectic substances), 2% proteins, 1% soluble sugars and 30% moisture on fresh fruit basis. Thus, the mature oil palm mesocarp was basically a reservoir of lipids and the supporting structural constituents. In contrast, mature oil palm seeds have fat and proteins as the major constituents, with very little structural components (Hartley 1977).

Histochemical aspects : Oil palm fruit takes about 24 weeks after anthesis to reach full maturity (Hartley 1977). The fruit undergoes several histological and biochemical changes during development. The present studies indicated that the mesocrap cells were thin-walled and oval-shaped, with intercellular spaces during early stages (12 weeks after anthesis). The mature mesocarp (24 weeks after anthesis) contained densely packed thick-walled polygonal cells, filled with number of oil bodies (termed as oleosomes), which, in turn, were distributed in a fibrous matrix. Histochemical localisation of lipids indicated that lipid constituents of the mesocarp started appearing from 16 weeks after anthesis as fine droplets in the middle mesocarp cells, with their distribution advancing to the outer and inner mesocarp, as the fruit development progressed. In mature fruit (24 weeks after anthesis), the lipids were distributed uniformly throughout the mesocarp cells (histological data not presented). Staining for carbohydrates showed that they were confined to cell wall constituents, both in early and mature mesocarp cells. Histochemical studies showed that the lignin got concentrated in the mesocarp fibres, which are known to be conducting strands of the fruit tissue. It is emphasized that the histochemical studies in oil palm fruit have not been reported so far, except for the association of lipase and lipids, reported from this laboratory (Mohankumar et al. 1990).

Changes in chemical constituents : The histochemical observations were supported with biochemical analysis of the mesocarp constituents in the developing oil palm fruit (Fig.1). The fruit mesocarp showed progressive rate of increase from

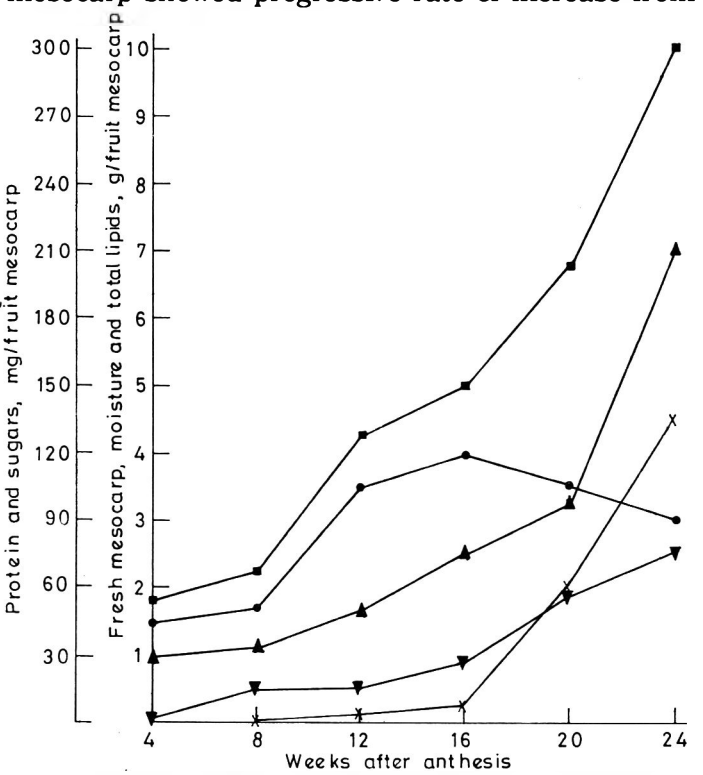

Fig. 1. Storage constituents of oil palm fruit during development (from 4 to 24 weeks after anthesis)
—■— : Fresh mesocarp, —●— : Moisture, —▲— : Protein, —▼— : Sugars, —X— : Total lipids

8th week after anthesis, with a rapid rise from 16th week after anthesis. The moisture content also showed a similar trend, except that it started decreasing towards the end of the maturation time. The lipid content was not very significant at early stages of fruit development (4 to 12 weeks after anthesis). However, it recorded a steep increase from 16th week, till the 24th week after anthesis (7 to 48%). The general pattern of lipid synthesis was, therefore, a rapid phase of lipid synthesis which occurred in the last quarter of oil palm fruit maturation. The minor constituents like proteins and sugars showed a progressive increase from 4th to 24th week, on par with fruit mesocarp(Fig. 1).

The structural carbohydrates (comprising pectin, hemicellulose, cellulose) exhibited a slow rate of

increase during the early stages, followed by a steep rise; the trend being similar to those of other constituents (Fig. 2). Lignin was the major supporting constituent, as shown by histochemical staining. The quantitative increase in the lignin content during fruit development was observed. The

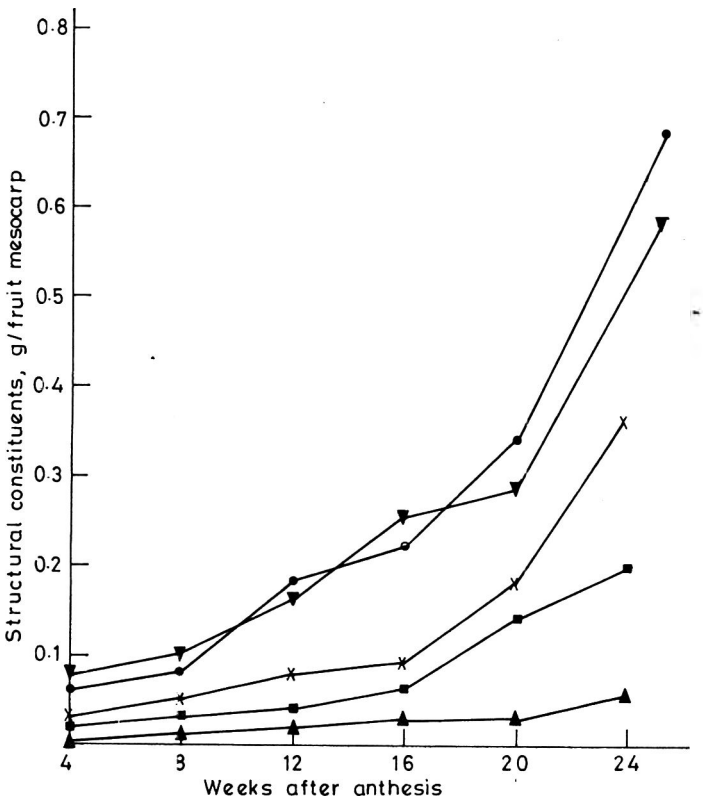

Fig. 2. Storage constituents of oil palm fruit during development (from 4 to 24 weeks after anthesis)
—●— : Lignin, —■— : Pectic substances, —▼— : Hemicellulose, —X— : Cellulose, —▼—: Ash

interesting feature of lignin was its almost exclusive localization in the conducting strands.

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Production of Pectinase from Coffee Pulp in Solid State Fermentation System : Selection of Wild Fungal Isolate of High Potency by a Simple Three-step Screening Technique

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Screening of 248 cultures, isolated from Mexico's coffee growing areas, was carried out to select potent culture for production of pectinase from coffee pulp by solid state fermentation. A pectolytic strain of *Aspergillus niger* CH 4 was used as reference to evaluate the potential of the wild strains. The screening involved three simple and rapid steps. The first step involved the qualitative evaluation of pectolytic activities of 248 fungal strains on a selective solid agar medium, while the second one consisted of the quantification of the pectolytic activities of selected 13 isolates in a submerged fermentation medium, with pectin as the sole carbon source. The third step involved the assay of four selected fungal isolates for their capacity to produce pectinase from coffee pulp by solid state fermentation. *Aspergillus niger* V 22 B 35, a wild strain, was found to produce 4 times more pectinase than the reference strain.

Keywords : Coffee pulp, Solid state fermentation, Wild fungi, Pectinase, Simple three-step screening technique, *Aspergillus niger.*

Wet processing of coffee cherries, involving solid state fermentation and the action of pectolytic microorganisms, has been the preferred method over the dry method in several countries, as it leads to the final product of better quality. The wet method, however, leads to generation of huge quantity of coffee pulp, which poses severe disposal problems. For example, the coffee plantations in Mexico, Central America and Columbia generate coffee pulp to the tune of approximately 40% of several million tonnes of coffee cherries processed (Martinez - Carrera et al. 1989). The coffee pulp is barely used in these countries for want of practical and economic avenues. Due to presence of 23-27% fermentable sugars on dry weight basis (Zuluaga-Vasco 1989), the coffee pulp forms a major source of the pollution of rivers and lakes, located near the coffee processing sites, as well as the environment.

Due to its rich organic matter content, the coffee pulp can form an excellent substrate for production of value-added microbial metabolites by solid state fermentation (SSF) system. Microbial enzymes constitute one of the industrially important groups of microbial metabolites. In fact, one such microbial enzyme, i.e. pectinase, plays an important role in the processing of coffee cherries. In the wet fermentation method of coffee cherries, the natural pectolytic microflora present on the cherries are allowed to grow and metabolize for facilitating pectinase production. This enzyme, then, hydrolyses the mucilage, that envelops the coffee bean and consists mainly of pectins. The degree of hydrolysis of this pectic envelope has an ultimate economical importance to the coffee processing industry, due to its significant role on the yield and the quality of the end product. The modern practice is to use externally added microbial pectinase for hydrolysing the pectic envelop of the coffee beans. Consequently, a large quantity of microbial pectinase is consumed by the coffee processing industry.

There will be a tremendous gain to the industry, if some by-product from coffee processing is used as substrate for microbial production of pectinase. Such an approach will be highly economical and may lead to cost reduction of the end product. Such possibility may also prove useful in overcoming the present economic difficulties faced by coffee processing industry. The presence of around 6.5% pectin in coffee pulp on dry basis (Pulgarin et al. 1991) and its moist solid nature allows such possibility, as pectinase is an inducible enzyme, which requires pectin as an inducer (Fogarty and Kelly 1983). The use of coffee pulp as a substrate for pectinase production may also lead to economy in the enzyme production, as isolated pectin is a rather costly chemical. In addition, pectinases also

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find extensive applications in fruit processing industries for clarification of fruit juices and wines, in the manufacture of pectin-free starch and in the curing of cocoa and tobacco (Ghildyal et al. 1981; Joshi et al. 1991). It has been estimated that the pectinase market for various kinds of industrial processes is about 165 million pounds per annum (Fogarty and Kelly 1983).

It is worth mentioning that SSF system is preferred for production of pectinases due to a number of beneficial reasons (Ghildyal et al. 1981). Production of pectinase by SSF of wheat bran has been studied extensively in India (Ghildyal et al. 1981). It has been earlier reported that the pectinase productivity by SSF is much higher than that in submerged fermentation (SmF) process (Trejo-Hernandez et al. 1991). These facts justify the selection of SSF technique in the present studies.

Screening of large number of microorganisms is an important step in selecting a highly potent culture for developing efficient fermentation process. It, however, is highly time-and resources- consuming. A simple and rapid screening method will be of high utility. Since no such method is available, the present three-step approach has been designed by us for screening of potent pectolytic cultures.

The objectives of the present investigations, therefore, were to test the potential of using coffee pulp for production of pectinase in SSF system and to evaluate the efficiency of three-step approach for rapid screening of wild fungal isolates as well as selection of the potent strain.

Materials and Methods

Microorganisms : A total of 248 fungal cultures were isolated directly from coffee plants and the soil samples from coffee plantation areas in Mexico, as reported elsewhere (Aquiahuatl et al. 1988). A pectolytic strain of *Aspergillus niger* CH 4, which is used as a reference culture, was provided by Dr Carlos Huitron, Department Biotechnologia, Instituto de Investigaciones Biomedicas, UNAM, Mexico, and it was isolated from agave pulp (Saval et al. 1981). All these cultures were maintained on potato-dextrose-agar (PDA) slants at 4°C by subculturing every alternate month in the ORSTOM-UAM collection, ORSTOM, Montpellier, France. For inoculum development, the cultures were grown on PDA slants at 25°C or 35°C for 96 h and the conidia were dispersed in 0.01% Tween 80 solution. All the experiments were carried out in quadruplicate.

First-step screening technique : Agar medium, designated as medium I, was used and it contained (g/l) : pectin citrate 2.0, urea 0.05, ammonium sulphate 0.15, agar 20.0 and distilled water 1000. The pH of the medium was adjusted to 5.5 and it was autoclaved at 121°C for 15 min in 4 Erlenmeyer flasks of 500 ml capacity. The medium was allowed to cool to about 55°C, poured in 16 ml quantity in sterilized petri dishes and allowed to solidify. The medium in plates was streaked with the test cultures and the plates were incubated at 25°C and 35°C for 72 h.

At the end of the incubation, the diam of the hydrolyzed zone of pectate around the colony was measured, as an indicator of the pectinase activity (Antier et al. 1992). This activity was measured through a coefficient called A'. It represents % higher activity with respect to *A. niger* CH 4 (reference culture) and is defined as below :

$$A' = (Hi/Ci - Hc/Cc)/Hc/Cc$$

Where A' = coefficient of pectolysis; Hi = pectolysis zone diam of wild strain; Ci = growth diam of wild strain; Hc = pectolytic zone diam of *A. niger* CH 4; Cc = growth diam of *A. niger* CH 4.

The A' value of the reference strain was taken as zero. As a confirmative test and also to determine the apical growth, the selected isolates (13 Nos) were grown on the agar medium to note their apical growth, in comparison with the reference culture.

Second-step selection technique : A liquid medium, designated as medium II, was used. For its preparation, 50% of pectin citrate solution was pasteurized twice at 70°C for 30 min. In addition, stock solution of urea (5%) was sterilized by membrane filtration. Both the solutions were mixed to contain a final composition, consisting of 20 g pectin citrate and 0.5 g urea in 1l medium. The pH of the mixture was adjusted to 5.8 with 1 N NaOH. The medium was, then, inoculated at a rate of 2 x 10^7 spores/ml medium, using the spore suspension from freshly grown PDA slants. The flasks were incubated at 25°C or 35°C (depending upon the results of the first-step screening technique) for 40 h on rotary shaker (220 rpm). At the end of incubation period, the culture broth was filtered through Whatman No. 1 filter paper to obtain cell-free liquor for assay of the enzyme by viscosity reduction method (Ghildyal et al. 1981). Pectinase activity was determined by using 18 ml of the culture filtrate in a Brookfield R. V. rotational viscometer at 45°C for 10 min. The substrate used was 1 ml of 2% pectin solution prepared in

0.1 M citrate-phosphate buffer (pH 5.5). One enzyme unit (U) was defined as the amount of the enzyme necessary to reduce the initial viscosity by 50%.

Third-step final selection technique : Coffee pulp-based solid state fermentation medium, designated as medium III, was used. It consisted of 34 g dry coffee pulp, 0.8 g urea, 3.3 g ammonium sulphate and 30 ml distilled water. Thoroughly mixed medium was autoclaved at 121°C for 20 min. as described elsewhere (Raimbault and Alazard 1980). After cooling to ambient temperature, it was inoculated with the spore suspension at a rate of 2 x 10^7 spores/g dry coffee pulp. The inoculated medium was packed in 2.0 cm diam and 15 cm long glass column fermenters, which were placed in a temperature controlled water bath (Raimbault and Alazard 1980). Humidified air, at the rate of 60 ml/min was passed through each column. The fermentation was carried out at 25°C or 35°C, based on the requirement of the culture under study. For extraction of the enzyme from moist fermented solids, 30 g portion of the fermented material was mixed with 30 ml of water and pressed in hydraulic press for 2 min at 1000 psi (Roussos et al. 1992). The extract, thus obtained, was filtered and used for enzyme assay, as per the methodology described above.

Statistical analysis : All the experiments were conducted in quadruplicate and the significance of data was tested by the analysis of variance (Snedecor and Cochran 1968).

Results and Discussion

Fungal isolates : Work on isolation of fungal cultures for their ability to grow on coffee pulp was undertaken earlier, with a view to isolate wild fungi capable of degrading organic matter and caffeine, with a a view to reduce the pollution potential of the treated coffee pulp. A novel isolation strategy was designed for this purpose and is described elsewhere (Aquiahuatl et al. 1988). It resulted in isolation of 248 wild fungi and the success of the strategy can be judged by the fact that it yielded a few cultures which were able to completely degrade caffeine in coffee pulp under SSF technique (Aquiahutal et al. 1988). Morphological study of these 248 isolates permitted to group them in ten different filamentous genera viz., *Acremonium, Aspergillus, Drechslera, Fusarium, Geotrichum, Mucor, Penicillium, Rhizopus, Trichoderma* and *Zygomycetes* (Roussos et al. 1989). These isolates have been screened in the present studies to select the potent fungi, capable of producing pectinase in moist coffee pulp medium in SSF system.

Result of first-step screening programme : Data on the growth and enzyme production of the isolates on pectin-containing agar medium are summarized in Table 1. Out of 248 wild fungal isolates screened, 5% did not grow (Ci = o) on this selective medium.This might be due to the absence of pectolytic enzyme system in these isolates, which otherwise, is essential for growth on this medium, because of the use of pectin citrate as sole carbon source. Further, 47% of the total strains screened were able to grow on this selective medium, but they did not produce any visible pectin hydrolysis zone (Hi/Ci =1). Such behaviour is attributed to the ability of these isolates to use pectin citrate as a sole source of carbon to produce pectolytic enzymes, albeit at extremely poor levels. The remaining isolates (48%) grew profusely and produced a clear zone of pectolysis (Hi/Ci > 1). Among these good pectolytic enzyme producing isolates, the coefficient of pectolysis (A') values were < 0, in case of 97 isolates (39% of total isolates screened). The value of these cultures for industrial exploitation was, therefore, ruled out in comparison with the reference culture. A total of 9 isolates showed the coefficient of pectolysis values, which were equal to those of the reference strain (A' = 0) and these cultures, therefore, do not offer any advantage over the reference culture. In contrast, 13 isolates showed the coefficient of pectolysis values, which were > 0, thereby indicating their better pectolytic abilities, as compared to the reference culture.

TABLE 1. SCREENING OF 248 WILD FUNGAL ISOLATES IN FIRST-STEP SCREENING TECHNIQUE INVOLVING CULTURING ON SELECTIVE AGAR MEDIUM IN PETRI DISHES

Attribute	Wild fungal isolates	
	Number	Approximate % of total isolates screened
Growth		
Ci = 0	12	5
Hi/Ci = 1	117	47
Hi/Ci > 1	119	48
Coefficient of pectolysis, A'		
A' < 0	97	39
A' = 0	9	4
A' > 0	13	5

It is, thus, evident that the strategy used for first-step screening programme proved highly efficient, as it was able to select a group of better enzyme producers, which amounted to merely 5.2% of the total isolates, for further studies. Rest of the 94.8% isolates were eliminated by the strategy, based on their worth, as compared to the reference

culture. The strategy is also quicker, simple and much less laborious, as compared to the conventional screening methods.

The selected isolates (13 Nos) were found to belong to two fungal genera (Table 2); *Aspergillus* (7 isolates) and *Penicillium* (6 isolates). These genera of filamentous fungi have been also reported to produce pectolytic enzymes (Fogarty and Kelly 1983). When the apical growths of these selected 13 strains were compared to the reference culture, it was found that only two isolates (*A. niger* C 28 B 25 and C 16 A 25) colonised the solid surface more rapidly and better than that by the reference culture (Table 2). These characteristics are of importance in industrial fermentation, as the economy of the process is determined, to a large extent, by the batch time.

TABLE 2. GROUPING OF 13 ISOLATES, SELECTED AFTER FIRST-STEP TECHNIQUE, AND THEIR APICAL GROWTH AS COMPARED WITH REFERENCE CULTURE (*A. niger* CH 4)

Wild fungal isolates		Apical growth as compared to reference culture
Strain No.	Genus/Species	
V 16 A 25	*Penicillium* sp	Inferior
V 23 A 25	*Penicillium* sp	Inferior
V 34 A 25	*Penicillium* sp	Inferior
C 14 A 25	*Penicillium* sp	Inferior
C 16 A 25	*Aspergillus niger*	Superior
V 12 A 35	*Aspergillus niger*	Equal
V 22 B 35	*Aspergillus niger*	Equal
V 32 B 35	*Aspergillus niger*	Equal
C 15 C 25	*Penicillium* sp	Inferior
C 16 C 25	*Aspergillus niger*	Equal
C 15 B 25	*Penicillium* sp	Inferior
C 17 B 25	*Aspergillus niger*	Equal
C 28 B 25	*Aspergillus niger*	Superior

Results of the second-step selection technique: A total of 13 strains, selected after first-step screening programme, were further studied in liquid medium containing 2% pectin citrate under SmF for specific reasons. For example, the viscosity of the liquid medium will decrease significantly during growth of the selected culture, due to hydrolysis of pectin. Such decrease in viscosity by the selected isolates can be compared with that by the reference culture, with a view to select one or two cultures, which are most potent. The results of such studies indicated that only four isolates (three isolates of *A niger* : C 16 C 25, C 28 B 25, V 22 B 35 and one isolate of *Penicillium* sp. : C 15 B 25) displayed a significant decrease in viscosity of the medium, than that by the reference culture (Table 3). Among these four isolates, the greater reduction in viscosity was given by two strains of *A. niger* i.e. V 22 B 35 and C 28 B 25 (Table 3).

TABLE 3. ABILITY OF THE SELECTED 13 WILD FUNGAL ISOLATES TO REDUCE THE VISCOSITY OF LIQUID MEDIUM IN SUBMERGED FERMENTATION DURING SECOND-STEP SELECTION TECHNIQUE

Genus/Species	Comparative pectolytic activity*	Comparative difference in final pH**	Classification***
Aspergillus niger			
V 22 B 35	+ 1 ± 0.17	0.2	1
C 28 B 35	+ 1 ± 0.15	0.1	2
C 16 C 25	0 ± 0.17	0.2	3
V 17 B 25	0 ± 0.14	0.1	4
V 32 B 25	- 0.5 ± 0.17	0	6
V 12 A 35	- 6.0 ± 0.13	- 1.0	10
Penicillium sp			
C 15 B 25	0 ± 0.14	0.1	4
C 15 C 25	- 0.5 ± 0.14	0	5
C 16 A 25	- 1.0 ± 0.15	0	7
V 34 A 25	- 1.0 ± 0.14	0	7
V 23 A 25	- 1.5 ± 0.15	- 0.3	8
V 16 A 25	- 2.0 ± 0.15	- 0.6	9
V 14 A 25	- 54.0 ± 0.11	- 4.0	11

* Values reported are units produced by the strain under studies (-) units produced by the reference strain. ** Values represent the differences in final pH of the strain under studies (-) that by the reference strain. *** The strains are classified based on their performance, as compared to the reference strain. Class 1 strain scored highest difference and thus is most potent.

Though the second-step selection technique allows efficient and reliable selection of two isolates (*A. niger* V 22 B 35 and C 28 B 35) as most potent cultures among the whole of 248 isolates screened, all the four isolates, which gave significant decreases in the viscosity of the medium, as compared to the reference culture, were selected for third step final selection technique. This was done by keeping in view, the recent report that the cultures, which are the best producers of the metabolite in SmF are usually not efficient, when used in SSF and *vice versa* (Shankaranand et al. 1992). It was, thus, possible to eliminate more than 98% of the 248 isolates and zeroed down on selected four culture in a most efficient way at the end of second-step screening, due to the screening strategy employed in the present studies.

Results of third-step final selection technique : The data in Table 4 give the comparative production of pectolytic enzyme at 72 h in coffee pulp medium under SSF technique by the four filamentous fungi, selected after second-step selection technique. It is evident that all the four wild fungal isolates studied were able to produce more pectolytic enzyme than

TABLE 4. ABILITY OF FOUR WILD FUNGAL ISOLATES, SELECTED AFTER SECOND-STEP SELECTION TECHNIQUE, TO PRODUCE PECTOLYTIC ENZYME AT 72 h IN COFFEE PULP MEDIUM UNDER SOLID STATE FERMENTATION, AS COMPARED TO THE REFERENCE CULTURE (*A. niger* CH 4)

Culture	Pectolytic activity in coffee pulp medium under SSF at 72 h
A. niger CH 4 (Reference culture)	12.1 ± 0.3
Penicillium sp. C 15 B 25	14.9 ± 0.4
A. niger C 16 C 25	20.5 ± 0.5
A. niger C 28 B 25	43.3 ± 1.1
A. niger V 22 B 35	47.7 ± 1.2

the reference culture (*A. niger* CH 4). The highest enzyme production was shown by *A. niger* V 22 B 35, followed by the strains C 28 B 25 and C 16 C 25, in decreasing order. The enzyme production by *Penicillium* sp. C 15 B 25 was slightly higher than that by the reference culture. The enzyme production by *A. niger* V 22 B 35 was about 4 times higher than that by the reference culture. It, therefore, has most potential for industrial exploitation.

The trend of data allows to infer that the three-step screening strategy used is most suitable for screening a large number of isolates in a simple, reliable and quick manner. The isolate selected was subsequently mutated to obtain pectinase hyper producing mutants (Antier et al. 1992), which is being profitably used currently in the coffee curing industry (Roussos et al. 1989).

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Comparative Studies on Functional Properties of Soymeals from 'Kalitur' and 'Bragg' Varieties

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'Kalitur' (black) soybean variety was evaluated and compared with 'Bragg' variety for the functional properties of their meals. Water absorption capacities were 217 and 237 g/100 g of 'Kalitur' meal proteins and its heat processed meal, respectively. Fat absorption capacities were 112 and 141 g oil/100 g 'Kalitur' and its heat processed meal, respectively. Foaming and emulsification capacities were minimum at pH 4.5 and higher at alkaline and highly acidic pH. Foam stability increased with increase in concentration upto 0.4 M NaCl and then started decreasing. The data indicate high potential of 'Kalitur' meal protein for application in food system.

Keywords : Soybean, 'Kalitur' meal protein, Functional properties, Water absorption, Fat absorption, Foaming capacity, Emulsification properties, Potential application in food system.

A study of functional properties of protein is essential to determine the potential of any protein in food system (Kinsella 1976). Lot of literature is available on the assessment of functional properties of yellow soymeal and protein concentrate as well as isolate (Hutton and Campbell 1977; McWatters and Holmes 1979), but information on black soymeal protein is meagre. 'Kalitur', black soybean variety, a product of agronomic improvement programme on black soybean (Mehta et al. 1982) in Madhya Pradesh (M.P.), is extremely popular with cultivators of that state and is being grown on 0.8 million hectares, out of 1.8 million hectares of the total soybean area (Agricultural Statistics 1992). The chemical composition of this variety has been determined by various workers (Nilegaonkar and Agte 1989; Gupta et al. 1982). At present, the black soybean is utilized for oil extraction and defatted meal is exported as cattle feed. In the present study, the functional properties of 'Kalitur' meal (KM) were determined and compared with those of 'Bragg' (yellow) meal (BM) to explore the possibilities of its use in food system. The functional parameters studied were water and fat absorption capacities, foaming and emulsification properties.

Materials and Methods

Soybean source and processing : 'Kalitur' and 'Bragg' varieties, grown under identical conditions, were obtained from Experimental Farms of the University. Defatted soymeal was prepared by dehulling of soybeans using plate mill. The splits obtained were mixed with 10% water (w/v)., held for 24 h to equilibrate and then passed between twin rollers to obtain 2 mm thin flakes. The flakes were dried at 27°C in a current of air, the dried flakes were defatted by repeated extractions (six times) with solvent hexane (60-80°C) and then powdered to pass through 70 mesh (BSS). Heat processed meal was prepared by mixing the defatted meal with distilled water (1:8, w/v) to make slurry. This was autoclaved for 20 min at 1 kg/cm^2 to inactivate heat labile inhibitors and subsequently freeze-dried after powdering to pass through 70 mesh (BSS).

Analytical aspects : The nitrogen content of the meal was determined by microKjeldhal method and a factor 6.25 was used to convert nitrogen values to protein. The water absorption capacity (WAC) and fat absorption capacity (FAC) were determined by the methods of Sosulski et al (1976). WAC is expressed as the amount of water retained by 100 g meal, while FAC is described as the amount of oil bound by 100 g meal. For determining emulsification capacity (EC), the method of Beuchat et al (1974) was used and EC is expressed as ml of oil emulsified by 1 g of protein. The amount of protein used was kept constant for these measurements. EC was also determined as a function of pH and NaCl concentrations. For measuring foam capacity (FC) and foam stability (FS), 5.0 g soymeal was taken along with 100 ml water in a Bajaj electric blender and the suspension was whipped for 5 min, before pouring in 250 ml measuring cylinder. The volume of the foam was recorded after 30 sec. FC is expressed as % volume increase, while the FS was determined by measuring the FC at 5, 10, 30 60, 90 and 120 min. In

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addition, FC was determined as a function of pH and ionic strength.

Results and Discussion

Water absorption capacity (WAC) : It was found to be 217 and 231 g/100 g of 'Kalitur' and 'Bragg' meal proteins, respectively (Table 1). If the water absorption capacities were expressed on protein basis, the values were 418 and 462 g/100 g proteins, respectively for KM and BM, thereby indicating higher WAC for BM than KM. The values obtained are in accordance with the values of soymeal (Lin et al. 1974; Sosulski and Flaming 1977). Heat processed KM and BM gave higher values of WAC, as compared to the meals (Table 1). This is in agreement with the finding of Wu and Inglett (1974). Higher WAC values in case of heat processed meal may be due to dissociation of soyproteins into 11S and 7S fractions (Wolf 1970), which constitutes 70% of total soyproteins. Perhaps, the subunits of 7S and 11S have more water-binding sites. The complex carbohydrate in the meal may also play a role in water absorption. During autoclaving, gelatinization of starch and swelling of crude fibre may occur, leading to increased water absorption. Fat absorption values of KM and BM were found to be 112 and 130 (Table 1). The values per 10 g protein were 216 and 260 for KM and BM, respectively. BM showed better fat absorption than KM. These values are in accordance with the reported fat absorption values (Lin et al. 1974; Sosulski and Flaming 1977). Generally, lipophillic proteins show superior binding of lipids, thereby implying that the non-polar amino acid residues bind the paraffin chains of the fat (Kinsella 1976). Heat processing increased the fat absorption capacity of both KM and BM, the values being 141 and 172, respectively. This increase could be due to dissociation and denaturation of proteins occurring on heating.

