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Enzyme-linked Immunosorbant Assays in Detection of Species Origin of Meats - A Critical Appraisal

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Identification of origin of meat products presents a serious problem to food analyst, who is confronted with providing a proof of fradulent substitution of mere expensive meat with cheaper meat. Thus, identification of the species and quantification of level of adulteration is a prerequisite for the regulatory control of such products. Recent developments in enzyme immuno assay techniques for the detection of species origin of meat, are critically discussed in this review. It is stressed that new approaches to species identification may have to place more emphasis on data interpretation, such as the use of specialized multivariate analysis in order to discriminate specific components of meat from other tissues.

Keywords: ELISA, Speciation of meat, Raw meats, Processed meats, Thermostable antigens, Immunogens.

Identification of origin of meat presents a serious problem to food analyst, who is confronted with providing a proof of fraudulent substitution of more expensive meat with cheaper meat. Thus, identification of the species and quantification of level of adulteration is a pre-requisite for the regulatory control of such products. The identification of fresh meat has been achieved using immunodiffusion (Fugate and Penn 1971; Swarts and Wilks 1982; Mageau et al. 1984; Cooper 1985; Hvass 1985; Doberstein and Greuel 1985; Bansal and Mandokhot 1988 a, b; Cutrufelli et al. 1988, 1991, 1992; Mandokhot 1995) and electrophoretic methods (Scopes and Penny 1971; Tinbergen and Olsman 1976; Sinclair and Slattery 1982; Slattery and Sinclair 1983; King and Kurth 1982; Hofman 1985; Bauer 1990). Potential of enzyme immunoassay as method of choice for the purpose has been well recognized. Its application for detection of adulteration or accidental contamination of one kind of food with other, however, is still in its infancy. The radio immunoassay (RIA) reported by Porath (1970) was the first food enzyme immunoassay employed in food analysis. He recommended it for the detection of specific proteins in the food extracts present below 1 pg/ml. Further development in the field was rather slow, because special facilities were required for handling and disposal of radioisotopes. Engvall and Perlmann (1971) and Van Weemen and Schuurs (1971) showed that radio labels could be replaced by enzyme labels and thus, the radio isotopes and their related problems could be dispensed with. Since then, various immunoassay procedures have been developed for use in food analysis based on enzyme labels. Horse radish peroxidase (HRP) and alkaline phosphatase (AP) are the most commonly used enzymes. These enzymes, however, have disadvantages for a rapid, field type assay kits, as both of them use substrates, which are unstable in aqueous solution. Hence, the substrate and solution need to be freshly prepared just prior to their use. Besides, they have diffuse end points in titration experiments, particularly AP and neither have substrates, which give an easily recognisable colour change. Chandler et al (1982) introduced enzyme urease and a suitable substrate solution consisting of urea and a pH indicator, bromocresol purple for use in enzyme immunoassays (ELISA). The system overcomes many of the disadvantages encountered with HRP and AP. The urease gives a colour change highly visual (yellow to purple) with sharp end points, when used with the stable urea indicator substrate solution, making it particularly useful for mass screening and field type ELISA procedures, besides circumventing the problems associated with endogenous peroxidase present in animal tissues.

ELISA as a technique for speciation of meat

ELISA is a well established technique in clinical laboratories. It provides rapid analysis of biological fluids and tissues for the presence of antigens or antibodies. The credit for the first application of ELISA in foods should probably be given to Ljungstrom et al (1974) and Ruitenberg et al (1974), who used this technique to detect parasites in pigs intended for slaughter. The work led to the development of food immunoassays which, however, were restricted to detect the

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presence of bacteria, viruses, mycotoxins, bacterial toxins and hormones in the food. Hitchcock et al (1981) were the first to employ the ELISA to detect a particular food antigen (soy protein) in food products (meat). It served as a model for all food products and was applied in food control laboratories with some success (Crimes et al. 1984; Olsman et al. 1984). The technique for speciation of meat was first adopted by Kang'ethe et al (1982) and Whittaker et al (1982). Since then, solid phase indirect and capture ELISA methods have been developed to detect adulterant meat at 1% to 10% levels in artificial mixtures (Kang'ethe et al. 1982; Patterson et al. 1984). The capture procedure was reported to be the most consistent and sensitive of the two procedures and was found to give better resolution between closely related species (Kang'ethe et al. 1982; Patterson et al. 1984). The better performance of the capture procedure for meat speciation was considered to be due to the consistency in preparation of the solid phase capture surface and the two site species discrimination required in the format. On the other hand, the indirect assay prepares capture surface by coating meat extracts, which are variable in molecular weight and protein composition.