TABLE 1. WATER AND FAT ABSORPTION CAPACITIES OF SOYBEAN MEAL

Sample	Crude protein, g/100 g	Water absorption capacity, g/100 g		Fat absorption capacity, g/100 g	
		Flour basis	Protein basis	Flour basis	Protein basis
'Kalitur' meal	51.8	217	418	112	216
'Bragg' meal	50.0	231	462	130	260
Heat processed 'Kalitur' meal	51.8	237	457	141	272
Heat processed 'Bragg' meal	50.0	270	540	172	344

Effect of pH on foam capacity (FC) : All the samples of soybean gave a U shaped pattern with the lowest (minimum) at pH 4.5 (Fig. 1). The FC values at minimum were 132, 136, 122 and 125 for KM, BM and heat processed samples of KM, and BM, respectively. Minimum volume increase, after whipping in the isoelectric region, has also been reported in case of other proteins (Hermasson 1975). Minimum volume increase at isoelectric pH may be because of low solubility of proteins. Low FC at isoelectric point may also be due to strong intermolecular forces, which do not allow protein unfolding and spreading. In alkaline range, the FC values were found to be higher than those in the acidic range, probably due to increased net charge on the proteins. Maximum foaming of native proteins has been observed at pH values below 3, but also at values which are close to the isoelectric pH (Kinsella 1979). However, exceptions have been reported in fish protein concentrate (Hermasson 1975), soybean (Eldridge et al. 1963), leaf protein (Wang and Kinsella 1976) and guar protein (Nath and Narasinga Rao 1981). Heat processing considerably lowered the FC values of KM and BM. At pH 2.2, the FC values were 150 and 155% of unheated KM and BM, respectively. At pH 10, heat processed KM and BM had higher FC values, than those at acidic pH. Our data compared well with those reported by Narayan and Narasinga Rao (1982), in case of soybean meal as reference protein.

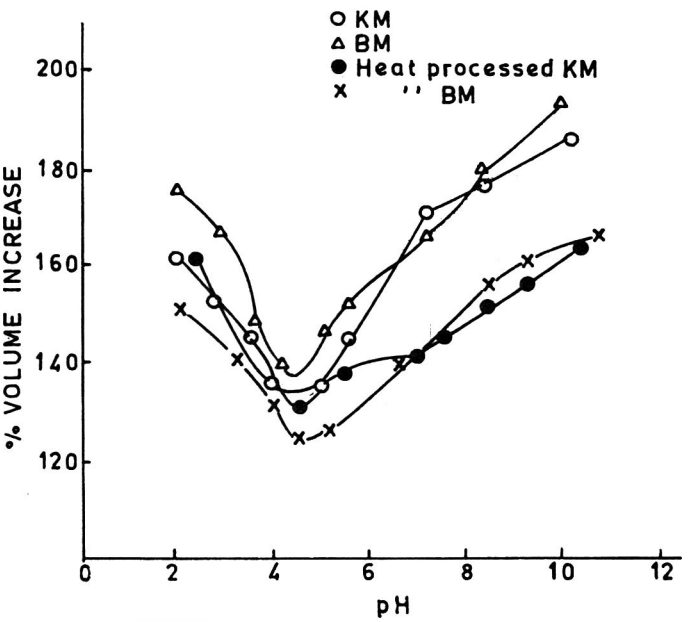

Fig. 1. Effect of pH on foam capacity of KM, BM and heat processed KM and BM.

Effect of NaCl on FC : Fig. 2 shows the effect of NaCl concentration, in the range of 0-1 M NaCl, on the FC of KM, BM and their heat processed meals. In all the cases, the FC increased with NaCl concentration upto 0.4 M concentration and then decreased. The FC values of KM, BM and their heat

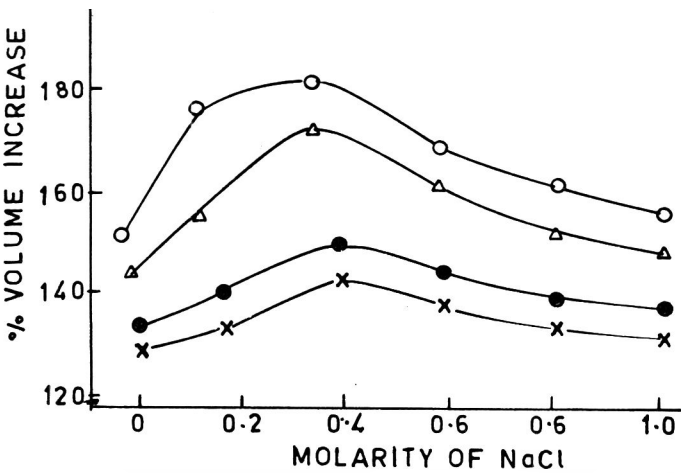

Fig. 2. Effect of NaCl concentration on FC of KM, BM and heat processed KM and BM.
— O — : BM, —Δ—: KM, —●—: Heat processed BM, — X — : Heat processed KM

processed meals were 150, 155, 130, 134, respectively. The FC values at 0.4 M NaCl concentration for KM, BM and their heat processed meal were 174, 180, 138, 142, respectively. These values decreased steadily at more than 0.6 M NaCl concentration. In these measurements, the pH values of the dispersions were maintained at 6.6. It was observed that the effect of NaCl concentrations on FC values was more evident in BM than in the KM.

Foam stability (FS) : FS of the KM, BM and their heat processed meals were determined by measuring the EC over a period of time. FS was also studied as a function of NaCl concentration (Fig. 3). For both the meals, the FS increased with increasing concentration of NaCl upto 0.4 M. Above this concentration, the FS decreased. The FS values for both KM and BM did not change appreciably beyond 30 min. This reduction in foam value ranged from 37.5 to 47.5% in KM and 40-44% in BM. In both cases, FS decreased steadily after 10 min. However, the FS of BM in water and NaCl solutions were better than those of KM. Heat processing considerably lowered the FS. The foam value also decreased steadily after 10 min. It has been reported that FS is related to denaturation. In addition, the native protein has given higher foam stability than denatured proteins (Yasumatsu et al. 1972).

Effect of pH on emulsification capacity (EC) : Fig. 4 shows the effect of pH on the EC of KM, BM and their heat processed meals. The EC *versus* pH profile of KM and BM showed a U shaped pattern with the lowest single point around pH 4.5. It is similar to the shape of FC *versus* pH profile, thereby showing that the emulsifying property was primarily due to dissolved proteins. BM showed higher EC values at alkaline and acidic pH, than

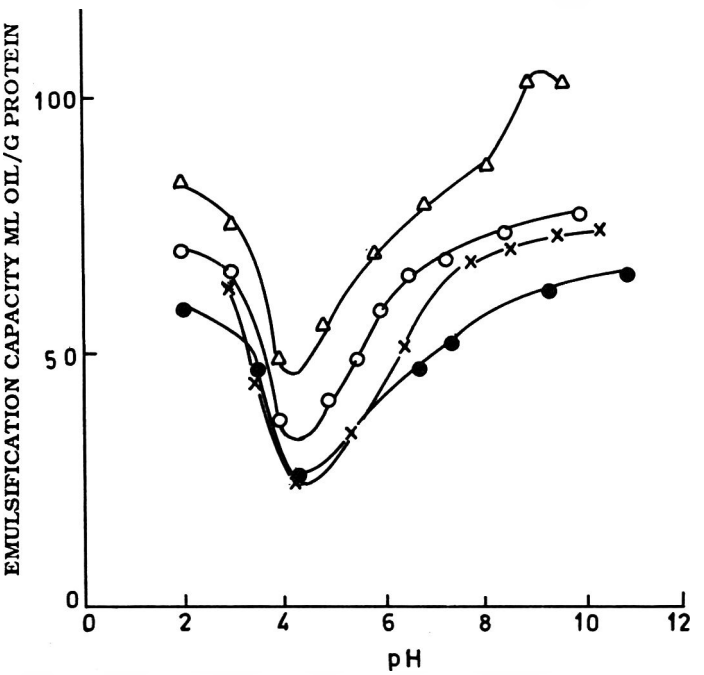

Fig. 4 Effect of pH on emulsification capacity of KM, BM and heat processed KM and BM.
— Δ — : BM, — O — : KM, — ● — : Heat processed KM, — X — : Heat processed BM

the KM. The values expressed as ml of oil emulsified/g protein were 33 and 45 for KM and BM, respectively. The EC of BM was better than that of KM, at all the pH values studied. The EC *versus* pH profile of soyflour resembled to those obtained with cottonseed protein (Crenwelge et al. 1974), groundnut protein (Ramanatham et al. 1978), and sunflower seed protein (Rahma and Narasinga Rao 1981). Heat processing markedly decreased the EC of both the meals at all the pH values studied. At pH 3, the EC of heat processed KM was 55 ml/g protein, as compared to the value of 71 ml/g protein of the raw meal. Similarly, heat processed BM had EC of 64 ml/g, as compared to that of 85 ml/g protein of raw meal at pH 3. Heat processing considerably reduced the EC of the meal. McWatters and Holmes (1979) have reported similar results in soyflour. Moist heating and its duration was stressed to be the primary determinants in the extent of the reduction of nitrogen solubility and consequently the EC.

Effect of NaCl on EC : Fig. 5 shows the results of EC *versus* ionic strength (NaCl) of KM, BM and heat processed KM and BM. The measurements were made in water containing appropriate concentration of salt at a pH 6.6. In case of KM and BM, the NaCl concentration upto 0.4 M had the incremental effect of EC. Beyond this salt concentration, there was practically little decrease

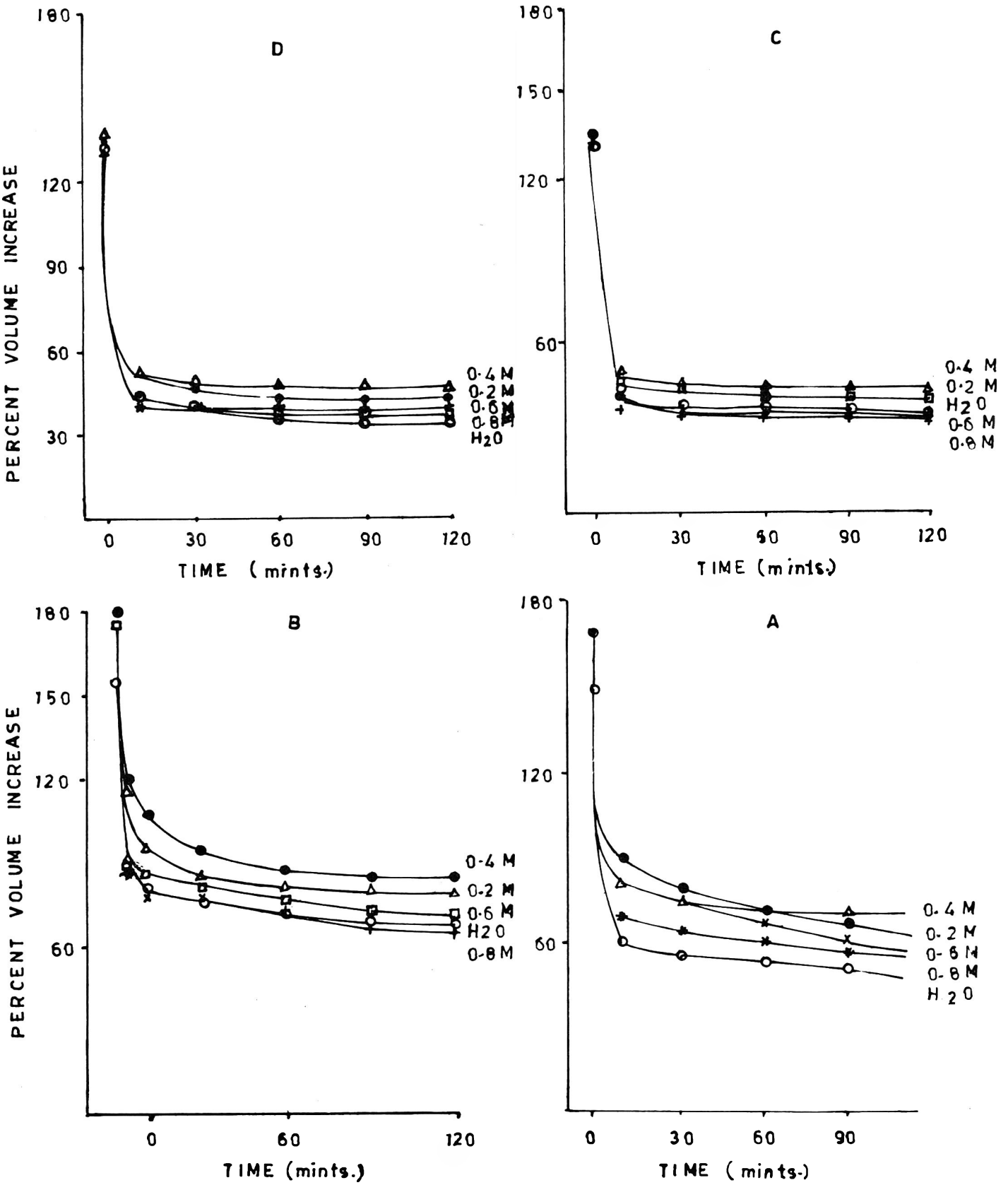

Fig. 3. Effect of NaCl concentration on FS of KM (A), BM (B) and heat processed KM (C) and BM (D).

in the EC. At all NaCl concentrations, the BM had higher EC than KM. In case of heat processed KM and BM, the addition of NaCl did not cause any appreciable change in EC. The nitrogen solubility characteristics of proteins in water and salt solutions differ and this may reflect in the EC also. EC is known to increase with moderate increase in salt concentration, because of the salting-in of the proteins. At higher salt concentration, the EC does not increase, as there is likely to be the salting-out of the proteins. Such effects have been observed in leaf protein concentrates (Wang and Kinsella

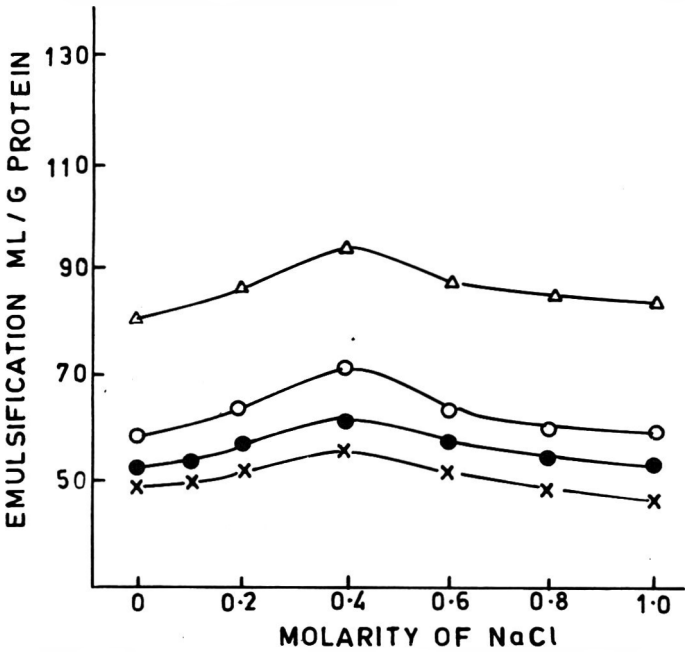

Fig. 5. Effect of NaCl concentration on emulsification capacity of KM, BM and heat processed KM and BM.
— Δ — : BM, — O — : KM, — ● — : Heat processed BM —X— : Heat processed KM

1976), groundnut protein (Ramanatham et al. 1978), and guar proteins (Nath and Narasinga Rao 1991).

Conclusion

Apparently, no significant differences were observed between the functional properties of meal proteins of black and yellow soybeans. 'Bragg' (yellow) meal protein was found to be superior in all the functional properties studied, in comparison to 'Kalitur' meal protein. The latter had all the desirable functional properties to be used in food system. Therefore, it is concluded that 'Kalitur' meal protein had the potential to be used in meat, ice cream, bakery and other food products, due to its comparatively good foaming and emulsification properties.

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Quality of Chicken Gizzard Pickle During Processing and Storage

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Vinegar-based chicken gizzard pickle was processed through 8 replicates. Its quality was evaluated, till 45 and 75 days of ambient storage during summer-rainy and winter seasons, respectively, and compared with refrigerated samples. Microbial counts of ingredients used in preparation of the product suggested the need for oven-drying the condiments, such as red chilli powder, turmeric, cumin, black pepper, caraway, aniseed, cinnamon and clove before use. Changes in pH, shear force, moisture, crude protein, ether extract, thiobarbituric acid and total plate counts in the pickled gizzards were faster during summer-rainy season. Data show that gizzard pickle could be consumed till 45 and 75 days of ambient storage in summer-rainy and winter seasons, respectively, and its quality is comparable with refrigerated samples.

Keywords : Chicken, Gizzard, Pickle, Storage quality, Sensory evaluation, Microbiological quality.

Gizzard constitutes nearly 3% of dressed weight (Charonpong and Chen 1980), but is not commonly consumed by the elite society. Considerable work has been done (Arfa 1977; Charonpong and Chen 1980; Sharma et al. 1986) on processing and storage stability of gizzard pickle under refrigeration. However, a need exists to determine the quality of ingredients used in the preparation of gizzard pickle and the product itself, when exposed to variable tropical environments, during its storage at ambient temperatures. Results of such a study are reported in this paper.

Materials and Methods

A total of 8 trials, including 5 in summer-rainy and 3 in winter seasons, were conducted. Chicken gizzards were collected from pilot poultry processing plant of the Institute. After removing adipose tissue, gizzards were sliced into small pieces and pressure cooked at 15 lb for 10 min. Recipe used in this study contained (g/kg gizzards) table salt 90, peeled garlic 32, ginger 32, cumin 6, red chilli powder 6, powdered aniseed 3, caraway 3, turmeric 3, black pepper 2, cinnamon 2 and clove 2. In addition, refined mustard oil 10 ml, vinegar 195 ml and water 195 ml were used per kg gizzard. Spices were fried and put into simmering solution of vinegar and water. This pickle solution was allowed to simmer for 10 min. Cooked gizzards were mixed with the pickle solution. The product was allowed to cool down and mature for 72 h before use. In two of the representative trials, raw gizzards, spices, utensils and workers involved in the preparation of vinegar-based gizzard pickle and air samples inside processing laboratory were examined for various microbiological parameters. Pickle made in each trial was subjected to ambient and refrigerated (4 ± 1°C, 81% RH) storage, in glass jars covered with plastic lid, till 45 and 75 days during summer-rainy (mid-March to mid-October) and winter (mid-October to mid-March) seasons, respectively.

Daily records of minimum, maximum temperatures and relative humidity (RH) were maintained during the experimental period. Three samples of pickled gizzards from each group were analyzed at zero day and at fortnightly intervals of storage for pH, moisture, crude protein, ether extract (AOAC 1985) and thiobarbituric acid value expressed as malonaldehyde, mg/kg (Taraladgis et al. 1960). Shear force of the samples was determined in lb/inch^2 by using Warner Bratzler shear press (Model 13806, G-R Electrical Mfg. Co., Kansas 66502, USA). Total plate counts (TPC) per 10 g, using appropriate medium and conditions, were determined in fresh as well as stored samples as per recommended methods (ICMSF 1982). Sensory evaluations were made in all the groups at the aforesaid stages of storage by using 10 - point Hedonic scale through minimum of 7 experienced panelists. Nutritional energy (calories/100 g) of gizzard pickle was calculated as per formula used by Shackeord et al (1989). After converting percentages into arc sine values, data were corrected

* Corresponding Author

for trial effects and statistical analysis was conducted (Snedecor and Cochran 1967).

Results and Discussion

Quality during storage : Means of microbial counts (Table 1), at various stages of preparing vinegar-based chicken gizzard pickle, revealed substantial number and variety of microbes in raw gizzards. But, no viable bacteria were observed in cooked (15 lb/inch², 10 min) gizzards. Among ingredients, table salt, garlic, ginger, mustard oil and vinegar also had no viable bacteria. Presence of few yeasts and moulds was noticed in turmeric.

TABLE 1. MEAN VALUES OF MICROBIAL LOADS IN COMPONENTS AND VINEGAR-BASED GIZZARD PICKLE

Sample	Per 10 g sample		
	Total plate count	Coliforms	Yeast and mould
Raw gizzard	93×10^5	14×10^3	23×10^2
Turmeric	-	-	3×10^2
Cumin	52×10^5	3×10^2	6×10^2
Red chilli powder	62×10^3	5×10^2	3×10^2
Cinnamon and Cloves	94×10^5	72×10^2	7×10^2
Black pepper	14×10^3	-	-
Caraway	31×10^6	54×10^5	12×10^2
Aniseed	7×10^2	-	10×10^2
Kitchen workers			
Nails**	NT	+	+
Throat*	NT	-	-
Hands-1	14.33/cm²	10/cm²	-
2	7.8/cm²	6/cm²	-
Jar	3.8/cm²	-	-
Spoons	7.8/cm²	35/cm²	-
Utensils	355/cm²	119/cm²	15/cm²
Vinegar-based gizzard pickle	-	-	2×10^3

- = Organisms not found; + = Organisms present, but not quantified; NT = Not tested; ** = Pathogenic organisms (*Streptococci, Staphylococci, E. coli*) isolated; * = *Streptococci* and *Staphylococci* isolated; None of these microorganisms were present in cooked gizzards, table salt, garlic, ginger, vinegar and mustard oil.

In powdered cumin, red chilli, black pepper, caraway, aniseed, cinnamon and clove, some of the microorganisms were seen (Table 1). This indicated that use of condiments as such in pickle preparations might cause early spoilage of product. Nevertheless, different steps of processing were able to reduce these counts to a remarkable extent, with the result, that gizzard pickle had none of the pathogens including *E. coli* and *Streptococcus* , with a meagre TPC. In view of these findings, the formulation ingredients needed decontamination, prior to their use, in such a way that their intrinsic properties remained unchanged. Based on such indication, spices as above were powdered and dried in the hot air oven at 65 to 70°C for 20 min, which is a well known method of giving heat treatment to condiments for removing all pathogens, except *Bacillus* or sporulating group of organisms, before use in other trials. Similarly, the utensils were also washed with boiling water prior to their use and utmost hygiene was maintained to avert the risk of contamination. However, the experiment was confined to its objective of assessing quality of pickled gizzards and it was not extended to verification of well known facts through evaluation of TPC in heat treated condiments or utensils. Risk of contamination through handling was averted by observing hygienic practices.

Storage quality changes : Mean minimum and maximum ambient temperatures and relative humidity in summer-rainy season were 27.3 ± 1.9°C, 34.2 ± 1.4°C, and 63.1 to 68.4%. These values in winter season were 16.6 ± 1.3°C, 25.6 ± 1.7°C and 61.2 to 71.2%.

Length of storage caused general rise in pH and decline in shear force values, but statistically no significant effects of temperature, seasons or periods could be observed (Tables 2 and 3). In some other experiments on storage of gizzard pickle, similar trends have been reported (Arfa 1977).

Moisture content of pickled gizzards reduced significantly on and after 30th day of ambient storage in summer-rainy season (Table 2). However, such changes were observed on 45th day in

TABLE 2. MEAN OBSERVATION ON STORAGE QUALITY OF VINEGAR - BASED GIZZARD PICKLE IN SUMMER-RAINY SEASON

Traits	Storage periods, days			
	0	15	30	45
pH				
Room	4.3±0.2	4.5±0.2	4.4±0.3	4.8±0.1
Refrig	4.3±0.2	4.6±0.2	4.5±0.3	4.6±0.2
Shear force				
Room	3.9±0.2	3.9±0.1	3.8±0.2	3.7±0.4
Refrig	3.9±0.2	3.5±0.2	3.4±0.2	3.6±0.3
*Moisture				
Room	50.5 ± 0.4^a	50.5 ± 0.6^a	49.2 ± 0.2^b	48.5 ± 0.4^b
Refrig	50.5 ± 0.4^a	50.3 ± 0.3^a	49.9 ± 0.2^a	48.2 ± 0.3^b
*Crude protein				
Room	27.8 ± 0.3^a	29.0 ± 0.3^b	29.5 ± 0.5^b	29.9 ± 0.3^b
Refrig	27.8 ± 0.3^a	29.8 ± 0.2^{bc}	29.70 ± 0.2^b	30.3 ± 0.2^c
*Ether extract				
Room	17.6±1.1	18.0±1.7	18.8±1.4	19.7±1.3
Refrig	17.6±1.1	18.4±0.1	19.3±0.9	19.5±0.9
Thiobarbituric acid				
Room	0.3 ± 0.1^a	1.0 ± 0.2^b	1.2 ± 0.3^b	2.0 ± 0.5^b
Refrig	0.3 ± 0.1^a	1.1 ± 0.3^b	1.0 ± 0.4^{ab}	1.6 ± 0.5^b

* Based on arc sine values; Figures bearing same or no superscripts did not differ significantly among storage periods.

TABLE 3. MEAN OBSERVATIONS ON PHYSICO-CHEMICAL CHANGES DURING STORAGE OF VINEGAR - BASED GIZZARD PICKLE IN WINTER SEASON

Traits	Storage periods, days					
	0	15	30	45	60	75
pH						
Room	4.8±0.1	47.±0.1	4.7±0.3	4.7±0.1	4.8±0.4	4.8±0.0
Refrig	4.8±0.1	4.8±0.1	4.8±0.1	4.8±0.2	4.9±0.2	5.0±0.0
Shear force						
Room	4.7±0.6	4.0±0.4	4.5±0.9	3.9±0.5	3.9±0.3	5.2±0.8
Refrig	4.7±0.6	3.5±0.1	4.2±0.4	4.3±0.6	3.6±0.6	5.2±0.6
*Moisture						
Room	50.2±0.6	49.9±0.4	47.9±1.1	48.7±0.9	48.9±0.8	48.2±0.5
Refrig	50.2±0.6	49.8±0.7	49.3±0.6	49.5±0.5	48.9±0.5	48.6±0.6
*Crude protein						
Room	28.6±0.3^{a}	28.5±0.7^{a}	30.4±0.6^{b}	30.6±0.6^{b}	31.9±0.5^{b}	31.7±0.4^{b}
Refrig	28.6±0.3^{a}	28.4±0.5^{a}	28.9±0.7^{a}	30.1±0.3^{b}	31.1±0.3^{bc}	31.9±0.3^{c}
*Ether extract						
Room	19.4±0.9^{a}	20.3±0.7^{a}	20.6±0.8^{a}	21.2±0.4^{a}	19.2±0.4^{b}	20.0±4.4^{a}
Refrig	19.4±0.9	19.9±0.4	21.4±0.5	20.4±0.2	20.8±0.3	19.8±1.0
Thiobarbituric acid						
Room	0.7±0.3	1.3±0.9	1.8±1.1	1.5±0.8	1.5±0.9	1.9±0.5
Refrig	0.7±0.3	1.5±1.1	1.8±1.0	2.1±0.8	2.3±0.7	1.6±0.6

* Based on arc sine values. Figures bearing same or no superscripts did not differ significantly among storage periods.

refrigerated samples. During winter, in room-stored pickle also, a significant reduction in moisture during storage had been reported (Singh and Panda 1984).

Significant increase in crude protein due to prolonged storage was noticed in both the seasons (Tables 2 and 3). Poultry meat pickle stored for 120 days at room temperature contained 24.2% protein against 24.35% in fresh samples, thereby indicating that there were no changes in protein values on storage (Chatterjee et al. 1969). However, the moisture content declined slightly from 53.2% to 52.3% in this product during storage.

Percent ether extract revealed significant differences only on 60th day of ambient storage during winter. For rest of the shelf-life in either season, significant changes in ether extract were observed (Tables 2 and 3). Reason for such significant observations at 60th day during winter season is not clear. In a separate study on oil-based gizzard pickle involving different recipe and different processing methodology, the insignificant effects of storage on ether extract values in hot humid and winter seasons were observed (Sachdev et al. 1992). Thiobarbituric acid values increased significantly during summer-rainy season (Table 2), but changes were insignificant in winter (Table 3). Higher thiobarbituric acid values in some of the refrigerated samples could not be assigned any specific reason.

Total plate counts in pickled gizzards showed (Table 4) comparatively slow rate of microbial multiplication during winter as well as under refrigeration temperature. In oil-based gizzard pickle also, similar trends have been reported (Sachdev et al. 1992). In both the seasons, the microbiological load counts, till the end of experimental periods,

TABLE 4. MEAN TOTAL PLATE COUNTS IN VINEGAR-BASED GIZZARD PICKLE

Period of storage, days	Storage conditions			
	Room	Refrigeration	Room	Refrigeration
	Summer-rainy season		Winter season	
0	17.0×10^{3}	17.0×10^{3}	7.0×10^{3}	7.0×10^{3}
15	36.0×10^{3}	9.0×10^{3}	10.0×10^{3}	NT
30	74.0×10^{3}	17.5×10^{3}	81.0×10^{3}	29.0×10^{2}
45	21.5×10^{3}	12.0×10^{3}	16.0×10^{3}	72.0×10^{2}
60	NT	NT	35.0×10^{3}	8.5×10^{3}
75	NT	NT	7.0×10^{3}	6.0×10^{3}

NT = Not tested

were well within the permissible limits (Elliott and Michener 1961).

Nutritional energy : Calculated calorific values in fresh and stored pickles ranged between 248 and 320. Gizzard pickle stored for longer periods rendered more energy due to comparatively less moisture and higher protein as well as lipid contents.