Polyclonal antibody-based indirect ELISA to detect species origin of fresh meat : The indirect form of ELISA is used in simplified form to analyse a variety of unprocessed meats and raw meat products. Most of the ELISA procedures currently available for raw meat speciation use polyclonal antibodies raised against blood proteins either whole serum or albumin (Griffiths and Billington 1984; Patterson et al. 1984; Jones and Patterson 1986; Kang'ethe et al. 1982) or soluble muscle proteins (Martin et al. 1986). The specificity and sensitivity, however, depended upon the availability of antisera made completely free of cross-reacting antibodies to heterologous species. This is achieved by immunoadsorption technique such as affinity chromatography. Kang'ethe et al (1982) and Whittakar et al (1982, 1983) independently developed indirect ELISA to detect residual serum albumin and serum proteins, respectively in the meats of cattle, horse, sheep (Kang'ethe et al. 1982) and horse, ox, sheep, camel, pig and kangaroo (Whittakar et al. 1982, 1983). The former raised the species specific antibodies in rabbits against the serum albumin of respective species, while the latter against the whole serum. The antisera were made species specific by affinity chromatography. Both used the HRP to label tracer reagents (HRP conjugated goat anti-rabbit antibody). Whittaker et al (1982, 1983) used sodium citrate (pH 5.5 extracts from raw muscles as test antigens. They obtained optimum binding of the antigen to the solid surface microtiter plates between pH 5.0 and 6.0, instead of the more usual pH 9.6. They further reported that the binding of extracts was non-linear at higher concentrations with dilutions ranging between 1:20 and 1:320 giving similar results. For the rountine testing, they used 1:50 dilution of the concentrated extracts or extract of 20 mg meat sample in 5 ml of citrate buffer for 5 min without further dilution. The test could detect adulterant meat at 10% level and above. They, however, expressed that a ten-fold increase in the sensitivity of test could be achieved, if capture antibody at the initial step was introduced in the assay.

Griffiths and Billington (1984) evaluated indirect ELISA procedure for quantification of beef in other meats, using commercially available polyclonal antibodies to bovine blood serum raised in rabbits and goat anti-rabbit IgG conjugated to alkaline phosphatase. They prepared model mixtures containing known percentage of leg of beef (reference material) in fat, hydrated husk and pork to determine the efficacy of the test in quantification of beef. A calibration curve was constructed for the beef reference material, plotting optical density (OD) vs log 10^1/beef for each dilution and the beef concentration in the mixed preparations was directly extrapolated from the calibration curve. However, more accurate results were obtained by using regression line formulae and a computer. The result suggested that a quantitative determination of beef in meat products using antisera against serum was not possible, because of the variation in quantity of blood serum from one joint to another, together with deterioration of blood serum. Evidently, the residual blood levels were too variable to correlate with corresponding meat. The test, though was not found applicable for the quantification of beef, provided a good qualitative test for detection of beef.

Patterson and Jones (1983) developed an indirect ELISA, based on detection and measurement of serum albumin present in the meat of sheep, horse, cattle, pig, goat and deer as residual protein after slaughter. They employed polyvalent anti-albumin antibodies raised in rabbits and made species specific by immunoadsorption by affinity column chromatography, using cyanogen bromide activated sepharose - 4B chromatography column. The immuno-adsorbed antisera in Ouchterlony test produced species specific reaction, but in ELISA,

they revealed cross-reactions. The adsorbed and purified anti-sheep serum cross-reacted strongly with goat and weakly with beef and deer antigens. The purified beef and horse antisera, however, did not reveal any cross-reactions. The horse meat thus could easily be differentiated from beef, pork and lamb, at the optimal dilution of the meat extracts (1:200 to 1:12800) and was also easily detected in mixtures with beef at levels above 3%.

Jones and Patterson (1986) improved upon the procedure to facilitate the use of non-specific (unpurified) anti-species antisera by integral assay inhibition of heterogeneous cross-reactivity. The antisera (anti-albumin) identifying beef, horse and pig produced in the laboratory and the four commercially produced antisera identifying beef, horse, pig, sheep/goat were stabilized on 6 mm dia discs of filter paper (Whatman No. 3) by applying 20 µl aliquots of each anti species antiserum (undiluted). After superficial drying at room temperature for 1 to 2 h, the discs were further dried overnight under vacuum and stored at 4°C in screw-capped bottles. These stabilized discs were given blocking treatment before use. The discs of anti-species antisera were soaked in 20 ml phosphate buffer, pH 7.2 with 0.05% Tween-20 (PBST), containing appropriate (1mg/ml) heterologous albumin (e.g., one anti-pig disc was added to 20 ml PBST, equivalent to 1:1000 working dilution in which was dissolved 20 mg each of bovine and equine albumin) and shaken gently for 1-2 h during incubation at 20-25°C. The optimum blocking conditions for each antiserum to give an appropriate species specific response and reduced cross-reactivity to a minimum (i.e., less than 20% of the homologous value) were determined by checker board titration assays, comparing the effect of heterologous treatment and antibody dilution.

Jones and Patterson (1986) compared tissue exudates (drip) and water or normal buffer extracts of meats of cattle, horse, pig and sheep either frozen in aliquots or freeze-dried as test antigens and found aqueous meat extracts to give consistent absorbance difference between the homologous (high colour) and heterologous (low colour) responses and provided reliable, accurate species identification without prior extensive purification of antisera by affinity chromatography. They found the ELISA to be a practical alternative to agar gel precipitation (AGPT) screening test. It provided results in less than 3 h either on a large or small scale. The test was reported to be more economical as compared to AGPT.