Sensory traits : A general decline in sensory characteristics such as colour, flavour, juiciness, tenderness, texture and overall acceptability was recorded with prolonged storage. Mean values for these attributes in samples, stored at ambient and refrigerated temperatures, ranged from 5.97 ± 0.49 to 7.12 ±0.44 over the entire storage period of 45 days in summer-rainy season. These values were in the range of 6.13 ± 0.09 to 7.51 ± 0.33 for the product stored in winter season. But, the changes

were insignificant in both the seasons and storage conditions. Thus, the gizzard pickle was quite acceptable, till the end of stipulated storage periods.

From the results obtained in the present study on the sensory evaluation, it may be concluded that vinegar-based chicken gizzard pickle could be consumed till 45 and 75 days of ambient storage in summer-rainy and winter seasons, respectively. The results also showed that shelf-life of this product at room temperature was comparable with that kept under refrigeration.

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Changes in the Functional Characteristics of Wheat During High Temperature Storage

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Storage of wheat at different temperatures showed considerable deterioration in grain quality. The deterioration was more in wheat stored for 5 months at 50°C. Storage of wheat at higher temperature for longer duration decreased both the 1000 kernel and hectolitre weights. Duration of storage also adversely affected the milling yield. Increases in colour grade value and ash content in flour were observed with increases in storage temperature and time. Storing of wheat at higher temperatures affected the α-amylase activity and this was indicated by an increase in falling number and amylograph peak viscosity values. Rheological studies showed a decrease in farinograph water absorption and an increase in resistance to extension in wheat stored for 5 months at 50°C. Baking tests revealed that, even though the quality of bread improved to some extent in wheat stored at 27 and 37°C, storage of wheat at 50°C considerably reduced the bread quality.

Keywords : Wheat, High temperature storage, Bread quality, Functional characteristics, Deterioration.

Wheat grain is generally harvested once or twice during the year and its cultivation is restricted to specific parts of the country. Considerable time usually elapses between procurement of wheat and its subsequent processing by the customer. During this period, wheat is stored at different storage conditions, varying in storage temperature and humidity. Storage temperature and humidity have been reported to bring about several changes in the functional characteristics of wheat (Anderson and Alcock 1954; Glass et al. 1959; Sawant 1985). In India, the ambient temperature varies from as low as 4°C to as high as 47°C, depending on the season and place. It has also been reported that the temperature of the grain could increase substantially when stored in silos or bins, due to the respiration process (Christensen and Kaufman 1969). However, a few studies have been reported on the storage of wheat flour under tropical conditions (Leelavathi et al. 1984; Premavalli et al. 1993).

The objective of this study was to determine various changes that occur in the functional characteristics of wheat grain, when stored at high temperatures. Reports available so far deal with storage of wheat grain at relatively lower temperatures.

Materials and Methods

Storage and milling studies : Ten kg lots of cleaned wheat (procured from local market) was fumigated (ethylene dibromide 32 g/m^3), packed in air-tight tin containers, and stored in incubators maintained at 27, 37 and 50°C, respectively. Wheat samples were drawn after 1, 3 and 5 months of storage for analytical purposes. Wheat was conditioned to 15% moisture for 24 h and milled in a laboratory Buhler mill (Model MLU-202). Yield of straight run flour was calculated and expressed as % based on the yield of total product.

Analytical methods : Estimations of moisture, gluten, ash, colour, sedimentation value, falling number, diastatic activity, alcoholic acidity and hectolitre weight were carried out according to standard AACC methods (1969). Hardness of wheat kernel was measured in hardness tester (KIYA Seisakusho, Ltd., Tokyo, Japan) and average value of 10 kernels are reported. To determine the germination capacity, 100 seeds, soaked overnight, were placed between damp filter papers in a petri dish and held for 5 days at room temperature (27±2°C). Seeds with normal shoots were counted and reported as % germinated. Dough raising capacity of flours was determined by ISI (1972) method. Farinograph, extensograph and amylograph characteristics were determined according to AACC (1969) methods. Amylograms were evaluated for peak viscosity (P), viscosity after 30 min holding at 95°C (H), cold paste viscosity (C), breakdown (P-H) and setback values (C-P) as per the method described by Bhattacharya and Sowbhagya (1979).

Baking characteristics : Breads were made according to 'Remix' baking test (Irvine and McMullan 1960) and 2% shortening was included in the formulations. Volume of breads was measured in loaf volume meter using rapeseed displacement

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method (Malloock and Cook 1930). The crust and crumb characteristics of breads were evaluated by a panel of six semi-trained judges. All the tests were carried out in triplicate and the average values have been reported.

Results and Discussion

Changes in the physical characteristics : Storage of wheat at room temperature (27°C) had minimal effect on its moisture content (Table 1). At higher temperatures, greater moisture loss was observed, and it continued to decrease as the duration of the storage period increased. This resulted in the wheat grains to be brittle, as indicated by the gradual decrease in the hardness value. Average grain hardness in wheat, stored at 27, 37 and 50°C, decreased by 21, 38 and 49%, respectively, at the end of 5 months storage period. Moisture loss in wheat grain on storage at high temperature was also reflected in a decrease in 1000 kernel and hectolitre weights. Storage of wheat at 27 and 37°C till 3 months did not have much effect on its germination capacity. However, the germination capacity of wheat reduced considerably by 5 months storage. On the other hand, wheat completely lost its germination capacity within 3 months, when stored at 50°C. Glass et al (1959) have also observed decrease in grain viability during 10 month storage period at 20 and 30°C.

Change in the milling yield : The milling yield increased slightly during the first month of storage. However, a gradual but slight decline was observed as the storage progressed further (Table 1). After 5 months of storage at 27, 37 and 50°C, the respective flour yield decreased to 67.9, 68.9 and 70.1%. Greater yield at higher storage temperature may be due to higher brittleness of the grains. This is reflected in higher ash content in wheat stored at 50°C (Table 2). Shellenberger (1939) observed insignificant change in milling yield, till three and half months, but reported an increase in the yield from 60.9 to 62.2% after 6 months of storage. In contrast, Glass et al (1959) noticed no change in flour yield throughout 10 months storage at 20 and 30°C.

Changes in the physico-chemical characteristics : The gluten content did not change significantly in samples stored at 27 and 37°C even after 5 months of storage, while it disintegrated after 3 months in wheat stored at 50°C (Table 2). The quality of the gluten protein, as indicated by sedimentation value, also showed an insignificant change during storage at 27 and 37°C, but by the end of 5 months storage at 50°C, there was a significant decrease in the sedimentation value. The colour of flour milled from stored wheat samples increased with storage temperature and duration. The extent of change in colour was higher in samples stored at

TABLE 1. CHANGES IN PHYSICAL CHARACTERISTICS AND MILLING YIELDS OF GRAIN DURING STORAGE

	Initial value	Storage temperature, °C								
		27			37			50		
		Storage period (months)								
		1	3	5	1	3	5	1	3	5
Moisture, %	11.22	11.16	11.05	10.70	10.67	10.09	9.18	10.30	9.00	8.55
Hardness, kg	10.62	-	10.55	8.38	-	10.22	6.63	-	6.88	5.38
1000 kernel weight, g	57.20	56.20	55.80	54.60	57.00	55.0	53.10	54.60	50.10	46.70
Hectolitre weight, kg/Hl	82.50	82.50	81.50	81.00	82.00	81.0	79.50	80.50	78.50	74.50
Germination capacity, %	85.00	83.00	78.00	70.00	82.00	76.0	58.00	52.00	0	0
Milling yield, %	70.50	71.10	69.50	67.90	72.90	70.0	68.90	72.20	71.00	70.10

TABLE 2. CHANGES IN THE PHYSICO-CHEMICAL CHARACTERISTICS OF WHEAT DURING STORAGE AT DIFFERENT TEMPERATURES

Quality parameters	Initial value	Storage temperature, °C								
		27			37			50		
		Storage period (months)								
		1	3	5	1	3	5	1	3	5
Gluten, %	10.8	10.6	10.8	10.8	10.5	10.4	10.4	10.3	-	-
Sedimentation value, ml	25	25	25	22.5	26	25	21	23	21	18
Colour grade value	4.1	4.4	4.7	5.1	4.7	4.9	6.1	6.9	7.6	8.9
Dough raising capacity, %	145	148	120	121	145	100	96	102	56	46
Falling number, sec.	546	552	522	585	572	580	621	703	705	745
Diastatic activity, mg maltose/10 g flour	348	360	350	302	344	302	267	300	282	257
Ash, %	0.44	0.45	0.47	0.51	0.50	0.48	0.52	0.51	0.65	0.72
Alcoholic acidity, % H_2SO_4	0.025	0.025	0.049	0.054	0.025	0.051	0.067	0.029	0.069	0.084

50°C for 5 months. This could be attributed to non-enzymatic browning and the presence of higher amount of bran particles in flour. This was substantiated by similar changes in ash content, which increased from 0.44 to 0.72%. The deteriorative changes in wheat stored at 50°C were also reflected in dough raising capacity, which decreased from initial 145% to 102, 55.5 and 46% after 1, 3, and 5 months storage, respectively. Storage of wheat at 50°C had more deteriorative effect on dough raising capacity, than those stored at 27 and 37°C.

Flour from wheat stored at 27°C had minimal effect on amylolytic activity. This was indicated by both the falling number and diastatic activity values. Storage of wheat at higher temperatures affected the amylase activity, which was reflected in an increase in the falling number value and decrease in the diastatic activity. The acidity increased during storage, depending on the period and temperature. The acidity rose by 118, 173 and 241% after 5 months storage at 27, 37 and 50°C, respectively. Shellenberger et al (1958) observed 3 to 4 times increase in fat acidity during 1 year storage at 38°C.

Change in the rheological characteristics : Rheological studies on stored wheat showed that the farinograph water absorption (FWA) remained unchanged till 1 month storage at 27 and 37°C (Table 3). Thereafter, it gradually decreased, as the storage period progressed. However, the reduction in water absorption during storage was much smaller in wheat stored at 50°C, probably due to the presence of higher amount of bran particle in the flour. Shellenberger et al (1958) have also reported higher water absorption capacity for wheat stored at 38°C, as compared to that stored at 4°C. However, Sudha Rao et al (1978), and Shellenberger (1939) observed insignificant increase in water absorption of wheat milled after 6 months of storage at 27±3°C. Flour milled from stored wheat recorded an increase in the dough development time and a decrease in the mixing tolerance index and the extent of change increased with increase in storage temperature. At the end of storage period, the dough development time was 3.5 min. for wheat stored at 27°C, as compared to 5.0 min observed for the wheat stored at 50°C. The stability of flour improved at higher temperature of storage, the increase being 3-fold at 50°C. Sudha Rao et al (1978) have also reported similar observations. Extensograph characteristics showed that resistance to extension decreased with storage, but increased with temperature of storage. At the end of 5 months storage at 50°C, the initial resistance to extension of 850 BU exceeded 1000 BU, as compared to that for sample stored at 27°C. On the other hand, dough extensibility in wheat stored for 5 months at 27, 37 and 50°C, increased by 50, 41, and 32%, respectively.

Changes in the pasting characteristics : Amylograph characteristics showed a gradual increase in peak viscosity with increase in temperature and duration of storage, thereby indicating a reduction in α-amylase activity (Table 3). Storage made the starch paste more fragile as indicated by gradual increase in breakdown value with increase in storage duration and temperature. Increase in

TABLE 3. CHANGES IN THE RHEOLOGICAL AND PASTING CHARACTERISTICS OF WHEAT DURING STORAGE AT DIFFERENT TEMPERATURES.

Characteristics	Initial value	Storage temperature, °C								
		27			37			50		
		Storage period (months)								
		1	3	5	1	3	5	1	3	5
FWA[1], %	65.8	65.8	63.8	62.6	65.8	64.6	63.8	66.4	65.0	64.8
Stability, min	4.0	4.0	4.0	5.0	5.0	6.0	6.0	6.5	8.0	12.0
DDT[2], min	3.0	3.5	3.5	3.5	3	3.5	4.0	4.0	4.0	4.5
MTI[3], BU	70	60	50	40	40	40	30	50	40	20
Resistance to extension (R), BU	850	760	740	540	885	805	620	1000	>1000	>1000
Extensibility (E), mm	110	125	130	165	120	125	155	120	120	145
Ratio fig, R/E	7.7	6.1	5.7	3.3	8.2	6.4	4.0	8.3	–	–
Area, cm²	121	132	139	124	145	142	146	158	–	–
Peak viscosity (P), AU	1460	1510	1590	1750	1510	1640	2160	1660	2090	2180
Breakdown (P–H)[4], AU	300	350	390	910	440	360	1030	490	720	1460
Set back (C-P)[5], AU	680	660	640	530	520	460	290	550	290	250

[1] FWA = Farinograph water absorption, [2] DDT = Dough development time, [3] MTI = Mixing tolerance index,
[4] H = Viscosity after 30 min holding at 95°C[5], C = Cold paste viscosity.

initial breakdown value was 5 times higher in flour sample milled after 5 months of storage at 50°C. Set back values of flour paste from stored wheat showed gradual decrease, indicating lower retrogradation of starch with increase in storage duration and temperature. Wheat, milled after 5 months of storage at 27, 37 and 50°C, recorded 22, 57 and 63% decrease in set back value. However, the storage duration and temperature had insignificant effect on onset of gelatinisation temperature.

Changes in the baking quality : Flour obtained from wheat stored at 27 and 37°C recorded 5.5 and 3.8% improvement in the specific volume of bread (Fig. 1). The crumb characteristics were not affected significantly on storage at 27 and 37°C.

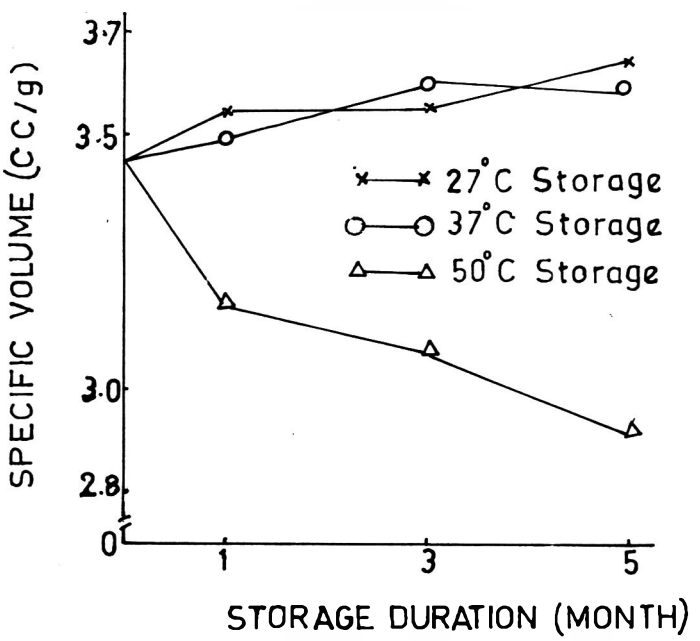

Fig. 1. Changes in specific volume of bread made with flour, milled from stored wheat.

Similar results were reported by Sudha Rao et al (1978) for wheat stored for 4 months at 27±3°C. However, Shellenberger (1939) observed initial improvement, followed by gradual decline, in loaf volume during 6 months storage at room temperature. On the other hand, Glass et al (1959) noticed marked deterioration in flour quality, resulting in dark crumb and poor dough handling characteristics in wheat stored for 10 months at 30°C, while it was stated that the change depended on the initial moisture content of wheat. Breads made from wheat stored at 50°C for 1, 3 and 5 months showed a significant decline in specific loaf volume. The breads made from such flours had pale crust colour and inferior crumb with coarse grain and hard texture.

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Manufacture of Freeze-dried Breakfast and Dessert Foods

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Quickly rehydratable and nutritious breakfast and dessert foods have been developed using freeze-drying. The freeze-dried wheat porridge (*dalia*), rice pudding (*kheer*) and vanilla custard contained 11.2-12.2% protein and provided 428, 447 and 403 Kcal/100 g, respectively. These are also rich in Ca^{+2} and P^{+5} (306 to 325 mg/100 g and 316.8 to 385 mg/100 g, respectively). These were stable for 6-9 months at 37°C, when packed in paperfoil - polythene-laminate flexible pouches. They exhibited a rehydration ratio of 1:3. *Dalia* and *kheer* could be rehydrated satisfactorily at 45°C, 70°C and in boiling water in 20, 10 and 5 min, respectively. Custard could be instantly rehydrated at 5°C and 45°C or at higher temperatures, if required.

Keywords: Freeze-drying, Dessert foods, *Dalia*, *Kheer*, Microbial profile, Chemical changes, Space foods.

Many disadvantages in drying of foods can be eliminated by application of freeze-drying (Chandrasekaran and King 1971; Peterson et al. 1973; Srinivas Rao et al. 1966). Freeze-drying is also applied to animal foods (Jasmine et al. 1970). In addition, freeze-dried foods can be reconstituted in chilled water as well as in warm or hot water, thereby eliminating the need to boil water. Keeping these advantages in view, attempts were made to prepare convenience forms of various traditional Indian foods for Defence use. Among many foods, wheat porridge (*dalia*), rice pudding (*kheer*) and vanilla custard were selected for their satisfying taste, sweetness, filling nature and familiarity to most people. Therefore, the freeze-dried versions of these products were prepared and assessed for their quality and shelf-life in the present study.

Materials and Methods

Broken wheat ('Bulgar'), short grained rice ('Bangarsanna'), vanilla flavoured custard powder and white free-flowing sugar (conforming to ISI specifications) were purchased locally. Pasteurized milk (solids- non-fat 8%, fat 3%) was skimmed from top, after boiling and cooling, and used in all preparations. Hydrogenated fat, saffron and cardamom were procured from the local retail shops. Microbiological media were freshly prepared from bactograde components (HiMedia Co., Bombay and Difco Ltd., USA). Dehydrated media (Difco) were used in the detection of *Salmonella*.

Wheat porridge (dalia) : *Dalia* or broken wheat (1 kg) was roasted in 100 g vegetable fat at 110°C ± 2°C in a shallow pan and then cooked in 3 l water at 15 psi for 30 min. Milk (10 l) was added to the softened *dalia* and the contents were cooked for 30 min at boiling temperature. Sugar (1.2 kg) was then added and the mixture was further cooked for 15 min, followed by the addition of 2 g cardamom powder. It was, then, cooled to room temperature.

Rice pudding (Kheer) : Raw washed rice (1 kg) was soaked in water at room temperature for 15 min. After draining, it was boiled in 10 l milk for 30 min. Sugar (1.2 kg) was, then, added to the cooking mixture and stirred for 15 min. Saffron (1 g) was dissolved in about 25 ml milk and then added to the mixture, after cooling to 60°C. The whole mass was cooled to room temperature.

Custard : Milk (20 l) and sugar (3.6 kg) were boiled together and slowly thickened by adding corn flour (vanilla-flavoured, yellow-coloured), which was dissolved earlier at 5% level in cold milk. The hot custard was poured into drying trays and allowed to cool to room temperature.

Freeze-drying : The cooled foods were frozen in a blast freezer (Hull Corporation, USA). *Dalia* and *kheer* were frozen to -20°C and custard to -30°C for 3 h . The frozen foods were cut into bits and thermocouples were inserted. These trays were, then, loaded into the freeze-drier (Hull Corporation, USA, Model 30F40) and refrozen for 30 min. The products were freeze-dried under a platen temperature of +60°C and a vacuum of 300 microns for 16 h.

Packaging : All foods were packed in 50-70 g portions in paper (45GSM)-aluminium foil (40 μ)-polyethylene (37.5 μ) laminate (PFP) pouches. All samples were stored for various periods upto 9 months at room temperature (RT 30°C ± 2°C) and 37°C.

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Rehydration : Different quantities of chilled water (5-10°C), tepid water (40-45°C), hot water (70°C) and boiling water (98°C) were added to weighed samples in stainless steel bowls with lids. Appropriate temperatures were maintained for the required period by placing the covered bowls in thermostatically controlled oven at preset temperatures. Rehydration quality was judged organoleptically.

Chemical analysis : The samples were analysed for moisture, proteins, fat, ash and carbohydrates as per standard procedures (AOAC 1984; Ranganna 1986). Analysis for Ca^{+2}, P^{5}, Na^{+1}, K^{+1}, Cl^{-1}, Fe^{+2}, Zn^{+2} and Pb^{+2} were carried out in an atomic absorption spectrometer (Shimadzu, model AA 670) as per the methodology of Ockerman (1978).

Shelf-life studies : Shelf-life of stored samples was observed by determining chemical and organoleptic changes. Initially and then after every 3 months, the stored samples (packed in PFP) were analysed for free fatty acids (Pearson 1968), peroxide value (AOAC 1984) and thiobarbituric acid content (Taraldgis et al. 1960).

Organoleptic analysis: All stored samples were subjected to sensory quality evaluation by a panel of 10 members for determining overall acceptability on a 9 -point Hedonic scale. The products receiving an overall score of 6 and above (9 for excellent) were considered acceptable.

Microbiological analysis : Microbiological quality of the foods was determined from random analysis of samples obtained from 5 pilot scale production batches of each food. The test procedures were those specified for space qualification of foods (NASA 1985). Sixty g samples (in duplicate) were macerated in 540 ml sterile trypticase-soy-broth. A resuscitation period of 1 h at room temperature was given to all samples. Sterile 0.1% peptone water was used as the diluent. Tests for standard plate counts, coliforms, *Escherichia coli*, yeasts and moulds, *Staphylococcus aureus*, *Clostridium perfringens* and *Salmonella* were carried out using the food macerate and its dilutions (NASA 1985).

Results and Discussion

The proximate composition of freeze-dried *dalia*, *kheer* and custard is shown in Table 1. All the foods have, more or less, the same content of various constituents, with the exception of fat. The moisture content of about 2% is commensurate with other freeze-dried foods, such as mango juice (Ammu et al. 1976). Moisture lesser than this has been found to adversely affect the stability of foods,

TABLE 1. PROXIMATE COMPOSITION AND ENERGY CONTENT ON THREE FREEZE-DRIED PRODUCTS

Composition (g/100 g)	*Dalia*	*Kheer*	Custard
Moisture	2.1 ± 0.2	2.5 ± 0.2	2.5 ± 0.1
Proteins	12.2 ± 0.3	10.5 ± 0.4	11.2 ± 0.8
Fat	9.3 ± 0.2	13.0 ± 0.3	4.3 ± 0.2
Carbohydrates (total)	74.0 ± 0.7	71.9 ± 0.9	79.8 ± 0.6
Ash (total)	2.4 ± 0.1	2.0 ± 0.0	2.2 ± 0.1
Energy Kcal	428	447	403

such as meats, which contain high proteins and fat. These foods (10-12% proteins) would partially contribute towards the maintenance level of protein of 0.8-1.0 g/kg body weight, as recommended by WHO and ICMR (Swaminathan 1981). Fat content (4-13%) is very much lower than that in other wheat semolina-based dessert and breakfast convenience foods, such as *sooji halwa* (26% fat) and *upma mix* (34% fat) (Premavalli et al. 1987; Arya and Thakur 1986). These other wheat semolina-based foods currently form a part of pack rations. It has been reported by troops and mountaineers that the fatty foods cause nausea and aversion to eating at high altitudes (Hannon et al. 1976). Since the troops and mountaineers also appear to develop a craving for sweet foods, the three food preparations, with their sweet taste and lower fat content, would be more suitable. In addition, the increased body energy expenditure at high altitudes could be compensated by the intake of these three foods, which have a high carbohydrate content (nearly 72 to 80%). In addition, these foods can provide the bulk of the energy (64 to 79%), from the total calculated values of 428 (*dalia*), 447 (*kheer*) and 402 (custard) Kcal/100 g. This is an additional desirable feature of these foods for high altitudes, as the energy derived from fat should be far less than that for the carbohydrate components.

Mineral content : The mineral analysis of the three products (Table 2) reveals that these products would provide nearly two-thirds of the recommended daily allowance (RDA) for Ca^{2+} for Indian adult males (ICMR 1984). Phosphorus content was also similar to that of Ca^{2+}. Among the electrolytes, concentration of Na^{+1} and Cl^{-1} ions varied; *dalia* having the maximum Na^{+1} (nearly two and four times that of custard and *kheer*, respectively) and Cl^{-1}. The latter was present in very insignificant levels in the other two products. Fe^{+2}, Cu^{+2} and Zn^{+2} contents were very low. Therefore, none of these could be considered significant in terms of the RDA. The concentration of Pb^{+2} (0.20 to 0.27 mg/100 g) was within the lower limit (2-5 ppm) prescribed by

TABLE 2. LEVELS OF CALCIUM, PHOSPHORUS, ELECTROLYTES AND SOME TRACE AND TOXIC METALS PRESENT IN THREE FREEZE-DRIED PRODUCTS

	Minerals, mg/100 g								
	Ca^{+2}	P^{-5}	Na^{+1}	K^{+1}	Cl^{-1}	Fe^{+2}	Zn^{+2}	Cu^{+2}	Pb^{+2}
Dalia	306±22	385±29	202±12	403±30	60±3.5	2.17±0.14	1.55±0.21	0.23±0.004	0.20±0.002
Kheer	350±26	316.8±16.4	57.3±4.2	459±28	0.072±0.003	6.75±0.33	2.06±0.17	0.42±0.006	0.27±0.006
Custard	325±19	341.4±12.6	112±12	468±36	1.46±0.21	4.98±0.42	1.85±0.12	0.38±0.008	0.24±0.005

the Prevention of Food Adulteration Act (PFA 1954, 1989) for various foods.

Rehydration characteristics : The rehydration characteristics of the three foods are shown in Table 3. Custard could be readily wetted, dispersed and gently stirred into a smooth mass, both in cold and hot water. In contrast, both *dalia* and *kheer* were satisfactorily softened at 70°C in 10-15 min or by simmering in boiling water for 5 min, with a rehydration ratio of 1:3. The products looked and tasted like the freshly made foods, except for custard, which did not set into a gel. The ability to rehydrate in water at different temperatures is a distinct advantage and imparts a versatility to the products, which is not found in other dehydrated products. Thus, it makes them useful in various situations, where either open cooking (such as in spacecraft) or cooking at higher temperatures is either not possible or not practical.

TABLE 3. REHYDRATION CHARACTERISTICS OF FREEZE-DRIED PRODUCTS

Parameter	*Dalia*	*Kheer*	Custard
Physical appearance before rehydration	Coarse whitish looking flaky powder, free flowing	Coarse, pale yellowish flaky powder, free flowing	Orange yellowish, fine powder, free flowing
Rehydration time (mins)			
Cold water (5-10°C)	Not rehydratable	Not rehydratable	2, gentle stirring
Tepid water (40-50°C)	20	20	-do-
Hot water (70°C)	10	10	-do-
Boiling water	5, simmering,	5, simmering	Not done
Appearance after rehydration	Creamy, thick, with soft grains or broken wheat	Creamy, thick, with soft broken grains of rice	Thick, creamy, smooth, does not set into gel

Rehydration ratio used was 1:3 in all the cases.

Microbiological quality : The data on the microbiological quality of the foods are presented in Table 4. The samples had low counts of aerobic bacteria, yeasts and moulds. *Salmonella* was absent in all foods, whereas coliforms ranged from 0 to 5 g^{-1}. The quality of the dried products reported in this paper conforms to the microbiological limits specified for space foods by NASA (1985), which

TABLE 4. MINIMUM AND MAXIMUM VIABLE COUNTS OF MICROORGANISMS DETECTED IN THE FREEZE-DRIED FOODS PRODUCED ON PILOT SCALE

Product	Batches tested	Microorganisms, g^{-1}	
		SPC	Yeasts and moulds
Dalia	5	115-200	5-10
Kheer	5	20-440	25
Custard	6	80-250	0-20

Coliforms were absent in *Dalia* and *Kheer* as against the count of 0-5^{-1} in custard.
Pathogens were absent in all the cases.

TABLE 5. CHANGES IN FFA, PV, TBA VALUES AND OVERALL ACCEPTABILITY SCORES OF *DALIA*, *KHEER* AND CUSTARD DURING STORAGE AT RT (32°C) AND 37°C.

Product	Storage period, months	FFA, % Oleic acid		PV, m.eq. O_2/ kg. fat		TBA, Mg MA/kg sample		Overall acceptability score	
		32°C	37°C	32°C	37°C	32°C	37°C	32°C	37°C
Dalia	0	0.62±0.02	0.62±0.02	8.2±0.4	8.2±0.4	14.0±0.3	14.0±0.3	8.0±0.8	8.0±0.8
	3	0.65±0.03	0.72±0.01	12.6±0.4	14.5±0.3	17.1±0.2	18.3±0.4	7.9±1.8	7.5±0.8
	6	0.70±0.03	0.75±0.01	13.6±0.7	16.7±0.5	18.3±0.4	19.0±0.2	7.5±0.8	7.1±0.6
	9	0.74±0.02	0.82±0.02	15.1±0.9	17.5±0.3	18.3±0.2	21.2±0.5	6.2±1.7	6.1±1.9
Kheer	0	0.65±0.03	0.65±0.03	9.5±0.3	9.5±0.3	13.0±0.4	13.0±0.4	8.0±0.8	8.0±0.8
	3	0.75±0.02	0.82±0.02	12.2±0.5	14.3±0.2	18.0±0.2	21.2±0.6	7.4±0.6	7.1±0.8
	6	0.80±0.01	0.85±0.01	15.5±0.2	15.5±0.3	19.1±0.6	22.0±0.1	7.1±0.9	6.6±1.3
	9	0.83±0.02	0.87±0.02	17.0±0.5	18.9±0.4	21.0±0.5	23.0±0.4	6.9±0.7	6.3±0.8
Custard	0	0.49±0.02	0.49±0.02	8.0±0.3	8.0±0.3	8.4±0.3	8.4±0.3	8.2±0.3	8.2±0.3
	3	0.59±0.02	0.61±0.01	10.5±0.4	11.2±0.2	10.3±0.7	12.1±0.3	7.9±1.1	7.7±0.6
	6	0.60±0.02	0.65±0.03	11.5±0.3	13.0±0.3	11.0±0.1	13.0±0.1	7.2±0.6	7.2±1.1
	9	0.63±0.03	0.71±0.02	12.7±0.3	14.2±0.6	13.0±0.2	15.2±0.4	6.8±0.0	6.3±0.8

are SPC < 10000/g, yeasts and moulds < 100/g, *E. coli* and pathogens - absent.