Polyclonal antibody-based capture (sandwich) ELISA to detect species origin of fresh meat : Patterson and Spencer (1983) developed a diagnostic kit, which gave results within 30 min for use in the field. The test was based on the use of polystyrene stick as the solid phase to which species specific antisera were coated in phosphate buffer (PBS, pH 7.0) by dipping the sticks at 37°C overnight. The species specific antisera were prepared against the serum of cattle, sheep, kangaroo, horse or goat by the methods of Whittaker et al (1982, 1983). The coated sticks were washed twice with PBST (0.05% Tween 20) and blotted dry before storage at 4°C. The stored sticks gave consistent results even after 6 months of storage at 4°C. These sticks were soaked in meat extract (test antigen) for 10 min, dipped in HRP-antibody conjugate for 10 min followed by 5-10 min dip in O-toluidine substrate solution. A positive reaction was characterized by a change from a colourless to deep blue solution, occurring after 5 to 10 min. The system detected beef, sheep, kangaroo, horse and goat meats at 1% level of contamination.

The method was improved upon by Patterson et al (1984) to enable to differentiate unprocessed beef, sheep, horse, pig, kangaroo, camel, buffalo and goat meats. The species specific antibodies produced in sheep/cattle were coated on to the microtiter plates instead of the sticks and then used to immunoextract soluble proteins from the prepared meat samples. Bound meat proteins were then detected by the addition of species-specific rabbit antisera followed by staphylococcal protein-A conjugated to HRP. Preparation of the test antigen was not found to be critical, because colour production was approximately constant between sample dilutions of 1000 and 10g/l. Increased sensitivity and selectivity allowed the bulking of the samples for screening purpose. In addition, the assay was faster and the testing could be carried out in less than 2 h. Species specific capture antibodies utilized in the test were raised in sheep or cattle. Primary and booster doses of multiple emulsions containing 4 mg of whole serum were administered to sheep (8 mg of goat antigen) and 10 to 20 mg to cattle (bleeding 8 to 10 days after booster doses). The second antibody was raised in rabbits and purified by affinity column chromatography.

The double sandwich ELISA procedure was further modified by Jones and Patterson (1985) so as to detect very low amounts of pig meat in beef and manufactured beef products made with husk

and other additives. The assay used a monospecific antiserum against albumin instead of the whole serum bound to solid phase support to sequester the porcine serum albumin (PSA) from amongst the many proteins co-extracted from the complex sample extracts. Further immunorecognition was made with a second antibody also specific for PSA, which was detected by a conjugated enzyme IgG that interacted only with second anti-PSA antibodies. Subsequent enzyme conversions of substrate gave clear visual differences between beef and beef containing 0.5% of pig meat and beef products with 1% of the lean replaced by lean pork, compared with reference samples.

Johnston et al (1983) developed commercial kit designed to detect contamination at 1% (w/w) within 2 h total test time. The kit comprised a 96 well flexible PVC plate coated with affinity purified antibody to which meat extracts were added and incubated for 30 min at 37°C. After washing, the wells were incubated with urease-labelled antispecies IgG antibody for 30 min at 37°C. Washing was followed by addition of urease substrate solution and observed for 15 min at room temperature. The presence of meat of the species under examination was indicated by the vivid yellow to purple colour change of the substrate solution. Standards of known meat composition were assayed simultaneously to provide a semi-quantitative information.

Preparation of test antigens for polyclonal antibody-based ELISA: The test antigens for use in the indirect assay were mostly prepared by muscle extraction either in sodium citrate (Whittaker et al. 1982, 1983) or in saline (Patterson and Jones 1983; Griffiths and Billington 1984; Jones and Patterson 1986) or as tissue exudate/drip (Jones and Patterson 1986). Whittaker et al (1982, 1983) prepared the test antigens by extraction of 1 g of coarsely chopped meat or meat shavings in 5 ml of 0.05 M sodium citrate buffer (pH 5.5) by gentle shaking for 5 min at room temperature. One ml of the extract was further diluted in 4 ml of citrate buffer to obtain 1:50 dilution for use in the indirect ELISA test. Alternatively, they also used approximately 20 mg of meat, extracted in 5 ml of buffer for 5 min without further dilution. They further observed that the clarification step was unnecessary, as presence of small tissue pieces and/or fat did not interfere with reproducibility of the assay. Patterson and Jones (1983) prepared meat extract antigens of raw meat or their mixtures by homogenization in saline of finely minced material, followed by centrifugation at 10,000 rpm for 30 min at 4°C. Supernatant was filtered through a Whatman No. 3 paper to get rid of fat particles and stored frozen in aliquots of 3 ml or in freeze-dried form. This extract was used as test antigen by diluting 1:50 w/v in coating buffer.

Later, Jones and Patterson (1986) simplified this procedure by deleting the centrifugation of the homogenized mixture. A 20 g of trimmed, comminuted sample was homogenized in 80 ml of water or normal saline (w/v), allowed to settle after filtration through a Whatman No. 3 filter paper. The filtrate was used immediately or frozen for later use. A portion of the extract from the authentic samples (reference material) was freeze-dried for future use as standard after reconstitution at a concentration of 60-65 mg per 10 ml of water. The absorbance, when water or buffer extracts of pork comminuted samples were used, fell in the similar range, though water extraction gave slightly higher values. The absorbance bore relationship to the variable albumin content in the samples. In addition, they also prepared authentic extracts to use as test/standard antigens from the tissue exudate of fresh or thawed meat (drip) after dilution (1:1) with distilled water and filtration (Whatman No. 1 filter paper) and 20 µl of this were applied to the paper disc (6 mm dia) of Whatman No. 3 spread out on flat petri plates. The discs were freeze-dried overnight under vacuum and stored in screw capped bottles at 4°C. The fresh drip absorbed on the discs, however, produced lower absorbance, as compared to the filtered meat extracts from the authentic meat samples. They however, considered the tissue exudate (drip) to be sufficiently sensitive for screening whole meat samples, but recommended thorough extraction by water to detect adulterant or contaminated meat by indirect ELISA, developed to detect residual serum proteins in the meats.

Griffiths and Billington (1984) extracted serum from meat sample for use as test antigen. The extraction was done in ice cold saline solution (0.9% w/v) by mixing 25 g of minced meat (fat removed before mincing of meat) and exact 50 g each of ice and saline solution. The mixture was homogenized for 1 min and centrifuged at 3000 rpm for 10 min. Fat, if any formed on the top of the liquid, was discarded and the supernatant was filtered through a Whatman No. 542 filter paper. The extract (1:5 dilution), thus, obtained was used as test antigen to coat the microtiter plates. They reported that the ELISA, based on residual meat as test antigen, provided good qualitative test for

beef, but not quantitative because of variation in the quantity of blood serum from one joint to another and of deterioration of the blood serum.

The test antigens for use in capture (sandwich) ELISA were mostly phosphate buffer Tween-20 extracts (Patterson and Spencer 1983; Johnston et al. 1983; Patterson et al. 1984). Patterson and Spencer (1983) extracted the meat antigens from fresh frozen meat in PBST (0.05% Tween 20, 1 g/ml) at room temperature for 5 to 10 min and found the mechanical extraction unnecessary, while Johnston et al (1983) prepared meat extracts by vortex mixing of chopped meat with PBST (0.5%) Jones and Patterson (1985) homogenized thoroughly 40 g of comminuted meat in 360 ml of water for about 2 min, filtered through Whatman No. 3 filter paper and stored frozen in small aliquots, till used. At the time of assay, the extracts were further diluted at 1:5 and 1:25 proportions with phosphate buffer containing 1.5% Tween-20.

Monoclonal antibody-based ELISA to detect species origin of raw meats

The hybridoma technology (Kohler and Milstein 1975) has an advantage of using impure antigens for the production of monoclonal antibodies. Johnston et al (1983) and Jones and Patterson (1988) discussed in detail the application of this technique for the production of monoclonal antibodies for the meat species testing in the field to solve the problems, related to antibody quality (specificity and avidity of polyclonal antibodies) and to remove their requirements of tedious, expensive affinity purification and extensive testing for species specificity.

Till date, Martin and associates (Martin et al. 1989, 1991; Morales et al. 1994; Garcia et al. 1994) are the only ones, who have exploited hybridoma technology for the purpose of speciation of meats. Martin et al (1989) produced hybridoma cell lines by using chicken specific proteins (CHSP), isolated from chicken meat extracts (sarcoplasmic proteins), purified by column chromatography and affinity chromatography. The cell lines secreted monoclonal antibodies specific for chicken and turkey muscle proteins and failed to cross-react with pork, beef, lamb, horse or rabbit muscle extracts as well as with casein, gelatin or soy proteins. One of the cell lines secreted a monoclonal antibody (mAb) that was specific to chicken muscle proteins and was capable of distinguishing chicken meat from turkey in an indirect ELISA.

Later, a sandwich ELISA, based on monoclonal antibodies secreted by the hybridoma cell lines was developed (Martin et al. 1991) for quantification of chicken meat in beef and pork meat mixtures. The assay used mAb specific to chicken muscle soluble proteins to capture this protein from the complex meat mixtures. Immuno-recognition of the captured protein was attained with rabbit polyclonal antibodies against chicken muscle proteins (anti-CHSP). A commercial goat anti-rabbit immunoglobulin conjugated to peroxidase was used to detect anti-CHSP antibodies bound to the chicken proteins. Subsequent enzymic conversion of substrate gave clear optical density difference, while assaying mixtures of beef and pork meats, containing variable amounts of chicken meat, the test could quantify 1 to 100% chicken meat in the prepared meat mixtures.