Storage stability : The stability of the foods stored at different temperatures is shown in Table 5. At RT, slight increases in FFA, PV and TBA were detected in all the foods over 6 to 9 months. The increases in PV and TBA were more pronounced in *dalia* and *kheer* than in custard, though there were no discernible off-odours. A similar trend was observed in samples stored at 37°C. Organoleptic evaluation revealed that the products remained acceptable (score of 6 and above) throughout the storage period of 9 months.

In the accelerated storage study, 3 months at 37°C are equated to more than one year at ambient room temperature (Hanson 1961). For space foods, the maximum stability period was required to be 3 months at 37°C. For other foods required by the Indian Armed Forces, it is at least one year at room temperature, i.e., 30° ± 2°C. The demonstrated storage life of the present foods, therefore, meets this criterion. Based on the data presented in this study, freeze-dried *dalia, kheer* and custard will meet the requirements of defence forces, expeditions and other similar missions.

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Effect of Ingredients and Processing Conditions on Fat Absorption and Texture of *Mysorepak*

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The effects of process parameters and functional properties of the ingredients on fat absorption and texture of *Mysorepak* were studied. The results revealed that the processing conditions, such as syrup strength, mixing time and temperature of the fat have considerable influence on fat absorption and texture of *Mysorepak*. The type of fat suitable to impart the desirable texture was found to be a mixture of hydrogenated fat and liquid oil. The fat absorption by cereal flours, such as *maida*, rice and corn starch, was found to be less than that of *besan* (*Bengalgram* flour). Rice flour and corn starch were not well suited for the preparation of *Mysorepak*. Refined wheat flour could be used in the preparation, but it imparted harder texture. The hardness of the product was found to be inversely proportional to the quantity of fat in the product. *Mysorepak*, having a wide range of textural properties (hardness), could be prepared by altering process parameters and relative proportions of the ingredients. The product formulation and process parameters to obtain *Mysorepak* having uniform texture and fat content, have been specified.

Keywords : *Mysorepak*, *Besan*, Texture, Refined wheat flour, Corn starch, Wheat flour, Dilatometry, Sensory evaluation.

India produces a variety of traditional sweets, each having unique sensory attributes. These sweets are an integral part of the Indian social fabric and a must at social gatherings as well as in all festive occasions. Though it is an age-old small scale industry, there is no scientific information available on many of these products, which would help in developing this into larger industry on par with Western confectionery. Though scores of traditional sweetmeats are known, basically, they are prepared using only three or four base materials such as milk, cereal flours, wheat and legumes particularly *Bengal*gram (*Cicer arietinum*) and fat. Yet, the sweetmeat manufacturers, using their inherited art/skill and effecting subtle changes in processing conditions, have been able to provide a large variety of sweetmeats in the market place. For example, the products prepared using *Bengal*gram flour (*besan*), such as *laddu*, *sohne papdi* and *Mysorepak*. though have similar ingredients, the textures of these products are entirely different. A study has also been done on *Mysorepak* by substituting soya flour (Sharma et al. 1992) to the extent of 25-100%, without significantly affecting the textural properties. In order to provide scientific information to some of these products, *Mysorepak* was chosen for the study. *Mysorepak* is one of the popular sweets in the country. It is prepared from *besan*, sugar and fat, for sale in rectangular blocks.It has a porous, honey-comb texture (like cake), property to crumble readily in the mouth and has characteristic flavour. In common with any traditional sweetmeat, the quality, especially the texture and fat content of the product, differs from batch to batch and from one manufacturer to another. This is mainly due to lack of optimization of product formulation and process parameters. Hence, in the present paper, results of a study on the effects of various processing conditions and role of ingredients on fat absorption and texture of *Mysorepak* are reported.

Materials and Methods

*Bengal*gram *dhal* (split), hydrogenated vegetable fat (*vanaspati*), refined groundnut oil and *ghee* (butter fat) were purchased from the local market. *Bengal*gram *dhal* was ground in a plate mill and the flour passing through 60 mesh (IS sieve) was used. Maize starch was procured from M/s Lakshmi Starch Ltd., Bangalore. *Phulwara (Madhuca butyracea)* kernels were procured from Khadi and Village Industries Commission, Pithorgarh, Uttar Pradesh. Fat was extracted from the kernels by Handler baby expeller and was refined using alkali (AOCS 1971).

Preparation of Mysorepak : The following traditional procedure was used to prepare *Mysorepak:* Sugar, taken in a SS pan, was dissolved by heating in a minimum quantity of water. The syrup was boiled to the required strength (70-85°C Brix).The strength of sugar syrup was checked with a hand refractometer. With the pan still on the stove, *besan* was added and contents mixed thoroughly (1-4 min) with the syrup, ensuring the absence of lumps. At this stage, the fat, heated separately (110-200°C), was added in small portions,

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while stirring the mass continuously. Addition of fat was discontinued, when it started separating out from the mass as a distinct phase. The hot mass was immediately poured on to a tray, allowed to cool and cut into rectangular blocks.

Studies on process parameters and role of ingredients : The effect of sugar syrup strength on fat absorption was determined at the constant ratio of *besan* to sugar (60:100) and 2 min mixing time. The fat (50/50 *vanaspati*/groundnut oil) was heated to 150°C, prior to its addition to the hot *besan*-syrup mass. In the next experiment, the mixing time was varied from 1-4 min at 60:180 ratio of *besan* to sugar, 78° Brix syrup strength and the use of *vanaspati*/groundnut oil (50/50) heated to 150°C. The effect of temperature of the added oil was evaluated in the range of 110 to 200°C, when other parameters such as 180/60 *vanaspati*/groundnut oil, 70° Brix syrup strength, 2 min mixing time and 50/50 *vanaspati*/groundnut oil were constant. For evaluating the effect of different ratios of *besan* to sugar, the other parameters were kept constant, i.e., 2 min mixing time, 150°C fat (50/50 *vanaspati*/groundnut oil) temperature. Under identical conditions, (180/60 sugar/*besan*, 78° Brix syrup strength, 2 min mixing time and 150°C fat temperature), the effect of various types of fats (groundnut oil, *vanaspati*, *phulwara* butter, *ghee* and 50/50 *vanaspati*/ groundnut oil) was evaluated. The effect of different cereal flours/starches was evaluated, when other parameters were constant (186/60 sugar/flour or starch, 78° Brix syrup strength, 2 min mixing time and 150°C fat (50/50 *vanaspati*/groundnut oil) temperature. In this experiment, sugar to *maida* ratio was also varied to 180:70 and 180:80. The effect of the quantity of fat was evaluated at constant parameters of 180/60 sugar/*besan* ,2 min mixing time and 150°C fat (50/50 *vanaspati*/ groundnut oil) temperature.

Physico-chemical analysis : Moisture content of the samples was determined by drying at 110°C for 5 h, in a hot air oven (AOAC 1960). Bulk density was determined by water displacement and also by measuring all the sides (length, breadth and height), weight of the rectangular pieces and expressed as g/cc. The fat content was determined (AOCS 1971) by Soxhlet extraction of dried samples, using petroleum ether (40-60°C).

Texture measurement : An Instron Universal Testing machine (model 1140) was used for measuring compression and WB shear. A flat, cylindrical plunger of 25 cm^2 cross section was used for compression. Shear was measured using horizontal blade. Cross head speed in both cases was 10 cm/min and chart speed was 20 cm/min. Depending on the hardness of the product, the full scale load was changed from 10 kg to 60 kg for compression and 5 kg to 50 kg for shear. The samples were cut into 2.5 cm cubes for measuring both compression and shear. The first peak was taken for calculation.

Dilatometry : Dilatation studies were carried out according to the standard procedure and the solid contents were calculated from the dilatation values (BS 1976).

Sensory evaluation : The sensory quality of *Mysorepak* was evaluated by a panel of 15 members. The hardness, melt-in-mouth, flavour and overall acceptability of the product were assessed and the opinion of the majority was taken for quality assessment.

Results and Discussion

The commercial samples of *Mysorepak* have a fat content varying from 40-50%, while more popular samples possess a still higher fat. With this in view, attention was paid to the quantity of fat taken up, when either the *besan :* sugar ratio was varied or the process parameters were changed.

Effects of process parameters

Effect of syrup strength : The effect of syrup strength on fat absorption at a constant ratio of *besan* to sugar and when the fat was heated to 150°C, prior to its addition to the hot *besan* -syrup mass, was evaluated. The results indicated (Table 1) that, in general, the fat absorption decreased and hardness increased as the strength of sugar syrup increased, especially above 78° Brix. However, the porous texture was not affected as indicated by bulk density. The moisture content of the sugar syrup-*besan* mass, prior to addition of fat, also appears to play an important role in fat absorption. As the moisture content decreased or the sugar syrup strength increased, there was a decrease in fat absorption (Table 1). However, at low syrup strengths (below 70° Brix), the *besan*-syrup mass absorbed the added fat quickly in the initial stages, but during the course of cooking (which lasted for 8-10 min), a large amount of the absorbed fat oozed out. The resulting product was hard and devoid of the characteristic texture of *Mysorepak*. The products prepared at syrup strengths of 74-78° Brix were found to be good and had desirable sensory attributes, whereas those prepared with 80° Brix and above were hard and had dry mouth feel (Table 1).

TABLE 1. EFFECT OF SUGAR SYRUP STRENGTH ON FAT ABSORPTION AND TEXTURE OF *MYSOREPAK*

Syrup strength, ° Brix	Fat absorption, g/batch*	Moisture content, %		Instron units, kg		Bulk density, g/cc	Sensory attributes
		Besan/syrup mix	Product	Compression	Shear		
67	162	ND	ND	3.8	ND	ND	ND
70	170	ND	2.5	4.9	ND	ND	ND
74	266	19.8	1.9	1.2	0.4	ND	Very good
76	210	15.5	2.3	1.6	1.0	0.78	Good
78	193	14.9	2.5	2.7	1.4	0.85	Good
80	155	5.0	3.0	3.6	1.3	0.85	Hard
85	153	ND	2.9	4.4	2.2	0.83	Not good

* Sugar:180 g ; *besan* : 60 g; Mixing time : 2 min. ; Fat used : *vanaspati*/groundnut oil (50/50) - heated to 150°C; ND : Not done

Effect of mixing time : The results in Table 2 indicate that longer the duration of mixing of sugar syrup and *besan*, lower was the fat absorption. This could be due to decrease in moisture content (as observed in the case of sugar syrup) with longer mixing time (Table 2). It was rather difficult to maintain moisture level constant under the conditions used. The products having lower fat content, resulting from longer mixing time (3 min and above), were found to be harder and not acceptable (Table 2).

viscosity of the system, possibly, contributes to greater ease of physical entrapment of fat/oil. Alternately, higher temperatures, perhaps, denature the proteins of *Bengal*gram, exposing polar groups (hydrophilic), that result in decreased fat absorption. Also, as temperature of the added fat increases, moisture is removed at a rate faster than is desirable, thereby destroying the water-protein, water-sugar solution properties, which are important for fat absorption. This was confirmed by increasing the rate of heating, just before addition of fat,

TABLE 2. EFFECT OF MIXING TIME OF SUGAR SYRUP AND *BESAN* ON FAT ABSORPTION AND TEXTURE OF *MYSOREPAK*

Mixing, time, min	Fat absorption, g/batch*	Moisture content, %		Instron units, kg		Bulk density, g/cc	Sensory attributes
		Besan/syrup mix	Product	Compression	Shear		
1	225	17.0	2.5	ND	ND	0.74	Very good
2	193	14.9	2.5	2.7	1.3	0.85	Good
3	171	13.6	2.8	2.4	1.5	0.81	Fair
4	134	5.1	1.9	8.4	2.4	0.76	Hard, not good

* Sugar: 180 g; *besan*: 60 g; Syrup strength : 78° Brix; Temperature of fat : 150°C; Fat used: *vanaspati*/groundnut oil (50/50); ND : Not done

Effect of temperature of added fat : The results in Table 3 show a definite relationship between temperature of the added fat and its absorption. With the moisture content of the mix, maintained constant, before addition of fat, the fat absorption decreased with increase in temperature of the added fat upto 200°C. This could be due to more than one reason. At lower temperatures, the high

which resulted in lowering of fat absorption. However, when the temperature of fat is very low (100°C and below), the fat absorption was slow and also it did not have any influence on fat uptake. The fat heated to about 150°C was found to be ideal. In general, the products having higher fat content were found to have better sensory attributes.

TABLE 3. EFFECT OF TEMPERATURE OF ADDED FAT ON ITS ABSORPTION AND TEXTURE OF *MYSOREPAK*

Temperature of fat, °C	Fat absorption, g/batch*	% fat based on dry wt. of product	Moisture, %		Instron units, kg		Sensory attributes
			Besan/syrup	Product	Compression	Shear	
110	207	46	ND	ND	ND	ND	ND
130	218	47	ND	ND	ND	ND	ND
150	193	44	14.9	2.5	2.7	1.3	Good
170	170	42	ND	ND	ND	ND	Good
180	164	40	14.5	2.4	4.0	1.6	Hard
200	150	38	13.9	2.8	6.0	1.9	Harder

* Sugar/*besan* : 180/60 g; Syrup strength : 78° Brix; Mixing time : 2 min; Fat used : *vanaspati*/groundnut oil (50/50); ND : Not done

Role of Ingredients

Effect of besan-*sugar ratio :* The results in Table 4 show that increasing the proportion of *besan* to sugar from 50:180 to 80:180 had little influence on fat absorption. However, when the proportion of *besan* was reduced to 40, the fat absorption was lowered. It is interesting to note that decreasing the proportion of sugar, lowered the fat absorption (Table 4). When the proportion of *besan* to sugar was 80:180, it imparted floury taste to the product and was not acceptable. The products prepared using lower *besan* ratios (40 and less) were found to be gritty, with lack of the characteristic flavour and were not acceptable (Table 4). The optimum ratio of *besan* to sugar was found to be 60-70:180.

Effect of type of fat : Fats having different solids content at various temperatures were used in the preparation of *Mysorepak* to assess their suitability. For this purpose, hard fats such as *vanaspati* and *phulwara* butter, liquid oils such as groundnut oil and a fat having solids content in between these two were chosen. The results in Table 5 show that the type of fat appeared to have no influence on fat absorption, but affected the texture to some extent. Hard fats such as *vanaspati* and *phulwara* butter were found to impart waxy mouthfeel, whereas, oil (i.e., groundnut oil), though imparted better appearance, gave oily mouthfeel. On the other hand, a mixture of *vanaspati* and groundnut oil (50/50) or *ghee* was found to impart the desirable texture and mouthfeel to *Mysorepak*. These results, therefore, revealed that a fat having an intermediate solids content, between those of *vanaspati* and liquid oil, was suitable for use in *Mysorepak* (Table 5).

Effects of cereal flours and starches : To find out the components responsible for fat absorption and texture of *Mysorepak*, *besan* was replaced by cereal flours such as refined wheat flour (*maida*), rice flour and corn starch. The results in Table 6 show that, under comparable conditions, *maida* absorbed less fat as compared to *besan*. This was not improved with increasing the ratio of *maida* (25 to 31% in fat-free mix) in the recipe. The fat absorption by rice flour or corn starch was much lower than that by *maida*. The order of fat absorption was *besan* > *maida* > rice flour > corn starch. Also, it can be seen from Table 6 that the bulk density of the products prepared with rice flour or corn starch was higher, when compared to those prepared with *besan* or *maida*. These results suggest that

TABLE 4. EFFECT OF SUGAR/*BESAN* RATIO ON FAT ABSORPTION AND TEXTURE OF *MYSOREPAK*

Besan/ sugar, (w/w) 'a'	Fat absorbed, g per 100 g mix 'a'	*Mysorepak*				Sensory attributes
		Fat, %	Moisture, %	Instron units, kg		
				Compression	Shear	
40/180	58.0	37.5	2.1	6.2	2.4	Lacks flavour
50/180	78.0	43.6	2.5	3.1	1.0	Good
60/180	80.4	44.3	2.5	2.7	1.4	Very good
70/180	82.0	ND	3.6	3.3	1.0	Good
80/180	87.7	46.5	ND	ND	ND	Floury taste
60/150	79.5	44.0	4.0	ND	ND	Fair
60/120	72.2	41.3	5.1	8.3	2.3	Not acceptable
60/120	55.5	35.0	5.7	Soft, like *halwa*		

Strength of sugar syrup : 78° Brix, except for last row, where it was 55° Brix; Mixing time : 2 min.; Temperature of fat : 150°C; Fat used : *vanaspati*/groundnut oil (50/50); ND : Not done

TABLE 5. EFFECT OF TYPE OF FAT ON TEXTURE OF *MYSOREPAK*

Fat type	Fat uptake, g/batch*	% fat on dry wt. basis	Moisture content, %	Instron units,kg		% Solids at °C						Sensory attributes
				Compression	Shear	20	25	30	32.5	35	37	
Groundnut Oil	195	44.4	3.7	1.4	0.5	0	0	0	0	0	0	Oily mouthfeel
Vanaspati	210	46.4	3.3	ND	2.4	35.0	25.9	22.0	17.0	13.0	8.0	Waxy mouthfeel
Vanaspati/ groundnut oil (50/50)	193	44.3	2.5	2.7	1.3	12.3	9.0	5.5	3.2	1.6	ND	Very good
Phulwara butter	184	43.1	2.9	ND	ND	68.8	62.6	38.4	29.0	10.0	6.7	Waxy mouthfeel
Ghee	190	44.0	1.7	ND	ND							Very good

• Sugar/*besan* : 180/60 g; Syrup strength : 78° Brix; Mixing time : 2 min.; Temperature of fat : 150°C; ND : Not done

TABLE 6. EFFECTS OF CEREAL FLOURS ON FAT ABSORPTION AND TEXTURE OF *MYSOREPAK*

Flour type	Fat uptake, g/batch*	Moisture content, %		Instron units, kg		Bulk density, g/cc	Fat content, % on dry wt. basis
		Besan/syrup mix	Product	Compression	Shear		
Besan	193	14.9	2.5	2.7	1.4	0.85	44.3
Maida	143	13.1	3.0	15.0	7.9	ND	37.0
	132	10.0	2.7	17.0	9.8	ND	35.2
*Maida***	121	ND	2.7	42.0	13.7	0.86	32.4
*Maida****	118	ND	3.6	38.0	ND	0.90	31.0
Rice	88	8.8	2.5	30.0	10.5	1.10	26.8
Corn starch	40	8.6	2.5	Very hard		1.10	14.0
Besan/corn starch							
(50/10)	179	NA	2.6	2.3	1.2	0.75	42.4
(40/20)	153	NA	2.6	4.3	2.5	0.76	38.6
(30/30)	122	NA	2.4	16.2	12.4	0.71	33.7

* Sugar/flour : 180/60 g; ** 70 g *maida* per batch; *** 80 g *maida* per batch; Syrup strength : 78° Brix; Mixing time : 2 min; Temperature of fat : 150°C; Fat used : *vanaspati* + groundnut oil (50/50); NA : Not applicable; ND : Not done

the amount of fat absorption and the texture were, perhaps, related to the protein content and quality of the cereal flours. Thus, gradually replacing *besan* with corn starch (Table 6) resulted in lowering of fat absorption. With comparable fat content, the product prepared with *maida* was harder than that prepared with *besan* (Tables 6 and 7). Likewise, the products prepared with rice flour or starch were rather hard. It has been reported that the proteins,

TABLE 7. EFFECT OF QUANTITY OF FAT ON TEXTURE OF *MYSOREPAK*

Fat Content		Moisture content, %	Instron units, kg		Bulk density, g/cc
g/batch* absorbed	% on dry wt. basis		Compression	Shear	
106	30.6	2.4	27.5	7.4	0.79
134	35.8	1.9	8.5	2.6	0.76
153	39.0	2.9	4.6	2.3	0.83
164	40.0	2.4	4.2	2.0	ND
193	44.3	2.5	2.8	1.4	0.85
210	46.6	2.3	2.0	1.0	0.78
266	52.0	1.9	1.4	0.4	ND

* Sugar/*besan* : 180/60 g; Mixing time : 2 min; Temperature of fat : 150°C; Fat used : *Vanaspati*/groundnut oil (50/50): ND : Not done

having more lipophilic side chains, are believed to bind hydrocarbon chains of fat/oil, thereby contributing to increased fat absorption (Hutton and Campbell 1981). Thus, the differences in fat absorption capacity may be due to the extent of lipophilic groups present in each ingredient (i.e., *Bengal*gram, rice, corn, *maida*, etc.). This may be of significance in preparation of *Mysorepak* also.

Effect of quantity of fat : Examination of the data in this study indicated that there existed a direct relationship between the fat content and texture of *Mysorepak*. The hardness of the product increased, as the fat content was lowered (Table 7). However, the porous honey-comb texture was not affected by the fat content of the product, as revealed by bulk density (Table 7).

Conclusion

From these results, it can be concluded that the process parameters and the relative proportions of the main ingredients play an important role in fat absorption and texture of *Mysorepak*. By controlling these, *Mysorepak*, having uniform fat content and texture, could be obtained.

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Effect of Natural Tannins on Canned Green *Bengal*gram (*Cicer arietinum*)

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Effect of process operations and natural tannins on the quality of canned green *Bengal*gram indicated that the tannins from green leaf tissue of *Bengal*gram are highly suitable as blanching and covering medium. It maintained the colour of the seeds and imparted acceptability to the product.

Keywords : Natural tannins, *Bengal*gram canning, Blanching, Covering medium.

*Bengal*gram, a well known multipurpose crop grown in India, contains 17-18% protein of good digestibility and assimilation properties (Singh et al. 1981). The regular intake of about 40 g green *Bengal*gram has been shown to reduce low density lipoprotein chclesterol quickly (Carper 1988). It was reported that both proteins and lipids in *Bengal*gram lowered serum cholesterol, while liver cholesterol was lowered by protein and not by lipid fraction (Murthy and Urs 1985). Potential also exists for export of green *Bengal*gram to gulf countries. However, no information on the canning trials is reported till now. Like other protein-rich green legumes, *Bengal*gram suffers from severe discolouration and heavy losses in nutrients, after drying and during storage. Therefore, the effects of processing operations and natural tannins on the quality of canned green *Bengal*gram were assessed.

Uprooted whole plants of green *Bengal*gram, with their leaves and pods, as well as the fresh and tender spinach leaves were procured from the local market. Green *Bengal*gram pods were hand-peeled and sound, uniform, semi-matured green seeds were subjected to different blanching and covering media, as per following details:

(a) blanching in tap water and the use of brine (2% salt + 4% sugar solution) as covering medium (T_1); (b) blanching in spinach extract (40%, w/v) and covering with (40% g w/v) *Bengal*gram leaf extract (T_2); (c) blanching in spinach extract (40%, w/v) and covering with brine solution of the composition as described in (a) above (T_3); and (d) blanching in spinach extract (40%, w/v) and covering with (40%, w/v) spinach leaf extract (T_4). After the blanching treatments, the seeds were filled in cans (470 g/can), covered with brine as specified in T_1 to T_4, exhausted for 10 min at 82.2°C, sealed, sterilized for 35 min in an autoclave at 115°C, cooled under tap water/fan to room temperature and stored at 30 ± 10°C.

Fresh and processed samples were analysed for total soluble solids (TSS), using refractometer at 20°C, and the pH was measured. AOAC (1984) methods were followed for determination of the proximate composition. The protein was estimated by the method of Khanna et al (1969), while free tyrosine and phosphorus were measured by the method of Folin and Ciocalteu (1927) and Fiske and Subbarao (1925), respectively. Estimation of sodium, potassium and calcium was as per Piper (1966). Direct current plasma emission spectrophotometer was used for the estimation of iron, magnesium and zinc. Organoleptic evaluation was carried out based on the responses of a panel of five judges, using numerical score method (Swaminathan 1979).

Cut-out examination of the cans containing treated sample showed no significant difference in drained weight (425-475 g). Vaccum inside the cans varied from 15-18 lbs and headspace ranged between 0.23 and 0.28 inch. A slight fall in vaccum and headspace was observed during storage, perhaps due to the liberation of hydrogen gas, as a result of Maillard reaction and corrosion (Cruess 1958). A gradual increase in pH and TSS was observed during storage (Table 1). This may be due to partial hydrolysis of the complex carbohydrates, acid contituent of the seed pulp and interaction between the organic constituents of the products. Ascorbic acid decreased invariably in the samples subjected to different treatments. The retention of ascorbic acid was better in samples T_2, followed by T_4, T_3 and T_1. These losses might be due to blanching in the covering medium and oxidation during heating. A slight increase in carbohydrate was observed in samples T_1 and T_3 during storage,

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TABLE 1. BIOCHEMICAL COMPOSITION OF CANNED GREEN *BENGALGRAM* DURING STORAGE

Constituent	Storage period								
	Fresh	30 days				60 days			
		T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Total soluble solids (°Brix)	8	11	9	10	9	12	11	12	11
pH	4.5	4.8	3.8	4.3	4.2	4.7	3.7	4.2	3.9
Ascorbic acid, mg/100 g	12	4	8	4.5	6	2.5	6	2	3.2
Tannin, as % tannic acid	0.42	0.06	0.62	0.08	0.53	0.04	0.67	0.04	0.56
Protein, mg/g	285.7	194	245	208	224	192	244	207	223
Total carbohydrates, mg/g	540	546	522	547	520	540	510	531	500
Tyrosine, g/g	2.23	1.96	2.17	2.03	2.15	1.85	2.17	1.91	2.04
Chlorophyll "a", µg/g	207.2	150	180	162	172	142	175	156	168
Chlorophyll "b", µg/g	74.3	42	55	48	51	30	52	45	45
Sodium, ppm	17	62	17	46	20	60	15	45	18
Potassium, ppm	60	22	29	12	20	19	26	9	19
Calcium, ppm	24	10	17	18	13	6	15	5	10
Zinc, ppm	26	20	20	16	19	14	18	10	14
Magnesium, ppm	98	97	90	98	89	90	86	93	85
Iron, ppm	90	100	85	77	79	105	84	75	77
Phosphorus, ppm	380	320	360	318	325	286	340	290	295

thereby indicating endosmosis of sugar of the covering media. The decrease in total carbohydrates in T_2 and T_4 might be due to degradation of carbohydrates by heat. Reduction in tyrosine content was observed in all the samples during storage, the loss being maximum in sample T_1, as compared to fresh gram (Table 1). This could be assigned to chemical reaction of amino acids and carbohydrates in the canned green *Bengal*gram.

Decreases in tannin contents in T_1 and T_3, as compared to the increases in T_2 and T_4, were observed (Table 1). The maximum tannin was found in T_2 and minimum in T_1. The probable reason for these changes in tannins may be attributed to the products into the covering medium, during the processing. In contrast, sodium increased significantly, while the decrease in iron was not significant.

Organoleptic quality of the canned green *Bengal*gram revealed that the sample T_2, blanched in 40% spinach leaf extract and covered by green *Bengal*gram leaf extract, scored significantly high acceptability, probably due to stabilization of the colour by tannin- protein complex (Table 2). The samples T_1 and T_3 were unattractive in appearance, thereby making the product difficult to market. These observations are further supported by the increase in the value of chlorophyll and minimum

TABLE 2. ORGANOLEPTIC SCORE OF CANNED *BENGALGRAM* DURING STORAGE

Quality	Treatment scores at the storage period of											
	Fresh				30 days				60 days			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Colour	60	85	68	75	54	82	60	70	50	75	55	65
Flavour	50	70	60	60	48	65	54	55	40	60	50	50
Taste	86	70	80	70	80	67	75	65	75	60	70	60
Texture	55	70	70	72	52	68	65	67	50	65	60	65
Average of total score	62.7	73.7	69.5	69.5	58.5	70.5	63.5	64.3	53.8	65.0	58.8	60.0

the hydrolysis of tannins into sugars, acids and other compounds (Salunkhe et al. 1968). A decrease in protein was observed, except in sample T_1. The maximum protein was found in sample T_2, probably due to formation of tannin-protein complex, and the prevention of denaturation of the proteins. The observations are in conformity with the findings of Lopez and Williams (1985). Calcium, magnesium, phosphorus, potassium and zinc decreased significantly, probably due to their leaching from losses in tyrosine in samples T_2 and T_4. These, in turn, indicate minimum degree of discolourations.

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Effect of Fungal Infestation on the Starch, Lipids and Dry Weight of Maize Seeds

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Infestation of three varieties of maize (*Zea mays* L.) by six dominant maize seed fungi, viz. *Alternaria alternata*, *Aspergillus flavus*, *Fusarium moniliforme*, *Penicillium expansum*, *Rhizopus nigricans* and *Trichoderma viride*, resulted in the reduction in dry weight, increase in fat acidity and depletion of starch. *A. flavus* increased fat acidity in, 'Local White' variety and 'Vijay' (53.5% and 69.2%) and *P. expansum* in 'VL-16' variety (74.2%), after 30 days of incubation. Starch depletion was found to be slow during the initial phase of incubation, but became pronounced after 30 days of incubation. *A. flavus* showed maximum depletion of starch (48.5%) and *A. alternata* the least (25.3%). Highest reduction in dry weight of the seeds was in case of *A. flavus*.

Keywords : Maize seeds, Infestation, Starch, Lipids, Dry weight, Fungi, *Aspergillus flavus*.

The role of fungi in seed deterioration has been studied by a number of workers all over the world (Goodman and Christensen 1952; Qasem and Christensen 1960; Lalithakumari et al. 1971; Krishna Reddy and Reddy 1986). One of the changes, associated with deterioration of seed, in general, and of oily seeds, in particular, is increase in their acidity (Milner and Geddes 1946). Milner et al (1947) reported increased fat acidity and loss of germination in the case of moulded wheat. Starch depletion was reported by Vidhyasekaran and Kandaswamy (1972) in *mung* and by Bilgrami et al (1979) in paddy. In this communication, the effect of a few dominant seed fungi of maize on the changes in dry weight, fatty acid and starch contents is reported.

Six dominant seed-borne fungi of maize, viz., *Alternaria alternata*, *Aspergillus flavus*, *Fusarium moniliforme*, *Penicillium expansum*, *Rhizopus nigricans* and *Trichoderma viride* were used. Spore suspensions of 10 days old cultures were prepared individually and the surface-sterilised seeds of three varieties of maize, viz., 'Local White', 'Vijay' and 'VL-16' were infested aseptically. The treated seeds were incubated at room temperature for 10, 20 and 30 days in alternating cycles of 12 h light (NUV) and darkness. Control set was maintained by adding three ml sterile distilled water, instead of spore suspension. Quantitative estimations were performed on the 10th, 20th and 30th days of incubation.

Analytical methods : Dry weight of 100 seeds was estimated by weighing the seeds, after drying at 100°C for 24 h. The free fatty acid content of the seeds was estimated by the standard AOAC (1960) method. Starch content of maize seeds was estimated by colorimetric method given by Snell et al (1961).

Dry weights : Slightly continuous and gradual fall of dry weight, during the incubation period in both treated and control samples, was observed (Table 1). However, the loss of dry weight in the treated samples was more pronounced. All the three varieties contaminated with *A. flavus* showed maximum reduction in dry weight, after 30 days of incubation (15.94% - 17.23%). The other test fungi also reduced the dry weights of the seeds in all the varieties. Statistical analysis (ANOVA) showed that there were significant variations in the dry weights of the seeds between the incubation periods ($F=31.669^{**}$, 23.466^{**} and 28.498^{**} for 'Local White', 'Vijay' and 'VL-16' respectively) as well as between the fungal species ($F=7.425^{**}$, 5.955^{**} and 6.052^{**} for 'Local White', 'Vijay' and 'VL-16', respectively). Several seed-borne fungi are reported to cause considerable losses in the seed contents (Goodman and Christensen 1952; Lalithakumari et al. 1971; Vidhyasekaran et al. 1973). The spoilage of seeds in storage becomes more pronounced under improper storage conditions, which provide ideal environment for the rapid colonisation of seeds by storage microorganisms (Christensen and Kaufman 1969). The loss of dry weight may be attributed to starch depletion (Bilgrami et al. 1979; Vidhyasekaran et al 1973).

Fatty acids : Both the infested and control seeds exhibited increases in fatty acid contents, with progress of time (Table 1). However, uninfested

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seeds showed negligible change in the fatty acid content. During the initial incubation, the rise in the fat acidity level was less pronounced than that in the last phase of incubation, in all the three varieties of maize. *A. flavus* caused maximum all varieties tested. During the initial phase of incubation, the starch depletion, in general, was slow, but became pronounced after 30 days of incubation period. *A. flavus* was responsible for maixmum depletion of starch and *A. alternata* the

TABLE 1. EFFECT OF FUNGAL INFESTATION ON THE DRY WEIGHT, FATTY ACID AND STARCH CONTENTS OF MAIZE SEEDS

Fungi	Incubation period, days 10			20			30		
	A	B	C	A	B	C	A	B	C
				Dry weight of 100 seeds, g					
A.alternata	15.1	18.1	20.0	14.6	17.8	19.8	14.7	17.3	19.3
A.flavus	14.2	16.8	19.1	13.8	16.2	18.5	13.3	15.4	17.4
F.moniliforme	14.8	17.4	20.4	14.3	17.3	19.3	13.8	16.6	18.7
P.expansum	14.6	17.1	19.2	14.2	16.8	18.6	13.7	16.1	17.7
R.nigricans	15.1	17.7	19.9	14.4	16.1	19.0	14.1	15.5	18.1
T. viride	14.7	17.6	19.7	14.1	17.3	19.1	13.9	16.3	18.2
Control	16.0	18.6	20.6	15.9	18.5	20.5	15.8	18.4	20.4
				Fat acidity, mg KOH required/100 g flour					
A.alternata	15.0	14.5	19.5	16.5	17.5	21.0	18.0	18.5	23.5
A.flavus	17.0	16.0	21.0	19.0	20.0	24.5	21.5	22.0	29.0
F.moniliforme	15.5	15.5	20.0	17.0	17.0	22.0	18.5	19.0	24.5
P.expansum	16.5	16.0	22.0	18.0	19.0	23.5	20.0	20.5	30.5
R.nigricans	14.5	15.0	18.5	15.5	17.5	20.5	17.5	19.0	24.0
T.viride	15.0	15.5	19.0	16.5	18.0	21.5	18.0	20.5	23.0
Control	14.5	13.5	18.5	14.5	14.5	19.0	15.0	15.0	20.0
				Starch content, g/100 g flour					
A.alternata	30.2	32.4	25.3	28.4	29.5	22.2	24.2	28.5	19.8
A.flavus	26.3	29.2	23.7	23.3	24.6	20.4	18.1	18.3	16.3
F.moniliforme	29.5	31.7	24.1	26.2	28.1	22.6	20.7	24.2	18.5
P.expansum	27.6	30.6	22.6	24.3	24.5	19.7	19.6	20.4	16.6
R.nigricans	30.4	32.8	27.1	27.7	27.3	20.9	20.3	21.7	17.1
T.viride	27.6	31.3	26.4	25.6	25.3	20.8	20.5	18.4	16.9
Control	32.1	35.1	28.8	31.5	34.7	28.3	31.1	34.3	27.6

A : Variety 'Local White', B : Variety 'Vijay', C : Variety 'VL-16'.
Control values were 16.0, 18.6 and 20.7 g for dry weight of 100 seeds; 14.0, 13.0 and 17.5 for fat acidity/100 g flour; and 32.4, 35.6 and 29.5 g starch/100 g flour, respectively, for A B and C.

increase in fat acidity level in the varieties 'Local White' and 'Vijay', whereas *P. expansum* showed maximum increase in the variety 'VL-16'. Statistical analysis (ANOVA) showed that there was significant variation in fat acidity between the incubation periods (F = 4.287*, 52.122** and 28.388** for 'Local White', 'Vijay' and 'VL-16', respectively). Significant variations between the fungal species were noticed only in 'Vijay' and 'VL-16' (F = 5.622** and 4.489**, respectively). Increase in fat acidity due to fungal infestation was also reported by Sauer and Christensen (1968) in corn, Lalithakumari et al (1971) in groundnut oil and Charya and Reddy (1981) in *mung*. According to Goodman and Christensen (1952), fatty acids were a product of fungal lipase activity upon corn oil and none of the fungi caused any increase in fatty acid in oil-free corn meal.

Starch content : All the test fungi were responsible for depletion of starch in the seeds of least. Variations in starch contents were found to be statistically significant between the incubation periods (F = 35.15**, 10.733** and 39.346**) in all the varieties, while variations between the fungal species were significant only for 'Local White' and 'VL-16' (F= 5.244** and 5.018**, respectively), but not for 'Vijay' (F = 2.253 NS). Starch depletion in seeds due to fungal infestation may be attributed to starch hydrolysing enzymes or its utilisation by the fungi as respiratory substrate. Similar results have also been reported by other workers (Vidhyasekaran and Kandaswamy 1972; Harris 1976; Baker 1965).

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Qualitative and Quantitative Analysis of Dust Pollution in *Dhal* mill

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In-plant air samples of *dhal* mills were analysed for their quantity, chemical makeup, particle size and fungal profile. The average concentration of suspended and settleable dust was 0.057 g/m³ and 0.065 kg/m²/day. The average particle size of the suspended dust was found to be 3.2 μ, while the major portion of the dust was chemically organic in nature. *Aspergillus* and *Penicillium* species were found to be associated with the *dhal* dust. In-plant air samples of all the *dhal* mills were found to be severely polluted. Several pollution control measures are suggested.

Keywords : Air pollution, Settleable dust, Suspended dust, *Dhal* mill, Particle size, Relative density.

Contamination of clean air by particulate material occurs through a number of sources such as soil, water, raw material of both plant and animal origins and human beings. The food and agricultural industries are one of the major contributors of particulate emission, because of the light weight and dry condition in which various unit operations, such as cleaning, sieving, grinding and drying are done. The particulate matters, alone or in combination with other pollutants, constitute a serious health hazard (Wark and Warner 1982). The particulate matter enters the human body mainly via the respiratory system, thereby causing respiratory diseases. Literature related to the environmental pollution, caused by different mills in which foodgrains are processed, is rather scanty. Qualitative and quantitative analyses of air-borne dust samples of corn and groundnut processing plants, wheat flour mill and convenience food factories were found to contain various microflora, that may cause occupational diseases (Devasthali 1978; Burg and Shotwell 1984; Shabbir and Sankaran 1991). No systematic study has so far been taken up to assess the level of air pollution in Indian *dhal* mills. Before any appropriate recommendations are made to the regulatory agencies for pollution control or to the manufacturer for modification and alteration in machines and systems to reduce pollution, it was considered necessary to undertake a study to determine the concentration of suspended and settleable dust in the plant atmosphere, as well as to analyse the dust for chemical and fungal profile.

Five *dhal* mills, located around Jabalpur, India, were selected and samples were collected in the months of June and July, 1989. The concentrations of the suspended dust particles in the in-plant atmosphere of the *dhal* mills were determined using a high volume sample (APM 410, Environtech, Delhi, India). The instrument was placed inside the *dhal* mill and operated for 2 h. The dust was collected on a preweighed glass microfibre filter paper (GF/A, Whatman, Wisconsin, USA). The volume of the air sample was measured using a manometer attached to the instrument. The difference in the initial and final weights of the filter paper was used for calculating the weight of suspended dust. The concentration of suspended dust was calculated as follows :

$$\text{Suspended particulate matter} = \frac{W \times 10^3}{V} \text{ g/m}^3$$

where V = volume of air sample (m^3), and
W = weight of suspended particulate matter (g).
The concentration of settleable dust was determined by following the method of Katz (1977) and expressed as kg/m²/day. The particle size of the dust was determined by measuring the individual diameter of the particle under optical microscope (14 x 45 magnification). The organic and inorganic contents of the *dhal* dust were determined by standard methods (AOAC 1976). Glucose content of the dust was determined by following the method of Chopra and Kanwar (1976). The mycoflora of dust was analysed in triplicate on Rose Bengal-agar-medium (Martin 1950), incubated for 120 h at 30°C. The fungi were identified using standard methods (Sutton 1980; Thom and Rapper 1985). The % relative density of each fungal species was calculated as described by Shrivastava and Jain (1992).

The concentrations of settleable and suspended

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dust in the *dhal* mills are presented in Table 1. These concentrations varied from 0.04 to 0.08 kg/m²/day and 0.03 to 0.09 g/m³, respectively. Suspended dust concentrations of five mills were much higher than the prescribed limit (500 µg/m³)

TABLE 1. QUANTITATIVE ANALYSIS OF *DHAL* DUST

Dhal mills	Suspended *dhal* dust concentration, g/m³	Settleable *dhal* dust concentration, kg/m²/day	Organic matter, %	Inorganic matter, %	Glucose, mg/g
A	0.04	0.04	91.29	8.71	0.10
B	0.09	0.08	90.30	9.70	0.07
C	0.03	0.04	87.52	12.48	0.07
D	0.04	0.08	91.74	8.26	0.08
E	0.07	0.08	90.60	9.40	0.09
Average	0.05	0.06	90.29	8.71	0.08

specified by the air prevention and control of pollution act, under section 16(2) (6) 1981 (Anon 1982). Similarly, the observed values were much higher than the primary (75 µg/m³) and secondary (60 µg/m³) standards fixed by Environmental Protection Agency (EPA) of the United States of America (Anon 1971).

Thus, such highly polluted in-plant atmosphere may cause severe health problems, leading to respiratory diseases among the workers.

The average particle size of the dust was found to be 3.20 µ, while the range of particle size of the *dhal* mill dust was 2.94 to 3.47 µ. Particulate matter larger than 50 µ settled quite easily and caused no problem, except the soiling. However, the particles of 0.5 to 2.0 µ size damaged the respiratory tract due to adhesion of the particles in the tract (Hunter 1975). The inorganic and organic matter in the dust varied from 8.26 to 12.48 and 87.52 to 91.74%, respectively. Similarly, glucose content of the dust varied from 0.07 to 0.09 mg/g (Table 1).

Different types of fungal species identified in the *dhal* dust are presented in Table 2. Species of *Aspergillus*, *Rizopus* and *Penicillium* were prominent and these fungi may produce allergic response in both the upper and lower respiratory tracts and cause broncho-pulmonal-aspergillosis (Hunter 1975). Colonies of *A. flavus*, *A. niger* and *A. nidulans* were more in the *dhal* dust collected in the month of July (Table 2). This may be due to prevailing rainy season. The compositions of air-borne fungal population were, more or less, same in the in-plant atmosphere of the *dhal* mill and the outside-plant atmosphere and mostly consisted of commonly known spoilage organism.

TABLE 2. MYCOFLORA OF *DHAL* DUST COLLECTED IN THE MONTHS OF JUNE AND JULY, 1989

Fungus	Relative density of fungal species, %			
	Dust from dehusking unit		Dust from outside of the dehusking unit	
	June	July	June	July
A. flavus	52.48	11.76	68.19	45.21
A. niger	32.58	54.41	5.45	27.82
A. nidulans	ND	16.17	ND	17.39
A. tamarii	ND	ND	9.98	3.47
A. versicolor	ND	1.47	ND	ND
Rhizopus sp.	6.78	7.35	10.91	2.60
Actinomycetes	ND	2.94	ND	1.73
Mucor sp.	ND	1.47	ND	ND
Absidia sp.	8.14	ND	ND	ND
Penicillium	ND	4.41	5.45	1.73

The major dust producing unit operations in the *dhal* mills might be the cleaning unit, the dehusking unit and the polishing unit. The area involving these unit operations and the machinery were not well enclosed and consequently, the dust from these unit operations enters into the air and causes air pollution. The following safety measures may prove helpful in controlling the air pollution in *dhal* mill: a) changes in the design of the cleaning, dehusking and polishing units so as to restrict the escape of the dust in the plant atmosphere and b) providing air cleaning device in the aspiration ducts. Thus, dust laden air will be sucked by this system for removal of dust particles from this polluted air in the subsequent operations. The resulting clean air can, then, be discharged into the atmosphere. Compulsory use of the dust-proof masks by all in the *dhal* mill, so as to prevent inhalation of *dhal* dust is yet another safety measure. In addition, the outlet of dehusker and bottom of roller should be provided with leak-proof enclosure.

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Development of Instant Dehydrated Wild Pomegranate *Chutney*

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The methods for preparing instant wild pomegranate *chutney* were evaluated and the products were analyzed for physico-chemical and nutritional characteristics. The product (T_3 combination) had good amounts of vitamin C, sugar, ash and fibre. It reconstituted well in cold water and possessed all characteristics of fresh *chutney*. The product had a shelf-life of more than 6 months.

Keywords: Wild pomegranate, Instant dehydrated *chutney*, *Anardana*, Sensory evaluation, Storage studies.

Wild pomegranate (*Punica granatum*) fruits are found in abundance in the dense forests of the hilly areas of Himachal Pradesh, Jammu and Kashmir and of Uttar Pradesh. The fruits are sour due to high acid content and are considered as significant importance in these regions. Being perishable in nature, the fruit is dried and preserved. Its dried seeds, called *anardana*, are collected every year from the hills, sold at various places throughout the country and also exported, for use in various industries like tanning, colouring etc. (Anon 1969). *Anardana* fetches a revenue of thousands of rupees every annum. One of the most important centres for wild pomegranate is Darlaghat, which is located about 50 km from Shimla in Himachal Pradesh. The word Darlaghat literally means a pass or place of wild pomegranate trees. The fruit is highly acidic, but with lesser pigments as well as total soluble solids. It forms a good source of nutrients, similar to other cultivated fruits and also has many medicinal values (Parmar 1981).

The wild fruit, due to its specific culinary taste and flavour, has good demand in rural as well as urban areas. Dried product is normally preferred because of reduced mass, lesser packaging as well as trasportation expenses and lower cost. Several fruits like apple, bamboo, tomato, coconut, and mango have been used for preparing different types of *chutneys* (Lal et al. 1967; Rao et al. 1991; Sethi 1991). However, the work on *anardana chutney* is scarce. Fresh *chutney* has very short shelf-life, unless preserved under refrigeration. Therefore, the present study was undertaken for the preparation of instant *anardana chutney* and the evaluation of its storability at room temperature.

Good quality wild pomegranate fruit, sugar, green chillies, salt, leaves of mint and coriander were procured from local market.

The wild pomegranate fruit was broken into halves the seeds were removed and kept in pan. Stalks of green chillies and roots of coriander as well as mint leaves were removed, while the other portions were washed in tap water. All the ingredients, including salt, were ground in an electrical blender for 3-5 min to uniform slurry. After screening of 14 different combinations of *chutney* by a panel of 16 judges for sensory quality, only five best combinations were selected (these gave good or very good ratings) for further optimization of the methodology of *chutney* preparation and the quantities of the ingredients. These combinations contained 250 g wild pomegranate, 5 g coriander leaves and 10 g mint leaves. Salt (10 g) was added to each combination, except for addition of 15 g salt to combination T_3. Sugar in 10 g and 15 g quantities was added only in combinations T_4 and T_5. Green chillies were used in 5, 10, 15, 15 and 20 g quantities in combinations T_1, T_2, T_3, T_4 and T_5, respectively. In all the cases, 10 ppm sodium benzoate was added to prevent any microbiological spoilage during the longer drying period of the *chutney*. The slurry was spread uniformly on aluminium trays of 30 x 45 cm size, at the rate 0.5 kg/tray, and dried in mechanical cabinet dehydrator at 65 ± 2°C for 10-14 h. Each sample (100 g) was packed in polyethylene pouches (300 gauge thickness) and stored at room temperature and 37°C.

Moisture, acidity, fat, total and reducing sugars, vitamin C, total ash, pH, and crude fibre were determined as per methods given by Ranganna (1986). Protein content of the products was determined by microKjeldahl method. All the determinations were made in triplicate and mean values are reported. The calorific values were calculated based upon the proteins, carbohydrates and fat contents in the products. The overall sensory quality of the reconstituted *chutney* was determined

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by composite scoring method, using Hedonic scale, and results were statistically analyzed by randomized block design method (O'Mohany 1986). For reconstitution of dehydrated *chutney*, cold water was added in the ratio of 2:1 and mixed well to get the desired consistency of the fresh *chutney*.

Table 1 indicates the physico-chemical characteristics of fresh fruit and dehydrated wild pomegranate *chutneys*. It is evident that the *chutney* is a good source of vitamin C, protein, fat and total sugars. The dehydrated *chutney* had an initial moisture content 3.0-6.2%. As the quantity of green chillies increased, the vitamin C, total sugars and pH, also increased, while the protein and acidity decreased. On the basis of sensory evaluation (Table 1), all the combinations are similar, the combination T_3 being with maximum sensory score, in respect of flavour, colour, taste and consistency. Therefore, combination T_3 was selected for storage behaviour at room temperature and 37°C.

The combination T_3 had an initial moisture content 3.5%, acidity as CA 6.5%, pH 3.2 and vitamin C 13.4 mg/100 g. These values remained more or less unchanged during the six months of storage at 37°C and room temperature, except for minor changes in vitamin C. The reduction in vitamin C was to 11.6 mg after 3 months at 37°C and 11.9 mg after 6 months at room temperature from the initial value of 13.4 mg/100 g product. Decrease of vitamin C was marginally higher at 37°C than at room temperature. The organoleptic score was rated very good (8) for the fresh dehydrated *chutney*. The score decreased to 7 (good) after storage at RT for 6 months and to 6 after storage at 37°C for 3 months. Except for decrease to score 7 for the product stored at 37°C for 2 months, no change was noted in sensory quality for intermediate storage periods. The trends of the results are similar to those reported by other workers (Lal et al. 1967; Rao et al. 1991). The dehydrated instant *chutney* has good palatability and sufficient storage (6 months) life under ordinary conditions.

TABLE 1. PHYSICO-CHEMICAL, NUTRITIONAL AND SENSORY CHARACTERISTICS OF WILD POMEGRANATE, INSTANT WILD POMEGRANATE *CHUTENYS*

Characteristics	Wild pomegrante	Green chillies	Combinations				
			T_1	T_2	T_3	T_4	T_5
Yield, %	-	-	56.0	65.0	68.0	72.0	79.0
Moisture, %	76.8	83.7	3.0	3.2	3.5	6.0	6.2
Acidity as CA, %	4.8	0.3	8.2	7.4	6.4	7.3	7.0
Protein (N x 6.25), %	1.8	3.4	12.3	11.3	10.3	9.9	11.2
Ash, %	0.5	0.7	6.2	9.6	12.0	25.0	30.0
Vitamin C, mg/100g	20.2	122.5	8.5	11.4	13.4	17.0	19.9
Total sugars, %	13.8	2.8	41.6	43.5	45.6	55.0	59.5
Fat, %	0.2	0.3	2.2	2.3	2.7	2.7	2.6
pH	2.7	ND	2.8	3.2	3.1	3.4	3.5
Reducing sugars, %	6.8	ND	27.7	33.3	31.7	35.7	41.6
Salt, %	NA	NA	17.4	15.6	12.8	19.0	15.0
Dehydration ratio	NA	NA	4.1:1	4.0:1	4.0:1	4.1:1	4.5:1
Redydration ratio	NA	NA	1:2	1:2	1:2	1:2	1:2
Total calorific value, Kcal/100g	66.0	30.0	256.2	261.7	270.2	313.0	335.9
Mean sensory evaluation (Reconstituted *chutneys*)							CD (P - 0.05)
Colour	NA	14.8	15.3	16.5	16.0	15.5	N.S
Flavour	NA	16.0	14.7	16.3	13.7	14.2	0.5
Taste	NA	15.7	15.7	17.2	14.7	15.5	N.S
Consistency	NA	15.3	15.2	16.8	16.8	16.5	N.S
Overall quality	NA	15.0	15.5	16.7	15.0	15.2	N.S

Max. score for each sensory attribute = 20; Rating 12 Not acceptable, 12-14 good, 14-16 very good and 16 excellent.
NA = Not applicable; ND = Not done;
± SD values for constituents were 0.02-0.83 for wild pomegranates and green chillies

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Recovery of Carotenes from Crude Palm Oil by Adsorption Method

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Acid-treated bentonite and alumina gel mixture, as adsorbents in varying ratios, recovered significant amounts of carotenoids from crude palm oil, without affecting its quality. As the clay level increased, the amount of residual carotenoids in the palm oil decreased. The maximum adsorption of 79% carotenoids was possible at 4:1 ratio of bentonite and alumina gel.

Keywords : Palm oil, Carotenoids, Bentonite, Alumina gel, Adsorption, Recovery.

Palm oil owes its distinctive orange - red colour to its relatively high content of carotenoids. Of all the vegetable oils that are consumed widely, the palm oil contains the highest known concentrations (500-1600 ppm) of agro - derived carotenoids (Ames et al. 1960; Jacobsberg 1974; Goh et al. 1985; Tan 1988; Arumughan et al. 1988). Unfortunately, all the carotenoids of the crude palm oil are destroyed by the presently used refining processes, which traditionally lead to the light coloured to colourless oil, as per the demand from major consumers. However, in the traditional process of extraction, when compared to mechanised process, palm oil retains about 3 times more carotene (Jideani 1992). If recovered, the value of the carotene concentrate would be close to that of the oil itself, excluding the processing costs. Carotene finds important use in manufacture of vitamin A. Hence, an attempt was made to recover carotenes from crude palm oil by adsorption method.

Alumina gel was obtained by preparing gelatinous aluminium hydroxide from aluminium sulphate (Mamuro et al. 1986a). Bentonite was acidified with 3 M sulphuric acid, filtered and washed several times with distilled water, until the filtrate was almost neutral (Nkpa et al. 1989). The washed bentonite was dried at 140°C overnight, ground to fine powder and mixed with alumina gel in desired proportions.

Prior to adsorption experiments, the crude palm oil was treated with 2% by weight of 85% phosphoric acid for 1 h at 80°C (Mamuro et al. 1986 a,c). Pretreated palm oil (20 g) was used along with 2 g adsorbent for each of the adsorption experiments. The mixture was heated at 50°C for 1 h in shaker water bath (Mamuro et al. 1986a). The procedure was repeated twice. The upper oil layer was separated by centrifugation and its carotenoid content was determined.

The adsorbent, separated from the mixture after the above treatment, was washed with pure hexane to remove residual oil. Then, the carotenoids were removed by using 20% acetic acid in 95% ethanol at 80°C for 30 min (Mamuro et al. 1986a). Carotenoids were, then, extracted in petroleum ether for spectral analysis. The carotene content was determined by spectrophotometry (Davies 1976). To ascertain any change in oil due to adsorption treatment, the iodine value of palm oil was determined before and after the treatment (Nkpa et al. 1989).

Data show that the carotenoid content decreases by 15 - 16% on pretreatment of the crude palm oil with phosphoric acid, the level of carotene in palm oil before and after such pretreatment being 737 and 619 ppm, respectively. Two possible reasons can be attributed to this. One is deterioration and/or removal of the carotenoids during pretreatment, and another is the removal of minor component (s), other than carotenes, which has same adsorption bands covering 448 nm, as that of the carotene. The latter possibility appears to be most applicable, since the shoulder in the vicinity of 420 nm, which appears in the spectrum of crude palm oil (Figs.1 and 2), changes into a peak and orange-red colour of the oil turns dark red after pretreatment. Moreover, Mamuro et al (1986a) have also shown that the pretreatment of oil with phosphoric acid, followed by washing to remove acidity, improves the recovery of carotenoids on adsorbents.

Table 1 shows the adsorption of carotenoids on bentonite-alumina mixtures of various proportions. The bentonite - alumina ratio of 4:1 was found to be the optimum. The % carotenoids

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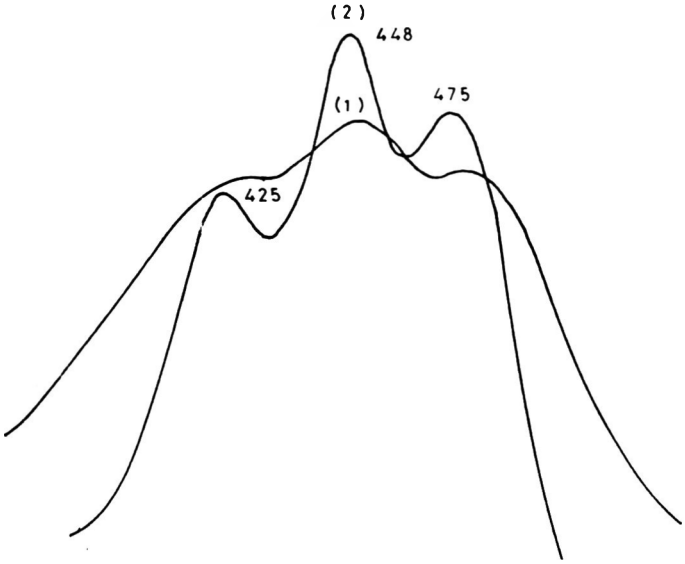

Fig. 1. Spectral analysis of 1) crude palm oil and 2) palm oil after pretreatment. Spectrum was taken using petroleum ether (40-60) as solvent.

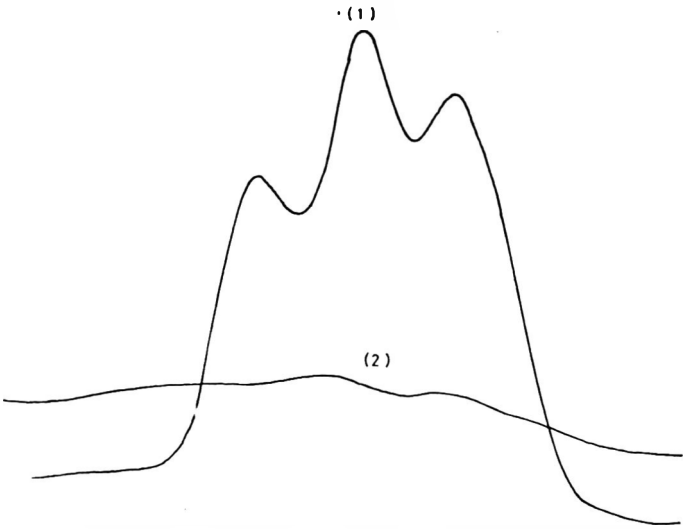

Fig. 2. Spectral analysis of 1) recovered carotene concentrate and 2) palm oil after carotene removal. Spectrum was taken using petroleum ether (40-60) as solvent.

adsorbed from pretreated palm oil with 30% level of adsorbent are shown in the same Table. The recovery of 76% carotenoids was achieved from the pretreated crude palm oil by using 4:1 ratio of bentonite : alumina. Alumina alone was unable to adsorb carotenoids, while bentonite alone gave 45% recovery.

The recovery of carotenoids from crude palm oil by the present method was far superior to that reported by other workers. For example, Nkpa et al (1989) reported 62% adsorption of carotenoids from crude palm oil on bentonite. Similar results were also reported with the use of other local Nigerian clays. Mamuro et al (1986b) used the mixture of alumina gel:clay and the highest recovery of carotenoids reported was 41% from the crude palm oil.

TABLE 1. EXTRACTION AND RECOVERY OF CAROTENES FROM CRUDE PALM OIL BY USING ACTIVATED BENTONITE : ALUMINA GEL AS ADSORBENT IN VARYING RATIOS*.

Adsorbent	Carotenes adsorbed, mg/g absorbent	Carotenes adsorbed, %	Carotenes released, mg/g adsorbent	Carotene * recovery, %
Alumina gel	0.00	-	0.00	-
Activated bentonite	1.47	60.00	0.66	45.00
Alumina : bentonite ratio				
1 : 1	1.03	42.00	0.38	37.00
1 : 2	1.35	55.00	0.65	48.00
1 : 3	1.67	68.00	1.02	61.00
1 : 4	1.94	79.00	1.47	76.00
1 : 5	1.77	72.00	1.20	68.00

* Values are average of three experiments.
* Carotene recovery is based on the amounts of carotene adsorbed.