Garcia et al (1994) produced a stable hybridoma cell line that secreted a mAb specific for horse muscle proteins. These cell lines were obtained by using horse specific soluble muscle proteins purified by Martin et al (1992) by immuno-adsorption chromatography, employing horse specific polyclonal antibodies against soluble muscle proteins, immobilised on a protein-A Sepharose CL-4B matrix. The mAb, when tested by indirect ELISA, did not show significant cross-reactivity against beef, chicken, pig and soy proteins or bovine caseins, gelatin and bovine serum albumin and could detect the defined amounts of horse meat (10 to 500 g per kg) in beef meat mixtures. Immuno-recognition of mAbs, adsorbed to horse meat, adsorbed on to the ELISA plate was made with rabbit anti-mouse immunoglobulins, conjugated to the enzyme horse radish peroxidase. Subsequent enzymic conversion of the substrate gave clear optical density differences, while assaying mixtures of minced beef, containing different amounts of horse meat.

This mAb, when used in an indirect ELISA format to detect the autoclaved horse meat extracts, revealed absorbance values of autoclaved preparations to be 80% lower than those of raw samples, thereby confirming the observations made by others (Johnston et al. 1983; Wijngaards and Van Biert 1985; Kang'ethe and Lindquist 1987a; Sherikar et al. 1988b) that the antibodies produced against heat labile proteins are not adequate to recognize proteins in heated meat products. They suggested that the target molecule or epitope to be detected in heated meat products must be the one to be used for developing mAb.

Preparation of test antigens for monoclonal antibody-based ELISA: Saline meat extracts were used as test antigens by Martin et al (1990), Garcia et al (1994) and Zade (1995). The former two extracted muscle proteins from beef, pork, chicken, pig and horse meat. Approximately 1 kg batches of lean meat were trimmed and well mixed and a representative 100 g sample was thoroughly homogenized in 300 ml of saline solution (8.5 g NaCl/litre) and the proteins were extracted by gentle agitation of these homogenates of 2 h at 4°C. Insoluble material was removed by centrifugation at 1500 x g for 5 min at 4°C and the supernatants were filtered through a Whatman No. 1 filter paper and stored at -20°C, until required for use. Zade (1995) prepared meat extracts by homogenising 75 g of meat/offal in 25 ml of NSS as antigen after centrifugation. He also used the pressed fluid extract of buffalo beef as test antigen in solid phase indirect ELISA by squeezing approximately 100 to 150 g of meat wrapped in muslin cloth. The fluid obtained was centrifuged (5000 rpm, 15 min) and the supernatant was used as the antigen.

Polyclonal antibody-based ELISA for detection of heated/processed meats

Reports on substitution of sheep, horse and kangaroo beef in Australian beef exports (Whittaker et al. 1983) and horse in British beef imports (Kang'ethe et al. 1982) have shown that processed meat products contain meats from animals other than those specified by the manufacturers. Speciation of meat in processed products, especially those that have undergone some degree of heating has not been successful, using the immunoassays based on detection of blood proteins commonly employed for raw meats indicating that assays of heated products must be based on the detection of heat stable antigens, which have retained their species specificity.

Milgrom and Witebsky (1962a) described the presence of the heat-stable antigens in adrenals. Hayden (1981) used antisera to such antigens to detect adulteration in thoroughly cooked beef sausages, using immunodiffusion test. Kang'ethe et al (1985, 1986) have shown that antisera to thermostable muscle antigens (TMAs) can be used to identify the species origin of fresh, cooked and autoclaved meat extracts from even phylogenetically closely related species in immunodiffusion test.

Kang'ethe and Lindquist (1987b) described an indirect ELISA for detection of beef, pork in raw and heat-treated meat products such as beef and pork sausages, beef bergers, cooked horse luncheon meat and corned beef, using antisera to TMA. Antigens from these products were extracted with phosphate buffered saline. The presence of beef and pork in these products was determined, using adsorbed goat antisera to cattle and pig TMAs, in an enzyme immunoassay. The assay was able to detect beef in pork sausages at the level of 10 and 5%, but not at 1%. Of the 44 commercial products labelled as containing only beef, 23 (52.3%) were shown to contain pork and 23 (50%) of the 46 pork products were shown to contain beef. Antisera to TMAs proved to be of great value in an enzyme immunoassay in species identification not only of heated (cooked), pasteurized and autoclaved meat products, but also of fresh unheated meats. Kang'ethe and Lindquist (1987b), however, reported that besides the species specific thermostable proteins, the TMA preparations made by the process of Milgrom and Witebsky (1962a) also contain some other proteins, which affect the ELISA adversely. Their ELISA experiments revealed inconsistent and irregular adsorption of TMA's extracted, as per the method of Milgrom and Witebsky (1962a) on microtiter plates. They further observed that most of these antigens gelled at 4°C. The gelling was considered to be caused by gelatin present in these antigens on account of conversion of muscle collagen on heating. Actually, gelatin formation is swift with pressure cooking and the step is an integral part in the extraction method of Milgrom and Witebsky (1962a). They attributed the presence of gelatin in TMA's to be the cause of poor adsorption of the antigen on the plates, because of the observation that there was 100% inhibition of adsorption on to the microtiter plate in the presence of 5 mg per ml of gelatin. Therefore, a method, which yielded antigen devoid of contaminating proteins, was developed. The TMA was dissolved in a small amount of saline and concentrated by ultrafiltration in an Amicon cell with PM-30 membrane, having a molecular weight cut-off of 30 KD. The concentrated fraction was loaded on a Sephadex G-200 or G-75 column and the fractions, containing the antigen as estimated by immunoassay, were pooled and concentrated by ultrafiltration for use in the test.