The iodine values of the crude palm oil, before and after recovery of carotenoids as per the present method, were 52 and 55 mg/100 mg, respectively. Such negligible differences in the iodine values, thus, indicate that the carotenoids were removed in the present process without detriment to the quality of crude palm oil. After recovery of carotene, the crude palm oil, thus, can be refined to desired level for marketing, while the recovered carotene can be used gainfully for additional returns.

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Effect of Roasting Process Variables on Hardness of *Bengal*gram, Maize and Soybean

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*Bengal*gram, maize and soybean grains were roasted at 12 ± 0.5% initial moisture content and 1:4 grain-to-sand ratio at 180°, 215° and 250°C sand temperature for 1.5, 2.0 and 2.5 min roasting time, to study the effect on hardness after roasting. Sand temperature was observed to be significant in all the cases. In general, the hardness decreased with roasting time. The optimum conditions for roasting were obtained by the canonical anlaysis of response surface methodology.

Keywords : Variability of hardness with roasting, *Bengal*gram, Maize, Soybean, Roasting time, Roasting temperature.

Roasting and grinding processes render the grain digestible, without the loss of nutritious components (Krantz et al. 1983) and hence such grains are consumed throughout the world. The puffing and roasting are almost similar processes, but the volume expansion in puffing is higher. Some such grains are consumed in powder form (Prasad and Srivastav 1984; Srivastav et al. 1989) and in such cases, the puffed grains pose problems. Consequently, the roasting is carried out at slightly higher initial moisture content to reduce the puffing of the grains. The preparation of roasted grain powder involves conditioning (moistening and resting), roasting, dehulling and grinding. Hot sand is commonly used for roasting.

Hardness of foodgrains is of interest for control of manufacturing operations. Grain hardness affects the particle size obtained from milling and sieving operations and energy consumption in milling (Symes 1969; Moss et al. 1980; Tran et al. 1981). Grain hardness is affected by size, direction of applied force, moisture content (Zoerb and Hall 1960; Bilanski 1966), chemical composition (Bennett 1950) and heat treatment (Abdelrahman and Hoseney 1984). Initial moisture content, sand temperature, time of roasting and grain-to-sand ratio are the commonly used heat treatment parameters that are varied to obtain the desired characteristics of the roasted grain. In this communication, the effect of sand temperature and time of roasting on the hardness of roasted *Bengal*gram, maize and soybean is discussed.

*Bengal*gram, maize and soybean were purchased from local market, cleaned and graded by sieving (BS 20 mesh) to obtain approximately same grain size. The sphericity (Mohsenin 1970) was calculated by measuring the length, width and thickness of the grains. Roasting was carried out, after adjusting the moisture content of grains to 12.0 ± 0.5%, by sprinkling predetermined amount of water and storing the grains overnight in moisture-proof containers. The grains (250 g) were roasted at sand temperatures of 180, 215 and 250 ± 2°C for 1.5, 2.0 and 2.5 min in sand (size : < 35 mesh or 0.417 mm), at 1:4 grain-to-sand ratio in an aluminium vessel (36 cm diam, 15 cm depth), placed over LPG gas burner. The contents were mixed with a stainless steel ladle. Mercury in glass thermometer (0-550°C) and stop-watch were used to measure sand temperature and time of roasting, respectively. The grains were separated from hot sand using a sieve (35 mesh size).

The sphericities of the grains were 0.79 (±0.02) mm, 0.81 (±0.05) mm and 0.84 (±0.02) mm, for *Bengal*gram, maize and soybean, respectively. For the measurement of hardness, the roasted grains were cooled to room temperature in desiccator, transferred in air-tight containers and hardness was measured (in triplicate) within 2 to 3 days. The grains were placed horizontally on the compression load cell of an Instron universal testing machine (Model TM-M, Instron Corporation, Massachusetts, USA), which was operated under 10, 20 kg full load scale, 20 cm/min chart speed and 2 cm/min cross-head speed. The hardness was noted as maximum force (kg) required to break the sample. Variabilities of the hardness values with the initial moisture content, sand temperature, time of roasting and grain-to-sand ratio were determined by developing a quadratic response surface model. The data were analysed by developing

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regression equations and testing the significance (Myers 1971).

The breaking strength of roasted *Bengal*gram, maize and soybean (Table 1) was found to decrease with increase in heat treatment given. The breaking strength of *Bengal*gram decreased at 180 and 215°C sand temperatures with respect to time (upto 2.0 min) in puffed grains, due to bursting of grains (Das and Srivastav 1989) and inactivation of the compounds responsible for grain hardness (Abdelrahman and Hoseney 1984). Further increase in time of roasting resulted in slight increase in the hardness, possibly due to reduction of grain moisture to < 4% (Srivastav 1989). The formation of a continuous protein matrix, which physically traps the starch granules, leads to difficulty in separating starch from protein and makes the grain harder (Stenvert and Kingswood 1977; Moss et al. 1980). This may also be responsible for the increase in hardness. In the case of maize, the breaking strength increased in the first part and then decreased at 215°C sand temperature, whereas the trend was reverse at 180 and 250°C. It might be due to the fact that surface gelatinization of maize starch took place initially and fissures developed on the grain, upon further heating, resulting in the reduced hardness. The breaking strength of soybean decreased with increase in time of roasting (Table 1). More or less the same trend was obtained with the increase in sand temperature, possibly because of inactivation of compounds responsible for the grain hardness. The role of protein matrix could be eliminated in this case, due to lack of starch.

TABLE 1. EFFECT OF ROASTING ON THE BREAKING STRENGTH

Temperature of roasting, °C	Time of roasting, min	Breaking strength, kg		
		*Bengal*gram	Maize	Soybean
180	1.5	14.9	9.0	5.8
	2.0	8.6	5.3	5.4
	2.5	9.0	5.6	3.1
215	1.5	10.0	9.6	8.7
	2.0	6.9	11.2	6.0
	2.5	8.5	10.3	3.4
250	1.5	3.3	8.0	2.5
	2.0	7.3	7.1	2.1
	2.5	5.6	6.8	1.6

Analysis of variance

Source of variation	Degree of freedom	Mean sum of square		
		*Bengal*gram	Maize	Soybean
x_1	1	28.6*	23.4*	12.5*
x_2	1	18.8^{NS}	2.5^{NS}	13.2*
x_1^2	1	0.3^{NS}	23.1*	13.7*
x_1x_2	1	16.8^{NS}	1.2^{NS}	0.8^{NS}
x_2^2	1	1.8^{NS}	0.3^{NS}	0.2^{NS}
Error	5	3.4	1.6	1.1

* Significant at 5%; ** Significant at 1% ; NS : Not significant

Regression equations for the hardness after roasting of *Bengal*gram (H_b), maize (H_m) and soybean (H_s) were :

$$H_b = 80.29 - 0.19X_1 - 42.09X_2 - 0.0003X^2_1 + 0.12X_1X_2 + 3.80X^2_2 \quad (1)$$

Correlation coefficient = 0.91

$$H_m = -98.50 + 1.14X_1 - 13.66X_2 - 0.003X^2_1 + 0.03X_1X_2 + 1.40X^2_2 \quad (2)$$

Correlation coefficient = 0.90

$$H_s = -72.28 + 0.82X_1 - 3.43X_2 - 0.002X^2_1 + 0.03X_1X_2 - 1.27X^2_2 \quad (3)$$

Correlation coefficient = 0.95

Analysis of variance (Table 1) shows that the sand temperature had a significant effect on the hardness of all the grains. Srivastav et al (1992) also observed the same effect of sand temperature on the sensory scores of roasted grain powders, prepared by following the same roasting process. The hardness values can be related to sand temperature (X_1) and time of roasting (X_2) as $H_b = f_1(X_1)$, $H_m = f_2(X_1, X^2_1)$ and $H_s = f_3(X_1, X_2, X^2_1)$, respectively, where the independent variables in functional relationships are presented in order of importance depending on their values. After deleting the non-significant terms, the regression equations could be reduced as :

$$H_b = 80.29 - 0.19X_1 \quad (4)$$

$$H_m = -98.50 + 1.14X_1 - 0.003X^2_1 \quad (5)$$

$$H_s = -72.28 + 0.82X_1 - 3.43X_2 - 0.002X^2_1 \quad (6)$$

To find the optimum condition of roasting, the regression equations were analysed to determine corresponding 1, 2 and 3 stationary points (Myers 1979; Khuri and Cornell 1987) and the characteristics of the stationary points were evaluated by writing the fitted surface in canonical form, for each of the grains, as : $y = y_o + \lambda_1 w_1 + \lambda_2 w_2$, where, y_o is the value of hardness at stationary point, λ_1 and λ_2 are characteristic roots of the symmetric matrix B (elements of which is estimated as coefficients of the second order terms), while w_1 and w_2 are the canonical variables (linear combinations of the original variables x_1 and x_2). The sign of λ-values determines whether the stationary point is a maximum (all '-ve'), minimum (all '+ve') or saddle (mixed i.e. one '+ve' and other '-ve'), points. The canonical equations for roasted *Bengal*gram, maize and soybean, respectively, can be written as :

$$y_B = 8.2 + 3.8\ x_1^2 - 0.001\ x_2^2 \quad \ldots (7)$$

$$y_M = 9.9 + 1.4\ x_1^2 - 0.003\ x_2^2 \quad \ldots (8)$$

$$y_S = 8.6 - 0.002\ x_1^2 - 1.267\ x_2^2 \quad \ldots (9)$$

From the magnitude and sign of the λ-values, the variability of the hardness with roasting process variables can be explained in a better way. If the stationary point does not lie in the experimental range of independent variables, the axes can be changed along the w_1 and/or w_2 directions to get the desired response.

The canonical analysis and the values of the independent variables, along with calculated values of hardness at transformed axes (different values of w_1 and w_2), are presented in Table 2. In the case of *Bengal*gram and maize, the stationary points are found to be saddle points. The λ_1-values, in the equations 7 and 8, are '+ve' and more than the λ_2-values. This implies that there will be a rapid change in the hardness, if the axis along the w_1 direction is changed (in +ve and -ve directions). In case of *Bengal*gram, if the axis is changed in the w_1 direction (i.e., temperature axis), the time increases rapidly and goes beyond the experimental range and also, results in the increase in the hardness (Table 2). Hence, the temperature axis cannot be changed at all. To get the desired hardness within the experimental range, only w_2 can be changed. The minimum hardness is desired in case of roasted grains, if they are consumed as such. So, we can select any temperature and time combination for $w_1 = 0$ and $w_2 = 10$-50 in the case of *Bengal*gram and $w_2 = 10$-30 in the case of maize. In the case of soybean, the stationary point is of maximum response, i.e., hardness as both the λ-values are '-ve'. From the developed canonical equations, it is obvious that any deviation from the stationary point will reduce the hardness. It is emphasized that the sensory qualities of the product must be taken into account, while selecting any temperature and time combination for roasting of grains.

TABLE 2. CANONICAL ANALYSIS AND DERIVED ROASTING PROCESS VARIABLES FOR OPTIMUM HARDNESS AT DIFFERENT w_1 AND w_2 VALUES

		***Bengal*gram**		
Stationary points		193.4°C	2.6 min	Estimated
λ-values		3.8	-0.001	hardness
w_1	w_2	X_1 (°C)	X_2 (min)	(Kg)
0	0	193.4	2.6	8.2
1	0	193.4	3.6	12.0
0	1	194.4	2.5	8.2
0	10	203.4	2.4	8.1
0	15	208.4	2.3	7.9
0	20	213.4	2.3	7.7
0	25	218.4	2.2	7.5
0	30	223.4	2.1	7.1
0	50	243.4	1.8	5.2
0	60	253.4	1.6	3.9
		Maize		
Stationary points		219.1°C	2.4 min	
λ-values		1.4	-0.003	
0	0	219.1	2.4	9.9
1	0	219.1	3.4	11.3
0	1	220.1	2.4	9.9
0	10	229.1	2.3	9.6
0	15	234.1	2.3	9.2
0	20	239.1	2.2	8.7
0	25	244.1	2.1	8.0
0	30	249.1	2.1	7.2
0	31	250.1	2.1	7.0
		Soybean		
Stationary points		197.9°C	0.7 min	
λ-values		-0.002	-1.287	
0	0	197.9	0.7	8.569
0	1	197.9	1.7	7.3
0	2	197.9	2.7	3.5
1	1	198.9	1.7	7.3
1	0	198.9	0.7	8.567
2	0	199.9	0.7	8.561
-1	-1	196.9	-0.4	7.3
-1	0	196.9	0.6	8.567
-2	0	195.9	0.6	8.561

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Surveillance on Artificial Colours in Food Products Marketed in Calcutta and Adjoining Areas

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Various types of prepared foodstuffs were analysed for detection and estimation of the added synthetic colouring matters. The quantities of permitted colouring matters in jam, jelly, and sweets were found to be within the range of 18-220 ppm. Only in few cases (6.6%), the statutory limit was exceeded. Instances of use of non-permitted colours and colours above permissible limits were higher in case of foods made by intinerant vendors, unorganised sectors, small and cottage scale industries.

Keywords : Non-permitted colours, Excess permitted colours, Toxic colours, Infringe P.F.A., Convenience food.

Non-permitted colours, excessive amounts of permitted colours (PFA 1992) and inclusion of colour in those foods which are not permitted to carry colour, may constitute serious public health hazards and also infringe regulatory provisions, besides being toxic (Khanna et al. 1973, 1980). The present survey was undertaken to find out the extent of fraudulent use of synthetic colours in a few items of food articles (convenience food), wherein only permitted synthetic colours in prescribed limits can be used.

The monitoring has been done for products manufactured by organised and unorganised sectors and the samples were collected from 430 shops in Central Calcutta, suburban areas and railway stations in Calcutta, over a period of one year. Samples included food items viz., confectionery, snacks, sweetmeats, beverages, jam, jelly and similar products. There was no statistical sampling as such. The sample collection involved a bias in that the suspicious samples (those suspected of having statutory infringements) were only lifted, as done by the food inspector for surveillance purposes.

The standard methods of Pearson (1990) and APA (1960) were followed for detection and estimation of colours in the samples. Foods, as examined in the present studies, are of different types and have diverse chemical composition. Dye-binding, being a complex process in food matrix, suitable modifications were made for isolation of the dyes quantitatively. In the present method, 5-10 g sample was extracted with petroleum ether to recover fats and fat soluble colours. The latter, if any, was analysed by colour reactions and chromatography. The residue was further extracted either with 70% acetone or was acidified with 1 ml of 2 M hydrochloric acid and treated with 50 ml of n-butanol or iso-amyl alcohol, shaken occasionally on water bath (sweetmeat, ice-cream, custard powder). The colour from the butanol layer was re-extracted with 1% aqueous ammonia solution. Butanol was completely removed by washing with diethyl ether and finally excess ammonia and ether were removed by gentle warming. Colour extracts, thus obtained by either of the method, were further clarified by using active alumina (BDH, standardised according to Brockmann) column. Elutes were used for separations and identification by paper chromatography. Absorbance values of the colour solutions in 0.1 N hydrochloric acid and neutral 0.02% ammonium acetate (blue and green colours) were used for quantitative determinations as per ISI (1985) method.

The observations are summarised in Table 1. This survey on 445 samples of convenience foods of diverse composition revealed that 62.9% of the samples contained permitted colours within prescribed limits of PFA (1992) rules, while 51.1% of the samples contained colours below 100 ppm. However, only 13.1% samples contained non-permitted colours. It is interesting to note that the manufacturers of the foods who admix colour above the permissible limits, are not the organised manufacturers or reputed establishments. The sweets from small shops, in general, contained colours above the permissible limit. In case of confectionery and beverages too, samples from some street hawkers or small shops were found to contain such undesirable quantities of the colours.

Sunset yellow, tartrazine, carmoisine, blue FCF, ponceau 4 R are the identified permitted synthetic colours, placed in an order of decreasing frequency of occurrence, in the samples examined. Amaranth (which has been deleted from the permitted list from August 1991 PFA 1992), orange II, metanil yellow, rhodamin B, Congo red, blue

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TABLE 1. SUMMARY OF SURVEY OF ARTIFICIAL COLOURS IN VARIOUS CONVENIENCE FOODS

Category	Samples analysed	Samples containing non-permitted colours	Samples containing permitted colours		Range of permitted colour concentrations, ppm
			Within PFA limit	Above PFA limit	
Toffees, chocolates, hard boiled sugar confectionery	60	7	42	2	50 - 225
Biscuits	30	2	14	0	25 - 152
Cake, pastry and like products	50	2	35	5	45 - 227
Fried snacks	30	3	8	0	25 - 122
Milk-based sweets	50	8	34	1	45 - 220
Besan and wheat-based sweets	50	21	22	0	35 - 112
Ready-to-serve beverages	40	3	30	0	15 - 135
Beverage concentrates	25	5	5	15	10 - 237
Ice-candy, ice-cream and like products	60	8	38	10	35 - 235
Jam and jelly	25	0	25	0	18 - 141
Custard powder and like products	25	0	25	25	300 - 730

VRS are the identified non-permitted colours in the samples examined. An unidentified chocolate brown colour was found in few hard boiled confectionery and pastry. A survey of the market for available colours revealed that the toxic non-permitted colours in small packings with ISI mark (textile colours) are abundantly available and these had been used by some vendors and manufacturers.

This short term comprehensive survey revealed the frequency of occurrence of permitted colouring matters as well as fraudulent use of non-permitted dyes in some convenience foods available in and around Calcutta. The results may be helpful to specify new range of permitted colours under the PFA rules. Used non-permitted dyes like metanil yellow, orange II and rhodamin B, are reported to be carcinogenic (FSC 1980). A systematic approach to evaluate the frequency of occurrence of toxic non-permitted colours and permitted colours in excessive amounts in various food products in India has already been undertaken by Indian Council of Medical Research and Director-General of Health Services. It is felt that proper programme should be undertaken to educate the people regarding these laws and the ill-effects of using non-permitted colours as well as permitted colours above the permissible limits.

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Antimicrobial Activity of Essential Oils from Spices

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Essential oils from spices viz., ginger, cumin, *ajowain*, coriander, basil, clove and mustard as well as eugenol showed various degrees of inhibition against *Aspergillus niger*, *Saccharomyes cerevisiae*, *Mycoderma* sp., *Lactobacillus acidophilus* and *Bacillus cereus*, as determined by paper disc agar diffusion method. *Mycoderma* sp. was the most susceptible, while *Bacillus cereus* was most resistant towards all the spice essential oils. Greater antimicrobial activity was observed in allyl-iso-thiocyanate (volatile oil of mustard), followed by oil of *ajowain* and eugenol, both at ambient temperature and 37°C. The oils from other spices were less effective.

Keywords : Spices, Essential oils, Microorganisms, Antimicrobial activity

India is known for its production of spices. These spices are generally used in a pulverised form as condiments for seasoning or garnishing foods and beverages. These are considered to act as preservatives, besides improving texture and flavour of foods (Ayres et al. 1980). Spices are also used in the Indian system of medicine for checking a variety of intestinal disorders (Krishnamurthy and Sreenivasamurthy 1956). The aromatic and pungent principles in spices are due to volatile oils and oleoresins (Pruthi 1980). The preservative quality of spices was attributed to the presence of some active antimicrobial principles contained in these oils (Sreenivasamurthy and Krishnamurthy 1959). Some of the spices, either in the form of powders, extracts or oil (Sreenivasamurthy et al. 1960; Saleem and Al-Delaimy 1982; Sethi and Anand 1984) are known to control microbial spoilage in foods. The information on the antimicrobial activities of the spice essential oils is restricted to merely a few spices. (Web and Tanner 1945; Anderson et al. 1953; Conner and Beachut 1984). In the present study, an attempt was made to find out the antimicrobial potency of selected spice essential oils against a few important microorganisms of spoilage and health significance.

Spice essential oils of cumin (*Cuminum cyminum*), *ajowain* (*Trachyspermum ammi*), ginger (*Zingiber officinale*), coriander (*Coriandrum sativum*), basil (*Ocimum basilicum*) and clove (*Eugenia caryophyllus*) were obtained from the National Bureau of Plant Genetic Resources, New Delhi. Allyl-iso-thiocyanate (volatile oil of mustard) and eugenol (Mayfair and Croydon Co., England) were obtained from the Division of Fruits and Horticultural Technology of the Institute.

Lactobacillus acidophilus, *Bacillus cereus*, *Saccharomyces cerevisiae*, *Mycoderma* sp. and *Aspergillus niger* were used as test organisms to study the antimicrobial activity of the spice essential oils. *Mycoderma* sp., *B. cereus* and *L. acidophilus* are from the collection maintained in the Institute. These have been isolated from spoiled fruits and vegetable products. *A. niger* was obtained from National Type Culture Collections of the Division of Mycology and Plant Pathology of the Institute. *S. cerevisiae* was obtained from Haryana Agricultural University, Hisar. These cultures were maintained at 4°C by subculturing every two months.

Before use, spice essential oils were tested for the presence of microorganisms. This test for sterility consisted of inoculating each spice oil (pure) (about 0.1 ml) on potato-dextrose-agar, mycophil-agar, lactobacillus-agar and nutrient-agar slants (Seeley and VanDemark 1970; Amonkar et al. 1978), incubation at 37°C and room temperature (25–30°C) for 3-4 days. Antimicrobial activity was tested by filter paper disc diffusion method as described by Lahariya and Rao (1979). Test organisms ($1x10^6$ cells or spores/ml) were inoculated into the specific plating agar media (Hi Media, Bombay) at 6% (v/v) level and poured into sterile petri plates containing 15 ml agar medium. The plating media used were lactobacillus-agar for *L. acidophilus*, nutrient-agar for *B. cereus*, mycophil -agar (glucose-yeast extract-agar) for *A. niger* and potato-dextrose-agar for both *S. cerevisiae* and *Mycoderma* sp. An appropriate number of small sterile discs of filter paper (3-4 mm dia) were dipped in spice essential oils (pure) for placing on the inoculated medium. In case of control plates, the discs were dipped in sterile water. One set of plates was incubated at room temperature (25-30°C) and the other at 37°C for 7 days. The observations for growth or inhibition were recorded at an interval of 24 h upto 7 days. The presence of definite zone (higher than 3 mm) of inhibition, surrounding the

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paper discs, was taken as an indicator of the antimicrobial activity. The diameter of the zone was measured in mm. All the tests were conducted in duplicate.

It is evident from the data (Table 1) that all the spice essential oils showed varying inhibitions of the test organisms. The spice essential oils, which exhibited highest antimicrobial activity, were allyl-iso-thiocyanate (mustard volatile oil), eugenol and oils from *ajowain* and clove. The oils of ginger, basil, cumin and coriander were less effective against the test organisms. Suresh et al (1992) found that eugenol possessed inhibitory effect against *Escherichia coli, Staphylococcus aureus, Bacillus* sp., *Micrococcus* sp., *Enterobacter sakazaki* and *Klebsiella pneumoniae,* which were all resistant to antibiotics.

TABLE 1. ANTIMICROBIAL ACTIVITY OF SPICE ESSENTIAL OILS AND EUGENOL AT 37°C AND ROOM TEMPERATURE (25-30°C)

Spice oil	Test organisms									
	A. niger		*B. cereus*		*L. acidophilus*		*Mycoderma* sp.		*S. cerevisiae*	
	RT	37°C	RT	37°C	RT	37°C	RT	37°C	RT	37°C
	(zone of inhibition*, mm)									
Ajowain	80	80	8	6	NS	NS	80	80	6	4
Mustard (Ally -iso-thiocynate)	6	8	80	80	80	80	80	80	80	80
Basil	8	9	9	7	5	5	NS	NS	6	7
Coriander	0	NS	NS	NS	5	4	9	8	0	NS
Cumin	NS	4	8	7	4	5	11	10	4	5
Clove	10	10	6	7	14	1	10	11	20	18
Ginger	0	10	NS	NS	4	NS	NS	NS	0	NS
Eugenol	80	80	8	7	16	15	16	16	30	28

* Inhibition zone does not include the diameter of the filter paper discs. NS indicates 1 to 3 mm zone of inhibition (non-significant)

Among the test organisms studied in the present study, *Mycoderma* sp. was most susceptible to the spice essential oils, while *B. cereus* was comparatively most resistant (Table 1). It is indicated that the oils may have various modes of action or that metabolism in some microorganisms is better able to overcome or adopt to an antimetabolic effect of a given oil, than that in other microorganisms. This degree of inhibition may be related to the cell wall composition and/or permeability characteristics (Conner and Beachut 1984). The sensitivity of microorganisms in ascending order is *B cereus, L. acidophilus, S. cerevisiae, A. niger* and *Mycoderma* sp. Each microorganism responded differently to various oils. Such differences in mode of action and the changes in the metobolism have been linked to the ability of microorganism to resist or become susceptible to the antimicrobial effect of a given oil (Conner and Beachut 1984).

Mycoderma has been found to be one of the microorganisms responsible for spoilage of lactic fermented fruits and vegetables during the storage studies (Mikki 1971; Sethi and Anand 1984). Some of the lactobacilli are also responsible for the spoilage of fruit juices (Sharma and Padwal-Desai 1988). The other test microorganisms employed in the present study are known to have food spoilage significance (Amonkar et al. 1978). The antimicrobial activity of the spice essential oils, as observed in the present study, thus, is of significance in preserving foods and food products. In spicy products such as pickles, *chutneys* and sauces, spice essential oils in very small amounts will, thus, not only increase the palatability of food, but will also enable in reducing microbial spoilage during storage.

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Compositional and Sensory Characteristics of *Kachcha churpi*

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Kachcha churpi, a traditional milk product, is prepared by coagulating skimmed or partially defatted cow or yak milk. *Kachcha churpi* prepared from skim milk contained more protein (31.7%) than the products from buttermilk (18.5%) and defatted milk (28.2%). On sensory quality, it was found to be superior to other products, with respect to flavour, body, texture, colour, appearance and overall acceptability.

Keywords : *Kachcha churpi*, Traditional milk product, Manufacturing methods, Proximate composition, Sensory profiles, Chemical-sensory attributes relationship.

Kachcha churpi, a low fat *chhana*-like traditional milk product, is commonly consumed either as a condiment or as an ingredient of curries in Darjeeling district of West Bengal, Sikkim and Bhutan (Tamang et al. 1988). Traditionally, it is prepared by churning cow/yak milk or *dahi* in a bamboo churn, warming (70-80°C) and the defatted milk, thus obtained, is coagulated with whey of previous day. The sour buttermilk is heated to 50-60°C for coagulation. The whey is drained immediately and the coagulum is tied in a cloth and hung overnight. Now-a-days, skim milk is also used for its preparation. The keeping quality of *kachcha churpi* is poor and hence needs to be consumed within 2-3 days. Its market price varies from Rs 20 to 40 per kg. Limited information is available in literature on the production methods and quality attributes of *kachcha churpi*. The present investigation was, therefore, undertaken to study the effect of manufacturing methods on chemical and sensory attributes of this product.

A total of 30 samples, prepared from defatted cow milk, buttermilk and skim milk (10 samples each), were purchased locally and transported to the laboratory within 6 h in iced stainless steel containers, with tightly closed lids. The samples were kept overnight at 15°C before analyses.

The samples were analysed for proteins, lactic acid, lactose, moisture, fat and titratable acidity, using standard methods (Meneffee and Overman 1940; Harper and Randolph 1960; Nickerson et al. 1976; ISI 1979; AOAC 1990). The ash content was estimated by drying the sample at 105°C, igniting gently on a flame and ashing at 550±20°C for 3 h (ISI 1980). The samples were assessed for flavour, body and texture, colour, appearance, and overall acceptability using a 9-point Hedonic scale (ISI 1971) by a panel of seven trained judges. In order to determine the relationship between proximate composition and sensory attributes, the data were subjected to correlation and regression analyses (Croxton et al. 1975).

As shown in Table 1, samples of *kachcha churpi* prepared from skim milk had a lower ($P<0.05$) fat, but a higher ($P<0.05$) protein content, than those prepared from buttermilk and defatted milk.

TABLE 1. COMPOSITION OF *KACHCHA CHURPI* PREPARED BY DIFFERENT METHODS

Constituents (%, fresh weight basis)	Product prepared from: Skim milk	Buttermilk	Defatted milk
Moisture	68.5^b (0.7)	74.5^a (0.6)	69.0^b (0.4)
Proteins	31.7^a (0.8)	18.5^c (1.0)	28.2^b (0.7)
Fat	0.4^c (0.1)	3.8^a (0.4)	2.0^b (0.1)
Ash	1.6^a (0.1)	1.5^a (0.1)	1.6^a (0.1)
Titratable acidity	0.9^b (0)	1.5^a (0.1)	0.9^b (0)
Lactic acid	0.2^b (0)	0.3^a (0)	0.2^b (0)

Values are means of 10 replicates, with SEM in parentheses. Means with similar superscripts, within rows, are not significantly different ($P<0.05$).

However, with respect to moisture, ash and acidity, there was no difference ($P<0.05$) between the samples prepared from skim milk and defatted milk. On the other hand, the product prepared from buttermilk had higher ($P<0.05$) moisture, fat and acidity, but lower ($P<0.05$) protein content. In all the samples, lactose content was less than the detection limit. The coefficients of variation for fat and lactic acid contents of individual samples were as high as 51 and 37%, respectively, in contrast

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to 18% for proteins, 29% for ash and 17% for titratable acidity. *Kachcha churpi*, prepared from skim milk and defatted milk, met the legal requirements of skim milk *chhana* for moisture (not more than 70%) and fat (not more than 13% on dry matter). Moderately high lactic acid content in the samples prepared from buttermilk was possibly due to low lactose content in the milk used for *dahi* making. The considerably higher ($P<0.05$) titratable acidity in those samples could be due to extensive protein and fat hydrolysis, during and following production of *kachcha churpi* with higher moisture content.

Data on sensory attributes of *kachcha churpi* are presented in Table 2. Mean flavour scores of the samples prepared from skim milk were superior to those for other products. A variation ($P<0.05$) in flavour score of the samples prepared from buttermilk was observed. Body and texture scores of the samples made from skim milk were higher ($P<0.05$) than the scores of other products. Similarly, the colour and appearance scores of the samples made from skim milk were higher ($P<0.05$) than those for the samples from buttermilk. However, these were equal for the samples from defatted milk. *Kachcha churpi* made from skim milk was considered by the judges as a compact mass of hard rubbery body with a mild acidic flavour. On the other hand, the samples made from buttermilk were rated as highly acidic, rancid and having weak body. The samples made from defatted milk were rated in between these two.

TABLE 2. SENSORY SCORES OF *KACHCHA CHURPI* PREPARED BY DIFFERENT METHODS

Attributes	Product prepared from		
	Skim milk	Buttermilk	Defatted milk
Flavour	7.3^{a} (0.3)	4.8^{b} (0.4)	6.9^{a} (0.3)
Body and texture	8.4^{a} (0.2)	5.2^{b} (0.4)	6.4^{b} (0.4)
Colour and appearance	8.2^{a} (0.3)	5.5^{b} (0.3)	8.1^{a} (0.3)
Overall acceptability	8.1^{a} (0.3)	5.2^{b} (0.4)	7.2^{ab} (0.3)

Values are means of 10 replicates, with SEM in parentheses. Means with similar superscripts, within rows, are not significantly different ($P<0.05$).

The relative contributions of fat (F), proteins (P), ash (A), titratable acidity (TA) and lactic acid (LA) to flavour (Fl), body and texture (BT) and overall acceptability (OA) of *kachcha churpi* were studied. The following multiple linear and log-linear regression equations were obtained :

$Fl = -0.997+0.381F+0.285P-0.614A+0.065TA;$ $R = 0.80$

$Fl = 2.330+0.469F+0.253P-0.465A-10.671LA;$ $R = 0.81$

$BT = 1.669-0.588F+0.134P-0.265A+1.999TA;$ $R = 0.70$

$OA = 3.971-0.012F+0.132P+0.577A-1.252TA;$ $R = 0.75$

$OA = -0.395F^{0.029} \cdot P^{0.496} \cdot A^{0.181} \cdot LA^{-0.463};$ $R = 0.80$

Fat, proteins, ash and titratable acidity explained 63, 49 and 56% variability in flavour, body and texture and overall acceptability scores, respectively of *kachcha churpi*.

It was, thus, evident that the market samples of *kachcha churpi* had a varying proximate composition with respect to moisture, fat, proteins and titratable acidity, which, in turn, affected the overall sensory acceptability. Highly desirable *kachcha churpi* produced from skim milk corresponded with low moisture, fat and acidity, but with higher protein content.

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Saponin Content and Trypsin Inhibitor of Pea Cultivars: Effect of Domestic Processing and Cooking Methods

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Two cultivars, each of field and vegetable peas, were found to contain considerable amounts of saponins (109-251 mg/100 g) and trypsin inhibitors (922-989 TIU/g). Processing methods such as sprouting, soaking, soaking plus dehulling as well as ordinary and pressure cooking of unsoaked, soaked-dehulled peas could bring about a significant ($P<0.05$) reduction in these antinutrients. Pressure cooking of soaked-dehulled pea seeds reduced the trypsin inhibitors by 90-95%, whereas germination for 48 h lowered the saponin content of peas by 67-84%.

Keywords : Peas, Saponins, Trypsin inhibitor activity, Dehulling, Sprouting, Pressure cooking.

Pea (*Pisum sativum*) is an excellent source of proteins, digestible carbohydrates and dietary essential minerals, but its utility to humans is limited by the presence of antinutrients such as phytic acid, saponins, trypsin inhibitors and polyphenols (Savage and Deo 1989). It is important to reduce these antinutrients so as to improve the biological utilization of the food legumes. In India, legume grains are generally processed and consumed in different forms, depending on culture and taste preferences. The most common domestic processing and cooking methods include soaking, dehulling, germination, ordinary and pressure cooking. These methods may reduce the antinutrients in legumes, including peas. With this perspective, an attempt has been made to find out such effects on the saponins and trypsin inhibitor activity of field as well as vegetable peas.

Seeds of two varieties each of the vegetable ('Bonneville' and 'Arkel') and field peas ('HFP4' and 'Rachna') were procured from the Department of Vegetables and Plant Breeding of the University. Selection of these varieties was done on the basis of high yield. After removing the extraneous matter, seeds were soaked in double distilled water for 6, 12 and 18 h at 30°C in an incubator, using seed to water ratio of 1:5 (w/v). For dehulling, the hulls were removed manually from the seeds soaked overnight for 12 h. In ordinary cooking, the soaked seeds (12 h) were rinsed in double distilled water and placed along with water (three times the weight of soaked seeds) in a round mouthed tall beaker, fitted with condenser, which was connected to cold running water. The seeds were cooked on a hot plate, until they became soft. Unsoaked seeds were also cooked similarly, using seed to water ratio of 1:4 (w/v). The time required for cooking varied from 83 to 106 min for different pea cultivars. In case of pressure cooking, both soaked (12 h at 30°C) and unsoaked seeds were pressure cooked at 121°C for 10 min. In this case, the dry seeds to cooking water ratio was 1:3 (w/v), whereas it was 1:2 (w/v) for soaked seeds. For sprouting, the soaked seeds (12 h) were placed in sterile petri plates, lined with wet filter papers, and kept in an incubator at 30°C for 12, 24 and 48 h.

All the processed, i.e., soaked, soaked-dehulled, ordinarily cooked, pressure cooked and sprouted, samples were dried in oven at 60°C to a constant weight. The dried samples were ground in an electric grinder (Cyclotec, M/S Tecator, Höganäs, Sweden), using 0.5 mm sieve, and stored in air -tight plastic containers at room temperature for analytical purposes. Saponin was extracted and determined by the modified method of Gestetner et al (1966). Trypsin inhibitor activity was determined by the modified method of Roy and Rao (1971). One unit of trypsin was defined as the amount of enzyme, which converted one mg casein to TCA soluble components at 37°C and pH 7.6 in 20 min. The data were subjected to analysis of variance for determining significance of differences among various cultivars and treatments according to standard methods (Panse and Sukhatme 1961).

Saponins : Saponin contents varied from 240 to 251 and 109 to 119 mg/100 g in vegetable and field pea varieties, respectively (Table 1). After soaking for varying periods, the saponin contents decreased significantly ($P<0.05$), reduction being higher in field peas than in vegetable peas. Dehulling of the soaked seeds further brought about significant ($P<0.05$) losses of saponins, the losses being higher in field peas as compared to vegetable pea varieties (Table 1). The losses of saponins during soaking may be due to leaching of saponins into the water

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TABLE 1. EFFECT OF DIFFERENT PROCESSING AND COOKING METHODS ON SAPONIN CONTENT OF PEAS (mg/100 g, ON DRY MATTER BASIS)*

Treatments	Vegetable peas		Field peas	
	'Bonneville'	'Arkel'	'HFP4'	'Rachna'
Control	251.0±0.05	240.0±0.05	109.0±0.07	119.0±0.07
Soaking (h)				
6	241.2±0.05	232.6±0.11	99.0±0.03	108.6±0.02
12	230.9±0.08	222.5±0.01	90.5±0.01	98.2±0.02
18	186.5±0.02	182.4±0.02	60.5±0.03	76.5±0.04
Soaked (12 h) + dehulled	220.5±0.02	212.7±0.07	80.6±0.01	84.4±0.02
Ordinary cooking				
Unsoaked	236.9±0.05	227.0±0.01	95.3±0.01	103.5±0.02
Soaked (12 h)	229.3±0.02	220.5±0.01	88.5±0.01	96.2±0.02
Soaked (12 h) + dehulled	181.5±0.01	177.6±0.01	55.3±0.02	75.2±0.02
Pressure cooking				
Unsoaked	232.3±0.02	223.4±0.02	91.4±0.04	99.1±0.08
Soaked (12 h)	180.5±0.02	176.7±0.02	54.9±0.01	70.7±0.01
Soaked (12 h) + dehulled	160.5±0.02	156.4±0.03	34.5±0.07	55.2±0.02
Sprouting (h)				
12	229.2±0.01	220.5±0.01	86.5±0.02	95.3±0.04
24	200.4±0.01	192.6±0.01	72.6±0.01	78.5±0.03
48	80.1±0.01	78.8±0.05	17.2±0.04	28.5±0.04
CD (P<0.05)	1.86	1.73	1.42	1.48
(Interaction between treatment and variety)				

* Values are mean ± SD of three independent determinations

through diffusion. Similar results have been reported by other workers in an amphidiploids of greengram x blackgram (Kataria et al. 1989) and pigeon peas (Duhan 1992).

Ordinary as well as pressure cooking caused further significant reduction in saponin content of unsoaked, soaked and soaked plus dehulled pea cultivars (Table 1), the losses being significantly higher in pressure cooked than in ordinarily cooked peas. Pressure cooking of the soaked and soaked-dehulled peas could reduce the saponins upto 50 to 68% in different cultivars. Hence, pressure cooking of the soaked and dehulled peas appears to be more beneficial over ordinary as well as pressure cooking of soaked or unsoaked seeds for bringing a greater reduction in saponins. Losses during ordinary or pressure cooking may, perhaps, indicate thermolabile nature of saponins. Not much is known about the formation of poorly extractable complex between saponins and sugars or amino acids upon cooking (Khokhar and Chauhan 1986). The results of the present study contradict the earlier report that saponins are not destroyed during cooking and processing (Khokhar and Chauhan 1986), but are consistent with those reported by Kaur and Kapoor (1990).

Germination for varying periods lowered the saponin contents (Table 1). Germination of peas for 48 h showed the maximum saponin losses in field pea cultivars 'HFP4' and 'Rachna'. The saponin losses during germination may be due to enzymatic degradation, but this has still not yet been well established. Many workers reported a reduction in saponins in sprouted moth bean (Khokhar and Chauhan 1986), amphidiploids of greengram x blackgram (Kataria et al. 1989) and pigeon peas (Duhan 1992).

Trypsin inhibitor activity of peas : Raw seeds of vegetable and field pea varieties contained varying amounts of trypsin inhibitor activities i.e., 922.4 to 989.5 TIU/g (Table 2). There were significant differences between trypsin inhibitor activities of vegetable and field pea cultivars. According to Savage and Deo (1989), the trypsin inhibitor content depends upon the type of peas. It was stated that wrinkled seeded peas had less trypsin inhibitor activity than smooth seeded types and spring types. Valdebouze et al (1980) stated that 90% of the trypsin inhibitor activity was found in kernel and 10% in the testa.

Soaking of seeds in water for 6 h could marginally reduce the trypsin inhibitor activity

TABLE 2. EFFECT OF DIFFERENT PROCESSING AND COOKING METHODS ON TRYPSIN INHIBITOR ACTIVITY IN PEAS (TIU/mg ON DRY MATTER BASIS)*

Treatments	Vegetable peas		Field peas	
	'Bonneville'	'Arkel'	'HFP4'	'Rachna'
Control	922.4±0.4	927.6±0.8	980.3±0.5	989.5±0.9
Soaking (h)				
6	913.3±0.5	920.2±0.5	970.2±0.2	981.3±0.2
12	888.0±0.3	893.1±0.2	946.2±0.8	955.2±0.6
18	525.1±0.4	540.2±0.7	588.3±0.6	608.2±0.6
Soaked (12 h) + dehulled	829.2±0.1	839.2±0.3	892.1±0.4	902.1±0.1
Ordinary cooking				
Unsoaked	905.1±0.5	910.2±0.1	963.2±0.62	972.2±0.4
Soaked (12 h)	249.2±0.2	299.2±0.7	342.1±0.4	331.1±0.8
Soaked (12 h) + dehulled	201.1±0.5	228.1±0.5	309.2±0.4	300.1±0.4
Pressure cooking				
Unsoaked	898.0±0.1	902.3±0.1	954.2±0.8	962.2±0.4
Soaked (12 h)	101.2±0.8	128.2±0.7	209.1±0.8	200.0±0.3
Soaked (12 h) + dehulled	50.2±0.1	62.2±0.1	100.1±0.8	99.1±0.4
Sprouting (h)				
12	858.1±0.2	863.2±0.6	920.2±0.6	930.2±0.5
24	475.0±0.2	490.2±0.5	538.1±0.3	550.3±0.6
48	350.2±0.9	370.2±0.1	432.3±0.3	420.3±0.6
CD (P<0.05)	1.24	1.34	1.21	1.38
(Interaction between treatment and variety)				

* Values are means ± SD of three independent determinations

(Table 2). As the period of soaking was increased, losses in trypsin inhibitor activity also increased. Trypsin inhibitor activity was reduced to almost half in all the four pea varieties, after soaking for 18 h. Soaking (12 h), followed by dehulling, also brought about a decrease in trypsin inhibitor activity to the extent of 9 to 10%, which is greater than that with soaking alone. Losses of trypsin inhibitors during soaking may possibly be due to leaching against concentration gradient. Generally, trypsin inhibitors are low molecular weight proteins and hence, they are likely to leach-out. Earlier workers also reported that soaking of dry legumes in water reduced the trypsin inhibitor activity (Deshpande and Cheryan 1983).

Ordinary and pressure cooking of unsoaked, soaked and soaked-dehulled seeds were effective in lowering the levels of trypsin inhibitors to different extents (Table 2). Trypsin inhibitors are heat labile, which may explain the destructive effect of cooking on diminished trypsin inhibitor activity. Similar results were also reported by other workers (Manorama and Sarojini 1982; Marickar and Pattabiraman 1988). Varietal differences were found to have a significant (P<0.05) effect on the destruction of trypsin inhibitor activity in sprouted peas. A decrease in trypsin inhibitor activity during germination may, perhaps, be due to the mobilization and breakdown of chemical constituents including trypsin inhibitors. Similar findings have been reported in case of different legumes (Bansal et al. 1988; Verma and Mehta 1988; Mulimani and Paramjyothi 1983). Bansal et al (1988) reported complete destruction of trypsin inhibitor activity in 8 days in sprouted chickpea.

As a result of soaking, dehulling, ordinary cooking, pressure cooking and sprouting, a significant reduction occurred in the levels of saponins and trypsin inhibitors. Germination for 48 h followed by pressure cooking of soaked-dehulled pea seeds was found to be the best method to lower down the saponin contents and trypsin inhibitor activities of field as well as vegetable peas.

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Age Gelation in Ultra Heat Treated Milk : Some Evidence for a Plastein Induced Mechanism

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Isolation of protease from whey and its role in age-gelation indicated the plastein induced type of reaction in micellar system. In contrast, casein fractions were completely hydrolysed in nonmicellar system.

Keywords : Ultra heat treated milk, Protease, Gelation, Plastein, Proteolysis, Micellar and nonmicellar systems.

Proteases are responsible for age-gelation in ultra heat treated (UHT) milk (Pande and Mathur 1989), but the exact mechanism of gelation is not known till date. In the present studies, whey obtained from UHT milk showed appreciable enzyme activity that led to the isolation of protease active fraction, which accompanied whey proteins.

The protease active fraction has been isolated and purified by conventional purification methodology with some modifications (Pande and Mathur 1992). The behaviour of such indigenous proteases towards different substrates has been studied. The protease activity was determined using the procedure of Dulley (1972) and PAGE studies were performed, using the procedure of De Jong (1975).

Table 1 shows that casein is the most common substrate, with β-casein being acted upon most extensively and k-casein the least. There is a remarkable difference as compared to acid protease and other milk clotting enzymes that hydrolyze k-casein to destabilize casein micelles and cause their aggregation. Hence, the gelation in UHT milk is altogether different and should preferably be called as nonclotting proteolysis. The electron micrograph of gelled UHT milk also showed extensive disintegration of casein micelles, but with no aggregation (Andrews et al. 1977). In this regard, the refrigerated storage of UHT milk bears special significance and explains the action of proteases on β-casein. The study of natural proteolysis in whey stresses the association of bacterial proteases with whey fraction, but no conclusive evidence has been given in this regard (Jost et al. 1976). Further, the effect of storage on proteases indicates that, during storage at 2-4°C, proteases migrate from casein micelles to whey serum, due to hydrophobic environment and changes in salt balance (Reimerdes and Klostermeyer 1976). On account of oozing out of β-casein as well as proteases to serum phase, the formation of γ-casein is easily accessible. Higher gelation in samples stored at 4°C, as compared to those stored at 37°C, has also been observed in the present studies.

TABLE 1. ACTIVITY OF PROTEASE, ISOLATED FROM UHT MILK, ON DIFFERENT SUBSTRATES.

Substrate*	Activity[1]
Cow casein	337.50
Buffalo casein	371.25
α - lactalbumin	135.00
β - lactglobulin	117.00
α_s - casein	319.50
β - casein	438.75
k - casein	33.75

* 1.0% (w/v) in 0.02 M sodium tetraborate buffer, (pH 8.6).

[1] units/ml. One unit = 1 μg/ml increase in tyrosine content under assay conditions

The action of isolated enzyme has been studied in a micellar system by incubating it with skim milk substrate at 37°C for different time intervals. The trichloroacetic acid (TCA) soluble supernatant was measured at 280 nm and TCA insoluble precipitate was subjected to electrophoresis. A reference experiment was run by using 2.5% acid casein (nonmicellar system) as a substrate.

Fig. 1 depicts a steady increase in TCA soluble peptides till 8 h, thereafter, their concentration declined. This indicated that, after sometime, activity can not be measured in terms of soluble peptides. Similar findings by Samel et al (1971), using stored UHT milk and studying its casein degradation, led to an inappropriate explanation that onset of gelation does not depend on extent of proteolysis. The corresponding PAGE analysis of TCA precipitated proteins, at different time intervals, revealed that there was continuous breakdown of casein fractions

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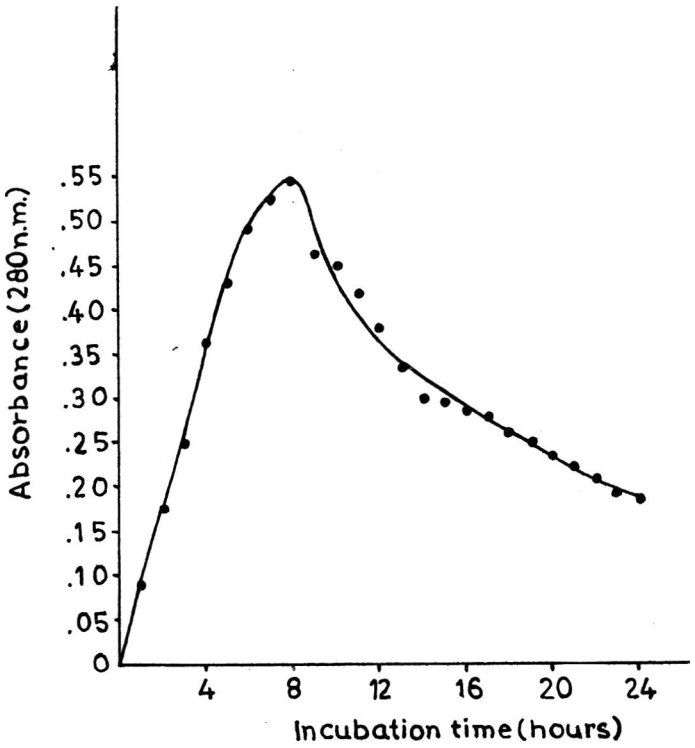

Fig 1. Concentration of TCA soluble peptides in the supernatant 1.0 ml of protease added to 2.0 ml skim milk and incubated at 37°C for 24 h. The TCA soluble supernatant was measured at intervals of one h at 280 nm.

(and therefore an increase in TCA soluble peptides) and a few TCA insoluble peptides appeared as separate bands (Fig. 2). However, there was a sudden increase in size and mobility of bands and some bands appeared in close association with casein after 8 h. The presence of such bands can be attributed to the formation of plastein-like products, which also explains the role played by smaller peptides and the concomitant decrease in

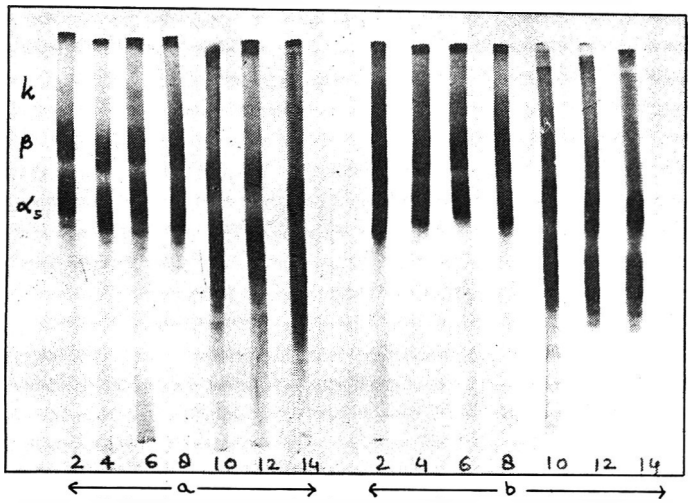

Fig 2. PAGE pattern of TCA insoluble fraction obtained after the action of protease for 2-14 h on (a) buffalo skim milk and (b) cow skim milk. 0.02 ml protein was loaded to gel and current of 3 mA/gel tube was applied at 150-200 volts.

TCA soluble fractions (Samel et al. 1971; Edwards and Shipe 1978). Such protein-like substances have been the result of physical aggregation between smaller peptides, mainly via hydrophobic bonding, but with ionic forces also playing a minor role. However, it has been observed in the present studies that the casein is almost completely digested after 2 h incubation with isolated protease (Fig. 3), thereby indicating that the formation of plastein products takes place only in a micellar system. In a non-micellar system, due to ready availability of

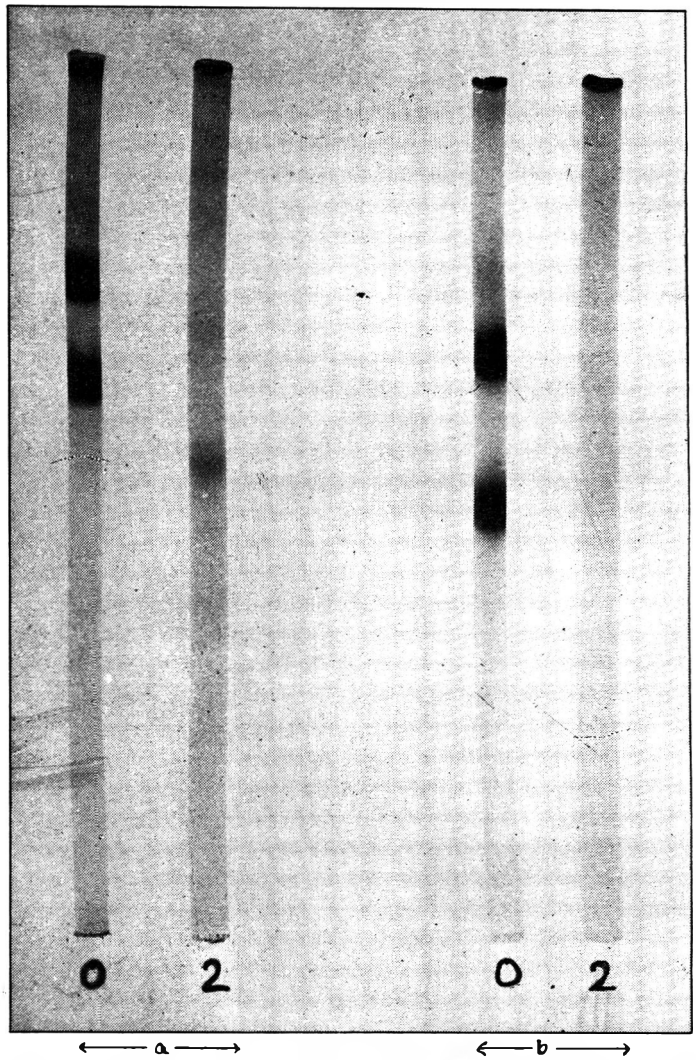

Fig 3. PAGE pattern of TCA insoluble fraction obtained after the action of protease for 0 and 2 h on (a) buffalo casein and (b) cow casein. 0.02 ml protein was loaded to gel and current of 3 mA/gel tube was applied at 150-200 volts.

caseins and nonspecificity of enzyme, there is over-digestion, thereby leading to the production of small peptides and amino acids, that do not enter into plastein formation (Hofsten and Lalasidis 1976). Such hydrophobic peptides that appear only in a micellar system may eventually aggregate, as a consequence of their increased hydrophobicity and this explains the formation of plastein gels.

In the light of above data, gelation can be hypothesized as a three stage process involving plastein intermediates that are analogous to the bridging material as observed by Carroll et al (1971). The first stage is purely enzymic and causes partial breakdown of casein, thereby forming peptides. At this stage, casein micelles can be observed as small disintegrated entities, on electron microscopy (Andrews et al. 1977). This also accounts for the distinct lag phase, prior to the onset of gelation (Guthy et al. 1985). The second stage involves formation of plastein products, due to enzymic resynthesis of these smaller polypeptides. The hydrophobicity of these polypeptides renders them TCA insoluble, thus reflecting low enzyme activity after 8 h (Fig. 1), as observed in the present investigation. This also explains the reports of Samel et al (1971) and Guthy et al (1985), who observed that samples with more gelation show less protease activity. The third stage is purely non-enzymic and involves aggregation of these hydrophobic polypeptides by non-covalent bonds, which may also interact with hydrophobic zones on casein micelle surface to form a continuous gel mesh network, that can be seen as fibres joining casein micelles, on electron microscopy (Caroll et al. 1971) and is manifested as age gelation in UHT milk.

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Effect of Seed Treatment on Lipolytic Deterioration of Pearl Millet Flour During Storage

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Pearl millet seeds were subjected to soaking in dilute acid solution (0.05 N HCl), dry-heating (50°C for 60 min or 100°C for 10 min) and boiling water-blanching (98°C for 30 sec) treatments, before grinding to flour. The changes in fat acidity in the flour during storage at ambient temperature were monitored. Boiling water-blanching treatment was found to completely arrest the development of the fat acidity in the flour, during 30 days storage at ambient temperature. The acid-soaking of seeds was partially effective, while the dry-heating of seeds was found to be totally ineffective for this purpose.

Keywords : Pearl millet, Seed treatments, Fat acidity of flour, Storage at ambient temperature, Dilute-acid soaking, Dry-heating, Boiling water-blanching.

A rapid development of rancidity and bitterness in the flour has been a serious problem in the acceptability and utilization of pearl millet flour (Kaced et al. 1984), as it contains high concentration of lipids (Vakharia and Chakraborty 1984). This has been attributed to the development of fat acidity, due to lipolytic activity (Lai and Variano-Marston 1980) and accumulation of peroxides, due to oxidation of lipids in the meal during storage (Kapoor and Kapoor 1990). Kaced et al (1984) have clearly shown that the development of rancidity in pearl millet flour during storage is mainly owing to the release of free fatty acids upon the action of lipase. Reddy et al (1986) have implicated enzymatic oxidation of phenolic compounds as one of the causes for development of off-odour in pearl millet flour. Panwal et al (1989) studied the bleaching and functional properties of pearl millet flour by treating with acids. The earlier attempts to retard such changes and improve the shelf-life of flour included use of anti-oxidants, defatting and salting of flour or heat treatments (Kapoor and Kapoor 1990), milling of grains into different milling fractions (Carnovale and Quaglia 1975), use of different containers for storage (Dahiya and Kapoor 1983) and heating of conditioned grains at 97°C for 12 min (Bookwalter et al. 1987). The decortication and separation of milling fractions may not be economically feasible at domestic level. The use of anti-oxidants or different containers as well as dry heat treatments do not fully eliminate the rancidity. A simple method that can be practised at domestic level to restrict the fat acidity development in pearl millet flour during storage at ambient temperature is reported in this communication.

The pearl millet grains, cultivar 'RHRBH-8802', (500 g) were soaked in 0.05 N HCl (1:3, w/v) for 12 h at ambient temperature (27±2°C), washed thoroughly under tap water and dried at 40°C in hot air oven for 12 h to 10% moisture. The second lot of dry grains (500 g) were heated in hot air oven at 50°C for 60 min in one set and at 100°C for 10 min in another set. The third sample (500 g) was subjected to boiling water-blanching (1:5 ratio of seeds to blanching water, w/v) at 98°C for 30 sec and dried at 40°C for 60 min to 10% moisture. All the treated grain samples, including untreated grains, were milled to whole flour in a laboratory mill (Milcent Swastic Eng. Works, Anand, India) and the flour was stored in cloth bags at ambient temperature (27±2°C). The flour samples were analyzed (AOAC 1975) for changes in fat acidity (mg KOH/10 g flour) at every 5th day, during storage for 30 days. The oil was extracted from the flour samples in petroleum ether (40-60), using Soxhlet apparatus, and the peroxide value of extracted oil was determined by titration with standard sodium thiosulphate (AOAC 1975).

The fat acidity was found to increase by about 6-fold in untreated and dry-heated grain flour; by about 1.5-fold in acid-soaked grain flour; while it remained almost unchanged in the flour obtained from boiling water-blanched grains (Table 1). The blanching treatment raised the initial grain moisture content from 10 to 13%, which was reduced to initial level by drying of grains at 40°C for about 60 min, without any detectable loss in dry weight. The accumulation of peroxides in the extracted oil from stored flour was not detected in both untreated or treated samples. The results clearly indicated the effectiveness of simple boiling water-blanching treatment of grains in complete arresting of the lipolytic deterioration of the flour.