Kang'ethe and Gathuma (1987) prepared partially purified thermostable muscle antigens (PTMAs) from muscles, as described by Kang'ethe and Lindquist (1987b) from TMAs of several domestic and wild species like buffalo, cattle, goat, sheep, horse, pig, bushbuck, waterbuck, eland, oryx,

kongoni, wildbeast, topi, impala and gazelle. The antisera were produced in goats against TMAs extracted by the method of Milgrom and Witebsky (1962a) from these species. An indirect ELISA was performed employing the above antisera and PTMA. The results indicated the goat-antitopi, buffalo, camel, horse, and pig TMA sera to be monospecific. Goat antisera to cattle and eland TMA were, however, the poorest, as they could speciate only 4 and 2 PTMA, respectively. Other antisera afforded speciation of more than 10 PTMAs from their homologous PTMAs.

An antiserum to autoclaved porcine muscle extracts was raised by Sawaya et al (1990), in sheep and tested for pork in mixtures of pork in mutton (P/S) or beef (P/B) heated at 70, 100 and 120°C for 30 min using a competitive ELISA method. The results indicated that the antiserum detected a low percentage of pork (1%) mixed in beef and in mutton (0.5%), even when the meat mixtures were heated at 70, 100 or 120°C for 30 min, corresponding to heat treatment of commercially processed meat products. Regression analysis showed a high positive correlation ($r > 0.99$) between absorbance (OD) values and different percentages of experimental or commercially processed P/B meat mixtures. It was concluded that the combination of anti-porcine sera raised in sheep and the ELISA method can be used for the detection of low percentages of pork in processed meats.

Heat processed (thermostable) antigens: Watson (1990), while reviewing the stress proteins, observed that all the organisms i.e., bacteria, fungi, plants and animals responded to a wide range of environmental stresses including thermal by inducing synthesis of proteins called stress proteins, which can be grouped in 3 ranges, according to their molecular weights, e.g., 85-110 KD, 60-80 KD and <50 KD. Besides, one set of low molecular weight protein (8.5 KD) named as ubiquitin is present in all eukaryotic cells in response to stress. He further observed that most of these proteins were also produced in unstressed cells under normal physiological conditions.

Milgrom et al (1962a, 1963), while performing the studies on serological structure of adrenal and brain, came across such proteins, which were characterized by their remarkable thermostability. Besides, these proteins contained organ-specific antigens. They found the presence of these thermostable antigens in adrenals of porcine, bovine, equine and human origin. These antigens were found in organ fractions that resisted boiling at 100°C and were precipitable by ethanol (72%). They named these thermostable organ-specific antigens as BE (boiling resistant and ethanol precipitable) preparations. Later on, organ-specific BE antigens were also reported in testicle (Milgrom et al. 1963) and in grey and white matter of ox, pig and human brain (Milgrom et al. 1964a). Milgrom (1964b) further observed that the BE preparations of brain and adrenal also contained antigens that were non-organ-specific. Interestingly, these non-organ-specific antigens were characterized by their species specific character, since antisera to bovine BE preparations would combine with BE preparations of bovine tissues, but virtually not with preparations of human, porcine or equines. It was further observed that these species specific BE antigens were distributed throughout the animal body including blood, though their concentrations differed in different tissues e.g., liver, spleen, adrenal, kidney and testicles, which were found to contain them in abundance, but not brain and skeletal muscles (Milgrom et al. 1964a, b; Milgrom et al. 1967; Kaplan and Meyeserian 1962; Espinosa and Kaplan 1968; Intori and Milgrom 1968). Similar observations were also made by Jones and Mortimer (1985) and Bhilegaonkar et al (1990), who found more concentrations of BE antigens in adrenals as compared to skeletal muscles.

Milgrom et al (1964a, 1965) successfully used the antisera to organ BE to detect species origin of blood (including decayed blood), urine and decayed tissues. The antisera used were produced against the organ BE rather than serum BE, since, unlike the latter, the former contained all the species specific antigens present in all the various tissues and organs. Based on these results, they suggested the use of antisera to BE antigen for detecting species specific antigens in meat samples as well as the species origin of food that was consumed by testing animal (rat) excreta.

Kang'ethe et al (1985) demonstrated successful use of antisera to thermostable antigens to identify the species origin of fresh, cooked and autoclaved meat extracts from even phylogenetically closely related species in immunodiffusion test. The same group of workers, however, cautioned that the species specificity of BE antigens was not an absolute phenomenon, as some degree of cross-reactivity was observed in closely related species such as man and rhesus monkey. Since then, the

similar observations on cross reactions with closely related species have also been made by various other workers (Kang'ethe and Lindquist 1987a, b; Sherikar et al. 1988b; Kang'ethe et al. 1986, 1987; Sherikar et al. 1987, 1988a; Radhakrishna et al. 1988; Karkare et al. 1988; Saisekhar and Reddy 1995; Reddy et al. 1990), who used BE antigens from muscle tissues and organs to raise antiserum to detect species origin of tissues. Hayden (1981) has used antiserum to adrenal thermostable antigens to detect adulteration in thoroughly cooked sausages.

Characterization of thermostable antigens: As stated earlier, the thermostable antigens, whether organ or species specific, are resistant to heat treatment at 100°C for 20-30 min and insoluble in 72% ethanol and the very name BE for these antigens has been derived, based on these properties. Shulman et al (1964) tentatively classified these BE antigens obtained as greyish white powder to be mucoproteins because of their partial degradation by periodate and chymotrypsin, heat resistance and staining with Schiffs reagent/PAS reagents specific for glycoproteins.

Jones and Mortimer (1985), Radhakrishna et al (1988, 1989) and Bhilegaonkar et al (1990), however, did not corroborate the findings of Shulman et al (1964), since they did not find the presence of carbohydrate moiety in BE proteins. In their experiments, the sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) gels containing electrophoretically separated BE proteins failed to take up thymol blue sulphuric acid stain specific for the carbohydrates. Jones and Mortimer (1985) separated BE proteins from adrenal and skeletal muscle extracts from pig, sheep, horse and beef by iso-electric focussing and compared them with proteins from the respective raw tissues, which were soluble in dilute saline. They found that the BE residues common to adrenal and muscle tissues had mostly low isoelectric point, which on subsequent separation on PAGE plate of narrow pH range (4.0-6.5), gave at least 10 distinct bands. They further observed that the BE proteins were isoelectrically focussed at the pH 3.5-6.5. Similar findings were also made by Bhilegaonkar et al (1990). Earlier, Hartshorne et al (1969) and Hartshorne and Mueller (1969) had observed that it was the troponin, which was obtained, as isoelectric supernatant of the protein complex, isolated from the skeletal muscles over the pH range of 3.5-4.6. Schaub et al (1972) also reported the isoelectric pH of troponin to be between 4.5-5.5.

Radhakrishna et al (1988, 1989) compared the whole muscle thermostable proteins (TMP) of ox, goat, sheep and buffalo as well as the fractionated thermostable muscle proteins such as sarcoplasmic, myofibrillar and stromal from the corresponding species by SDS-gel electrophoresis and UV absorption. The TMP of all the 4 species revealed 4 distinct major bands in the low molecular weight range besides a few faint lines in the higher molecular weight range as well as some diffuse bands. The diffuse and the faint bands were common to all the species. The distinct bands appeared in the molecular weight range of 35-37,28, 26 and 16 KD and corresponded to the pattern and mobilities exhibited by the myofibrillar fraction of the respective species. They also compared by SDS-gel electrophoresis, the sheep TMP obtained by ethanol precipitation (TMPE) and by 10% trichloroacetic acid precipitation TMP (TCA) with the sheep BE adrenal protein (TAP) as well as pure troponin and tropomyosin fractions. Both the TMP (E) and TAP revealed similar patterns, but the former had greater intensity in the molecular weight range of 25000-37000 daltons, corresponding to tropomyosin-troponin complex. This ,major band was also detected in TMP (TCA). Simultaneous immunodiffusion studies with TMP, tropomyosin and troponin revealed the TMP to be identical to troponin and not related to tropomyosin.

The TAP exhibited UV absorption maxima at 260 ηm. but not TMP (E) and TMP (TCA). The latter two also did not exhibit UV absorption maxima at 280 ηm. The 260/280 absorption ratio of TMP (E) of all the four species studied was lower as compared to TAP of the corresponding species, but neither of these proteins revealed the ratio as low as 0.66, the characteristic of bovine serum albumin. It was further observed that the TMP (E) fraction with 35 to 37 KD molecular weight and similar to troponin can be precipitated with ammonium sulphate solution at 40-53% saturation level.

Bhilegaonkar et al (1990) also characterized the BE proteins prepared from the muscles and adrenals of goat, sheep, ox, buffalo and pig by SDS-PAGE and PAG isoelectric focussing (PAGIE) and found that the heat-stable adrenal antigens contained only one component of 35-36 KD molecular weight, corresponding to troponin-T, while the muscle proteins contained additional low molecular weight component as minor proteins, which were probably due to the occurrence of low molecular weight troponin present, due to heat treatment and complex nature of the muscle

proteins. Earlier, Greaser and Gergely (1971) had reported 4 fractions of troponin isolated from rabbit skeletal muscles as 35, 29, 21 and 14 KD. Later, Parisi and Aguiari (1985) reported three troponin components having molecular weights of 35.08, 32.25 and 20.63 KD from the myofibrillar portion of the muscles.

Bhilegaonkar et al (1990) also reported that it was troponin-T fraction alone, which was responsible for the production of species specific precipitating antibodies.