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TABLE 1. EFFECTS OF DIFFERENT SEED TREATMENTS ON CHANGES IN FAT ACIDITY (mg KOH/10 g FLOUR) IN PEARL MILLET FLOUR DURING STORAGE AT AMBIENT TEMPERATURE

Seed treatment	Fat acidity, mg KOH/10 g flour							Moisture after 30 days, %
	Flour storage, (days)							
	0	5	10	15	20	25	30	
Untreated (control)	4.3	10.9	16.8	20.7	21.7	23.8	25.2	11.6
Acid-soaked (0.05 N HCl)	4.2	10.6	9.8	9.5	6.6	7.0	6.7	10.2
Dry-heated								
50°C, 60 min	4.4	12.0	15.1	20.7	23.8	25.2	28.8	8.6
100°C, 10 min	4.4	12.9	16.2	21.6	23.6	25.2	26.6	8.5
Boiling water-blanched (98°C, 30 sec)	4.2	4.2	4.2	4.2	4.2	4.2	4.6	10.2

CD at 5% : Treatments - 0.184; Storage - 0.217; Interaction - 0.486. Initial moisture content of the flour = 10%

Kaced et al (1984) reported about 4-fold increase in fat acidity of pearl miller flour, after 10 days of storage and the increase in fat acidity has been attributed to the lipolytic breakdown of lipids and accumulation of free fatty acids. In the present studies, moist-heat was found to be highly effective in destroying the lipolytic activity, as compared to the dry-heat treatment. The acid-soaking of grains, although found to be effective in retarding the lipolytic degradation to a certain extent, imparted sour taste to the resultant flour. The hot water-blanching of seeds is simple, effective and it can be adapted at domestic level. Experiments on effects of boiling water-blanching of seeds on improving the shelf-life of flour of several other cultivars of pearl millet, changes on nutritional composition of flour and sensory properties of the unleavened pan bread, prepared from the flour of blanched grains, are in progress.

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2 Million Years of the Food Industry : Maguelonne Toussaint-Samat; Renaud Albernys, Ian Horman-Direction by Remy Montavon; Nestle S.A. Vevey, Switzerland-1991. p 255, Price : Nil

This superb compilation by Nestle S A is to offer a historic perspective at the 125th Anniversary of this world famous company in Switzerland. The treatise starts from the use of primitive stone tools to the present day sophisticated machines. The subject matter is covered in 14 main heads with left hand narrations of olden times state-of-art and the right side narrations giving comparative situation as today, on the various topics. Two hundred colour photo reproductions, in photo-lithography on art paper, trace on to the memory of Paleolithic age to the modern concepts in Food Science and Technology. In two hundred and fifty five pages (24 cm x 2.9 cm), the authors have brought out a commendable publication, tracing human imagery from early times to the 21st century, where need for 'best healthy foods', increased convenience and an ever-growing insistence on flavour and quality are the criteria, linked to the needs and desires of the modern day consumers.

For more than million years, we survived with flint tools and our skill, but we made progress. Fire came later. About half a million years ago, we created hearth and we began our hot dinners. Primitive cookery methods gave way to culinary arts. Cooking made foods more digestible and nutritious. With tools and fire power, the base of our food industry started. From domestication of animals in wild surroundings, and from the art of eating raw meats, we have now a variety of ways to enjoy eating meat. Our wide diversity of tools and our mastery over fire gave us a firm base for the modern food industry.

Water became vital to life and "all life is water" make water a universal item in diet. Methods of handling and making water safe and reach the public in tap, evolved gradually from early handling of water in broad leaves, bark, bamboo tubes, animal skin and pots to modern wares, have been developed due to the ingenuity of man.

From toasting, grilling, smoking, cooking in water, use of hot plates, ovens and to the present day microwave technology, the evolution has been traced clearly.

Taming cereals, making agriculture a science, processing them to get at the edible grain by stone grinding, chaffing to the present day milling, all came in succession as productivity increased. By crushing, toasting and grinding, the use of gruel was evolved. Combining of cereals with milk, Henri Nestle came out with milk food 125 years ago. Kneading, baking and using all sorts of culinary traditions, pita, pizza, tortilla, chapathis, pan cakes and flat breads gave us modern baking technology.

From fertile plains of Yang Tse Niang, Mekong and Gangetic India, rice came into the picture and it became the staple food of Asia. It went to Japan around 500 B.C.

Around 5000 years ago, early Peruvians and Mexicans were growing corn. Early 1900, John Hartog Kellog and his brother Keith Kellog started, the family business, based on corn.

Chinese, Japanese, Koreans, Germans, French, Italians and Americans knew about making noodles. Italians told many pasta stories.

Between 20000 and 10000 B.C, we were herding certain species of animals in North Africa and Ukraine. By mid 19th century, North America, Australia, Argentina and New Zealand were producing beef and mutton. Frozen meat went by ship in 1873. Essence of breeding came later to change wild animal husbandry to modern ways of handling.

From 2500 BC, milking of goat was known. Milk became a good thing in more ways. In 1856, Borden made sweetened condensed milk. In 1866, Henri Nestle started in Vevey, Switzerland, a plant for sweetened condensed milk. Milk was consumed in very many combinations with other foods. In Marco Polo time, the Tartars took with them milk products on rampage. Milk in powder form was used even at that time. Milk products technology developed fast in 19th century and milk as well as milk products are consumed today for health in many different combinations.

Even in Paleolithic times, shellfish was consumed. The treasures from the seas, lakes and rivers were exploited gradually. Today, the fishing has become a modern industry giving nutritious foods to consumers. In 5th century B.C., Greeks were fully aware of the inexhaustible marine resources. Canned fish and frozen fish of today have become common items of trade.

From Neolithic times, fruits were known in

different areas of the world. From wild nature orchards, the groves came in. Chinese mandarins were known 3000 years ago, but only in the past 30 years, we have used modern methods of handling, packing and transport. Spanish explorers brought tomatoes from South America in 16th century. It became a basic food for Italians and then spread world over. From the earth, came tubers and potatoes and have become immensely popular in nearly all over the world.

Around 7500 B.C., Thailand, Mexico and France harvested vegetables. Canning, bottling and bulk preservation came in later. In 1860, Heinz created market garden business. Today, vegetables form an important aspect of horticulture and biotechnology. Over 3500 years ago from China, came the soya bean, a nutritious product of modern times. This golden bean has become 'gold nugget of nutrition'.

Romans liked food and spicing became a part of delicacy. Ships from India carried spices to Egypt, Syria and Lebanon. Spices laid the foundation for 'pleasure eating'. Now, spices form the main flavour in foods.

From Kaffa in Ethiopia, came the coffee bean. In 18th Century, coffee became popular in Europe. Latin American countries took to cultivation and they are the largest producers now. The conventional instant coffee of today was developed in Switzerland in 1887 and coffee drink is the most popular beverage of today. By the 10th century, another popular drink, tea, came from China. In 1834, British planters introduced tea in India and Sri Lanka. Black tea, Oolong tea, green tea, flavoured tea and instant tea are the drinks of today. Even in 1523, another important beverage, cocoa came into being. Now chocolates have become a popular item in varied forms and combinations.

By 6000 BC, fruit juices were known. From fruit juices to wine became a controlled fermentation industry. The golden sugar from sugarcane, from the Gangetic Plains, was first exploited. This was introduced in West Indies. The sugar from beet, established itself from the German chemist Marggraf, finding that the sugar from beet and cane are one and the same.

Oil from olive was pressed 5000 years ago in Eastern Mediterranean and the present day production is still based on methods of artisan. In Brazil, peanuts were pressed to pulp and boiled with water to separate oil. Sunflower was processed in America. Sesame oil was pressed in India. Around Mediterranean, corn, rapeseed, soya, coconut, peanut, cottonseed etc., became sources of oil. Today, oil is bleached, filtered, winterised and deodorised to get a clear oil. Sumerians made crude butter from milk. Butter was common in Egypt. Butter was made from goat's milk, yak milk in Tibet and Celts made it from cow's milk.

Thousand years back, fermentation was known. From Neolithic times, fermented cereals, milk, honey and fruits formed a very much used processed food. Sumerians and Egyptians were the very first brewers. In 4000 BC, Babylonians brewed barley. Over 4000 years back, Egyptians began bread making, Greeks invented the oven and in 19th century, yeast came into bread processing.

Distillation is an ancient art used in Near East and Mediterranean. In 8th century, Persians knew the art of making alcohol from wine.

There are 75 different cheeses and 36 Camemberts in the world today. Sumerians, Greeks and Romans knew the act of cheese making. Huns and Mongols knew the art of fermentation.

In a 12000 year old fresco, honey was mentioned in Spain. Egyptians domesticated bees. Bees process their food to honey.

Microbes were the main cause of spoilage in food. Some microbes give useful products. To control bad microbes, several methods of preservation of food were developed. Drying, smoking, freezing, heating, pickling in acid, salt, fat, sugar or alcohol were applied. Many of these methods were then in crude form, known to early historic men. These applications became science after Pasteur's discovery in 19th century.

The fact that cold helped in preservation was known in Paleolithic times, Eskimo preserved food in snow. Romans built cold storage warehouses. Today, frozen foods have become a reality. Commercially, heating was used to check spoilage by empirical means till 16th century. In 1804. Nicholas Appert gave a scientific basis to sterilisation in cans and bottles.

From Neolithic times, acid soup was known in Europe. Saurkraut was a popular item in Central Europe and acid method of preservation became popular.

Around 500 B.C., Celts worked on salt mines. Salt became the most important item, we ever used for preservation and even today, it is used in refined manner.

Fats give a protective layer to foods. Dutch goude has been preserved with a layer of wax on

the surface. Pemmician is one of the oldest foods preserved in fat. Early 19th century saw fresh meat transported in barrels of fat.

Sugar coating to preserve foods was practised in China, India, Middle East and Egypt, since ancient times. Crystallised sweets came later in confectionery of today.

From ancient times, fruits, meat and fish were preserved in alcohol. Wine added new flavour and aroma. Geese, before immigration, ate gregariously to sustain flight. This idea was known to Egyptians and fatty goose too became a delicacy and has always been a symbol 'of lofty heights of the gastronomic arts'. Research on food science and nutrition used all these observations and applied nutrition was started. The means to adopt our foods to our needs and life styles are now established and will be a forerunner in the 21st century.

In 1830, Edmund Crosse and Thomas Buckwall made a business using old condiments and relishes. Tasty products resulted with the use of spices, herbs and exotic fruits. Use of mustard paste was known from Roman days and recipes were evolved using all food ingredients to enhance acceptability and convenience. Experienced chefs played a great role and even now, they are contributing to flavour field. Sensory science has now taken prominence in food fields. Recipes were adopted by food scientists and technologists to make safe foods and food engineers build machines and factories to make them. Thus, the culture of team work evolved itself to make present day products.

Romans and Gauls stuffed intestine to make sausage and the early packaging started with this. Leaves were used as wrapping of food from ancient times. Packages to keep food safe from microbes, water, insects and environment came in. Packaging engineers are now doing a remarkable job in giving the consumer elegant and protected food.

Food is as old as human beings. For millions of years, we have evolved ourselves and our food. We have made empirical solutions and traditional methods have been now refined to give better nutrition. This evolution has been the result of our changing life styles and fight for survival. Flavour, colour, nutritive value and safety are incorporated. With control of spoilage, insects and food poisoning, foods were preserved. From cottage industry, we are now in the industrial scale to cater to international food chain giving 'Quality and Safety'.

In spite of our progress, we see hunger and poverty in many parts of the world. Local productivity, coupled with local food industry, will be the answer for new food problems and new solutions will be found. But the urge of man to meet needs and desires in food will be the great motivation for progress. With modern knowledge of food science, nutrition and food safety, major advances can be seen in 21st century. Quantity, convenience of quality, and pleasure of eating will be our motto for times to come hereafter.

To the above ends, this publication has given us an insight into the past, expectations for the future with regard to food, health and prosperity. Food is as old as living things. Symbiosis of food and living things will govern the future.

The efforts put forth by Nestle in bringing out this wonderful, artistic and beautiful book are laudable. One glance at the book will earn the appreciation from every one.

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Evaluation of Certain Food Additives and Naturally Occurring Toxicants; 39th Report of the Joint FAO/WHO Expert Committee on Food Additives: WHO, Geneva: 1993, p. 49, Price. 6 Francs.

This report presents the conclusions of Joint FAO/WHO Expert Committee, convened to evaluate the safety of selected food additives and naturally occurring toxicants, and to recommend specifications for the identity and purity of food additives.

Section 1 gives the introduction regarding the function and scope of additives of JECFA, established on 1955.

Section 2 describes the general considerations regarding commonly employed terms, such as Acceptable Daily Intake (ADI), No Observed Effect Level (NOEL) and safety factor. It is suggested that the safety factor should comprise of two aspects i.e., pharmaco-kinetics and pharmaco-dynamics, whenever appropriate data are available. The committee stressed the need for data on inter-species and inter-individual differences in these parameters. Regarding safety assessment of flavouring compounds, the committee emphasized the need for data on structure-activity relationship, and exposure, in addition to factors considered earlier for safety evaluation of these substances.

On request of Codex Committee on Food Additives and Contaminants, the committee also reviewed naturally occurring toxicants-cyanogenic

glycosides and solanine along with other additives on agenda.

Section 3 includes the detailed comments of expert committee regarding evaluation of some food additives on the current agenda for the first time, as well as re-evaluation of some additives on the basis of additional toxicological data. Toxicological monographs have been prepared based on adequate data available. In some cases, clear cut or tentative recommendations regarding specifications and ADI have been either framed or revised in the light of new data, whereas additional studies have been recommended for the rest. The additives include emulsifiers such as sucrose esters, Tosol, Tosom, enzyme preparations, flavouring substances, thickening agents, solvents, waxes, miscellaneous compounds like curcumin and furfural as well as naturally occurring toxicants, cynogenic glycosides and solanine.

Total information of the current status of the selected additives and toxicants has been summarized in tabular form in Annexure 2. Recommendations of expert committee regarding lacunae in present knowledge and suggestions for further research have been included in section 4 and Annexure III. The report also includes a list of reports and other documents of the earlier meetings and list of recent technical reports from WHO. This report is a useful reference for those directly or indirectly involved in Food Toxicology, Analysis and Processing.

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Toxicological Evaluation of Certain Food Additives and Naturally Occurring Toxicants: WHO Food Additives Series : 30-Prepared by the Thirty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), IPCS-International Programme on Chemical Safety, World Health Organization, Geneva 1993. p. 399, Price : Sw. fr. 31.50

The monographs contained in this volume were prepared by the 39th Joint FAO/WHO Expert Committee on Food Additives (JECFA) which met in Rome from 3 to 12 February 1992. Monographs in this volume summarize the safety data on selected food additives and naturally occurring toxicants, reviewed by the Committee. Monographs on each of these substances are presented in a general format covering the review of biological data viz., biochemical aspects, toxicological studies in animals and humans, followed by comments, evaluation and references; spread over eight sections. The data reviewed include - two emulsifiers (thermally oxidized soyabean oil and thermally oxidized soyabean oil interacted with mono-and di-glycerides of fatty acids), two enzyme preparations (cellulose derived from *Trichoderma longibrachiatum* and lysozyme), two flavouring substances (limonene and quinine), three solvents (1,2-dichloroethane, dichloromethane and diethylene glycol monoethyl ethers), one thickening agent (alginic acid and its salts), six waxes (bees wax, candelilla wax, carnauba wax, micro-crystalline wax, paraffin wax and shellac), and two other additives (furfural and potassium bromide), as well as some naturally occurring toxicants (cyanogenic glycosides, solanine and chaconine).

For the first time, safety data on limonene-a flavouring agent and waxes (bees wax, candelilla wax, carnauba wax, shellac) have been presented, with expanded data on diethylene glycol mono ethyl ether-a solvent. The information presented in the annexures includes the list of reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on food additives and recommendations on compounds on the agenda.

The data summarized in these monographs serve as the basis for acceptable daily intakes established by the Committee. This volume and others in the WHO Food Additives Series contain information that is useful to those who produce and use food additives and veterinary drugs, government and food regulatory personnel, industrial testing laboratories, toxicological laboratories and universities.

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Low Calorie Foods: Handbook by Aaron M. Altschul, Marcel Dekker Inc., 270, Madison Avenue, New York. March 1993, Price $ 165.00

Undernutrition and overnutrition are two conditions encountered due to imbalance of nutrition in different geographical areas. Overweight and obesity are problems of industrialized societies, whereas deficiency diseases are rampant in underdeveloped countries. With advances in food science and technology, there are a variety of low calorie foods introduced into the market to combat overnutrition. This book, specially written with

reference to American population, presents a wealth of information contributed by different scientists and tries to answer most of the questions, a health professional would ask about low calorie foods.

The book has been divided into five different sections. Section A deals with overview of obesity and food trends which includes 5 articles on adult and childhood obesities, trends in eating pattern in United States and fat in foods. Section B is the major section of the book which includes 12 chapters on all aspects of low calorie foods. It traces the history of low calorie foods in United States with consumption data upto 1991. Current and future regulations by FDA have also been covered. This section also includes chapters on sugar substitutes, microparticulated proteins, fat substitutes, bulking agents, low fat and low calorie foods. The information given covers safety and regulatory aspects, importance, application, chemical properties, technical aspects and functionality. This could be useful to anyone looking for detailed information on any aspect of low calorie foods.

Section C written on impact of low calorie foods covers 3 chapters on food marketing, nutrition education and development of low calorie and low fat recipes. Section D examines the impact of diet composition on energy intake, impact of dietary fat and carbohydrates on body weight maintenance, effect of lowering dietary fat on health status and consequences of long term low fat diet regimens. Last section gives information on low calorie foods and obesity, psychological aspects of low calorie foods and role of low calorie foods.

This book serves the need of good reference book on low calorie foods for food scientists, technologists and health professionals. This could serve as a good handbook even for students training to be dietitians or nutritionists.

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AFST (I) NEWS

THE OBSERVANCE OF WORLD FOOD DAY BY THE ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA)

KHARAGPUR CHAPTER, LOCATED AT
AGRICULTURAL ENGINEERING DEPARTMENT,
INDIAN INSTITUTE OF TECHNOLOGY, KHARAGPUR 721 302 (W.B.), INDIA

The World Food Day-1993 was celebrated with the major theme of 'Harvesting Nature's Diversity'. The celebration was carried out in two phases. In the first phase, a number of activities involving participation of school and college students as well as Campus ladies were organised. The main programme of prize distribution and 'World Food Day' seminar was subsequently held in the second phase, on the World Food Day, the October 16, 1993.

The Phase-wise details of the events are as follows :

PHASE I : COMPETITIVE EVENTS

(1) Food and Nutrition Quiz: Entries were invited from students of Class VIII to XII. Seventeen teams comprising two members each competed in the Quiz. In the preliminary round, eight teams were selected, through an objective type written test, for the final oral questioning round. The Best and the Second Best teams (two teams jointly won the second place) were awarded the certificates of merit and consolation prizes.

(2) *Debate:* Debate contest was organised both at school and college levels, the topics being "Food for all by 2000 A.D. - A mere dream" and "India needs foreign investment in Food Sector", respectively. Six school level and two college level teams, comprising two members each, participated in the debate. The best and the second best speakers were awarded the certificates and prizes. A consolation prize was also awarded at school level.

(3) *Essay :* The topics for the school and college level debate were "Balanced Diet" and "Need of Food Processing for the Economic Growth of the Country", respectively. Eleven entries were received at school level, out of which one first and two second prizes along with the certificates of merit were given away. At college level, two entries were received and both were awarded consolation prizes.

(4) Poster : The topics for the school and college level Poster Competition were "Future Foods" and "Food Scenario for Third World Countries", respectively. Twenty-nine entries were received at school level. Certificates and prizes were awarded to the Best and the Second Best entries. Consolation prizes were given to three entries.

At college level, only one entry was received. Since no comparison was possible, a special prize was given to this entry.

(5) Cookery Competition : Potato-based recipes were invited from IIT Campus ladies for this competition. A spectacular variety of delicious potato dishes was displayed by twenty participants. The dishes were evaluated by four judges on the basis of selection of product, nutritional value, completeness of cooking, cost, garnishing and dressing. One First, two Second and three Consolation prizes were awarded.

PHASE II : WORLD FOOD DAY SEMINAR

The main programme of 'World Food Day' Seminar and prize distribution was held in the Second phase, on the afternoon of 16-10-93. The function was presided by Dr. N.G. Bhole, President of the Chapter, Prof. K.L. Chopra, Director, IIT Kharagpur graced the occasion as the Chief Guest, Dr. S. Bal, Officiating Head, Agricultural Engineering Department, IIT, Kharagpur, was a special invitee.

The function began with the opening remarks by the Secretary of the Chapter, Dr. S.L. Shrivastava, who informed the House about the objectives of celebration of World Food Day, its initiation and the theme of this year's celebration.

The President of the Chapter, Dr. N.G. Bhole, in his welcome address, narrated the state of hunger and malnutrition prevalent all over the world and suggested possible measures to face the

situation. He reviewed India's varied natural resources and expressed the hope that by harnessing the nature's diversity, especially the biodiversity, India will be able to tide over the problems of food shortage not only of her own, but also of other developing countries.

After President's address, Dr. S. Bal addressed the House. He stressed the need for observation of such days to make people aware of all the aspects of food production and consumption. Thereafter, prizes were distributed to the winners of various events by the Chief Guest. The prizes were partly sponsored by the only corporate member of the Chapter, M/s. Mahabir Rice Mill, Midnapur. Chief Guest, in his address, commended the performance of the participants in various events and congratulated the winners. He emphasized the role of food scientists and technologists in fighting out the increasing food shortage of the world. He also stated that Food Process Engineering is going to be very important branch of study in the near future.

The last event of the World Food Day celebration was a seminar talk by Dr. S. Bal on 'Status of Food Processing Industry in India'. Elaborating the need for food processing, he informed the House that even with a meagre utilisation of the potential, the food processing industry happens to be the largest industry in India, accounting for the highest fiscal outlays as well as employment opportunities. Development of food industry could go a long way in checking the food losses, thereby increasing the availability of food. At the same time, with its vast potential for employment generation, this industry could bring about a sea change in country's economy.

The function ended with a vote of thanks by the Secretary of the Chapter.

S.L. Shrivastava
Secretary

INDIAN FOOD INDUSTRY

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Contents of November-December 1993 Issue

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

INSTRUCTIONS TO AUTHORS

Manuscript, in triplicate, should be typed/printed in double-space on one side of A_4 size/bond paper, leaving 2.5 cm margin on all four sides of the page. The data reported in the manuscript must be original with clear definition of objectives, materials used, methods employed and without repetition. It should not have been published or offered for publication elsewhere. The manuscript must be as per format of the journal and authors should consult a recent issue of the journal for style and layout. The manuscript will be returned to authors, if it departs in any way from the required format and style. Papers essentially of an advertising nature will not be accepted. Foot - notes for text are to be avoided. All submissions will be reviewed by two referees and an appropriate editorial board member. The editor reserves the right to accept/reject the papers after reviews and make appropriate changes in the text and format.

Four different types of papers are published :

1. Research Papers with a maximum of 16 manuscript pages, including figures, tables and references.
2. Research Notes with a maximum of 8 manuscript pages, inclusive of figures, tables and references.
3. Rapid Communication with a maximum 16 manuscript pages (all inclusive) will be published rapidly, out of order of submission. Such communications must be based on new results of impact-making quality. The authors have to append a note, indicating novelty, implications of the results and urgency in publication. The editor reserves the right to decide on to what constitutes a Rapid Communication.
4. Reviews on specific topics of higher utility and current trends are also published. At present, these are by invitation only.

Materials and Methods must give sufficient details of the work to be repeated. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Any hazard must be mentioned and the relevant safety precautions described or reference made to safety procedures. The use of proprietary names should be avoided.

Each paper should be provided with an abstract of maximum ten lines, reporting concisely on the principal findings of the paper and in the form acceptable to abstracting agency. This should be followed by upto 6 keywords and this should cover all the main topics incorporated in the paper including any already given in the title.

The manuscript of the research paper should be divided into 5 sections viz., Abstract, Introduction, Materials and Methods, Results and Discussion and References. The research notes will be without these sections, under headings, except References. The chemicals are to be referred by names and not by formula in the text. All vernacular names e.g., *pulav, puree, chapati* must be underlined and briefly described when appearing for the first time. Similarly, all varietal names such as 'IR 150', 'Pb-56' must be put in single inverted commas.

The title of the paper is to be typed in capital and small letters for all types of papers. Authors' names should be in capital and the affiliation in capital and small letters. This should be followed by Abstract and Introduction (without heading).

Tables, numbered consecutively with Arabic numerals are to be typed on separate sheet and placed after references section. No vertical lines should be used and the table should not have more than 12 columns. Nil results should be indicated by using ND (not detected), while absence of data sign, '–'.

Graphs and line drawings must be in a style and standard of draughtsmanship. These should be drawn in Indian ink, with stencilled lettering, on tracing paper or white drawing paper or preferably art paper. The lettering should be twice the size of the printed letter. Photographs should be submitted as clear black-and-white prints on glossy paper and must have good contrast. High quality computer-generated line diagrams or glossy prints are also acceptable. Legends for all the figures are to be typed on separate page with details of symbols. The graphs, line drawings and photographs must be protected adequately against damage and bending of the envelope during transit. The manuscript will be returned to authors, if these requirements are not fulfilled.

In case of sensory evaluation results, the objectives should be stated clearly. Adequate details and references should be provided of the sensory evaluation methods used, along with the details of the collection, analyses and interpretation of data. The experimental designs used are to be clearly stated, e.g. randomized block, Latin squares, factorials, fractional factorials, incomplete blocks, etc. The source of panel, whether in-house or from outside is to be indicated. The number of panelists should be stated along with the composition (age, sex, status etc.) of the panel. It is necessary to state that same or different panel has participated in different sensory evaluation trials. The data involving less than 15 panelists and also where the panelists are not trained to function as a human analytical instrument (with periodic re-orientation) as well as the members without required sensitivity are not acceptable. For effective tests, the data involving less than 20 panelists and without information on the target population selected are unacceptable.

Sensory evaluation studies should report the data on physical and environment factors (taste booth type, colour of walls/separator, extent of distraction from external sound/odour, room temperature, relative humidity, illumination specification etc. The equipment and methods of sample preparation, testing temperature conditions, sample size, number of samples evaluated per session, nature of palate clearing agents used, time of evaluation, sequence of testing and data entry carriers should be reported. For effective test, the location of testing (stores,

homes, central location etc.), the instructions given regarding sample preparation/processing and the questionnaire used for collection of data should be specified.

Statistical procedure used for handling sensory evaluation data should be clearly indicated including any transformations or deviations that are carried out, e.g., assignment of numbers to intervals, categories and the like. The type of analysis carried out, categories, the level of significance and the decisions made are to be provided with appropriate tables and graphs. Appropriate and adequate data on the tests of significance, (like F, x^2, t, r, Rank sum, Mannwhitney, Rank Correction etc.) should be provided to justify conclusions and enable repeatability. The probability levels, degrees of freedom, the observed value of the test criterion, the direction of the effect and the decision based on these are to be indicated.

References should be cited at the appropriate point in the text by giving authors' names and year in bracket i.e. (Smith 1990; Smith and Gibbons 1990; Smith et al. 1992) as per Harvard System. A list of references, in alphabetical order, should appear at the end of Results and Discussion section. In case of single author reference, the alphabetical arrangement should be by date, while it should be by co-author when the reference with one co-author is cited. The alphabetical arrangement should be by date in case of the reference with more than one co-authors. Abbreviations such as *et al.*, *ibid*, *idem* must be avoided. The titles of all scientific periodicals should be abbreviated as in *Chemical Abstracts*, *Biological Abstracts*, *Annual BIOSIS List of Serials* and *Serial Sources for the BIOSIS Data Base*. If the journal is not included in these sources, then the name of the journal is to be given in full. Unpublished data or private communications should not appear in the list, but may be indicated in the text. No italic types should be used in the references, except for botanical and vernacular names. The examples of layout of typical references are as below :

Published Papers/Notes/Reviews

a) Tairu AO, Omotosu RA, Bamiro FO (1991) Studies on oxidative stability of crude and processed yellow nutsedge tuber and almond seed oil. J Food Sci Technol 28:8-11

Books/Approved Methods

a) Hacking AJ (1986) Economic Aspects of Biotechnology. Cambridge University Press, Cambridge

b) AOAC (1984) Official Methods of Analysis, 14th ed. Association of Official Analytical Chemists, Washington, DC

Chapters in Edited Books/Book Series/Papers in s Symposium Proceedings/Souvenir

a) Kurtzman CP, Phaff HJ, Meyer SA (1983) Nucleic acid relatedness among yeasts. In: Spencer JFT, Spencer DM, Smith ARW (eds) Yeast Genetics, Fundamental and Applied Aspects. Springer-Verlag, New York, pp 139-166

b) Gross E (1975) Subtilin and nisin: The chemistry and biology of peptides with $\alpha - \beta$-saturated amino acids. In: Walter R, Merenhoper J (eds) Peptides: Chemistry, Structure and Biology, Proceedings of the Fourth American Peptide Symposium, Ann Arbor, Michigan, USA, pp 31-42

c) Bhalerao SD, Mulmulay GV, Potty VH (1989) Effluent management in food industry. In : Souvenir, National Symposium on Impact of Pollution in and from Food Industries and its Management. Association of Food Scientists and Technologists (India), Mysore, pp 1-31

Reports by Specified Authors/Institutions

a) Andress EL, Kuhn GD (1983) Critical Review of Home Preservation - Literature and Current Research, Co-operative Agreement No. 12-05-300-553. USDA and Pennsylvania State University, Pennsylvania.

b) USDA (1977) Home Canning of Fruits and Vegetables, Home and Garden Bulletin 18, United States Department of Agriculture, Washington, DC

Patents

a) Schmidt GR, Means WJ (1986) Process of preparing algin/calcium gel-structured meat products. US Patent 4 603 054

Thesis

a) Ramesh MV (1989) Production of heat stable alpha-amylase. Ph.D. Thesis, University of Mysore, Mysore, India

Papers presented at Symposia

a) Stevens KA, Klapes NA, Sheldon BW, Klaenhammer TR (1991) Anti-microbial action of nisin against *Salmonella typhimurium* lipo-polysaccharide mutants Paper 7-501. Presented at 91st American Society for Microbiology, Annual Meeting, Dallas, Texas, USA, 5-9 May

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