Thermostable proteins as immunogens : The BE preparations from muscles and adrenals of domestic animals like sheep, goat, ox, buffalo, pig, chicken, horse, camel, dog and several wild animals have been successfully used for the production of antibodies to detect the species origin of cooked, processed meat and meat products mostly by immunodiffusion techniques (Sherikar et al. 1988a, b; Milgrom and Witebsky 1962a, b; Hayden 1981; Kang'ethe et al. 1985, 1986; Milogram et al. 1962b, 1964a; Karkare et al. 1988; Reddy 1986; Reddy et al. 1990; Reddy and Reddy 1995). It was, however, observed that the distantly related species like chicken, pig, horse, dog could be easily detected and differentiated from the species like ox or buffalo or sheep or goat. However, the closely related species like ox and buffalo or sheep and goat revealed cross-reactions and needed absorption of the antisera.

Saisekhar and Reddy (1995) used troponin isolated from cattle and buffalo skeletal muscles for the production of antibodies to detect and differentiate cattle and buffalo from each other and from sheep, goat, pig and chicken. They reported the species specificity of anti-cattle troponin antisera. The anti-buffalo troponin antiserum, however, revealed cross-reactions with cattle, sheep and goat antigens prepared from fresh, cooked and decomposed muscles.

Encouraged with the performance of BE antigen to raise antisera for use in immunodiffusion, Kang'ethe and Gathuma (1987), Kang'ethe and Lindquist (1987 a, b) and Sawaya et al (1990), employed these antigens for the production of antisera for use in ELISA for detection of raw/cooked processed meat/meat products of buffalo, bush buck, waterbuck, eland, oryx, topi, impala, gazelle, cattle, goat, sheep, camel, horse and pig.

This review has covered the developments in enzyme immunoassay techniques for detection of species origin of meats. New approaches to species identification as stated by Jones (1985), however, may have to place more emphasis on data interpretation, such as the use of specialized multivariate analysis in order to discriminate specific components of meat from other tissues.

References

Bansal A, Mandokhot UV (1988a) Studies on differentiation of beef from meat of other species of animals. I. Comparative specificity and sensitivity of serum raised in buffalo calves against cattle antigen. J Food Sci Technol 25(3): 146-151

Bansal A, Mandokhot UV (1988b) Studies on differentiation of cattle meat from the meat of other species of animals. II. Comparative efficacy of different serological methods. J Food Sci Technol 25(4):232-235

Bauer F (1990) Electrophoretic identification of species in raw and heated meats. Ernahrung 14(6):357-361

Bhilegaonkar KN, Sherikar AT, Khot JB, Karkare UD (1990) Studies on characterization of thermostable antigens of adrenals and muscle tissues of meat animals. J Sci Food Agric 51:545-553

Chandler HM, Cox JC, Healey K, Macgregor A, Premier RR, Hurrell JGR (1982) An investigation of the use of urease-antibody conjugate in enzyme immunoassays. J Immunol Methods 53:187-194

Cooper MG (1985) Meat species testing in Australia. In: Patterson, RLS (ed) Biochemical Identification of Meat Species. Elsevier Applied Science Publishers, New York, pp 80-85

Crimes AA, Hitchock CHS, Wood R (1984) Methodology for meat species identification. In: Patterson RLS (ed) Biochemical Identification of Meat Species. Elsevier Applied Science Publishers, London and New York, pp 1-8

Cutrufelli ME, Mageau RP, Schwab B, Johnston RW (1988) Development of porcine rapid identification method (PRIME) by modified agar gel immunodiffusion. J Assoc Off Anal Chem 71(2):444-445

Cutrufelli ME, Mageau RP, Schwab B, Johnston RW (1991) Development of a rapid equine serological test (REST) by modified agar gel immunodiffusion. J Assoc Off Anal Chem 74(2):410-412

Cutrufelli ME, Mageau RP, Schwab B (1992) Development of a deer rapid identification field test (DRIFT) by modified agar-gel immunodiffusion. J Assoc Off Anal Chem 75(1):74-76

Doberstein KH, Greuel E (1985) Identification of meat of African antelope species and kangaroo by the agar gel precipitation test. In: Patterson RLS (ed) Biochemical Identification of Meat Species. Elsevier Applied Science Publishers, London and New York, pp 65-79

Engvall E, Perlmann P (1971) Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochem 8:871-874

Espinosa E, Kaplan MH (1968) Antigenic analysis of human heart muscle. J Immunol 100:1020-1031

Fugate HG, Penn SR (1971) Immunodiffusion technique for the identification of animal species. J Assoc Off Anal Chem 54:1152-1156

Garcia T, Martin R, Morales P, Haza AI, Anguita G, Gonzalez I, Sanz B, Hernandez PE (1994) Production of a horse-specific monoclonal antibody and detection of horse meat in raw meat mixtures by an indirect ELISA. J Sci Food Agric 66:411-415

Greaser ML, Gergely J (1971) Reconstitution of troponin activity from three protein components. J Biol Chem 246(13):4226-4233

Griffiths NM, Billington MJ (1984) Evaluation of an enzyme-linked immunosorbent assay for beef blood serum to determine indirectly the apparent beef content of beef joints and model mixtures. J Sci Food Agric 35:909-914

Hartshorne DJ, Theiner M, Mueller H (1969) Studies on troponin. Biochem Biophys Acta 175:320-330

Hartshorne DJ, Mueller H (1969) The preparation of tropomyosin and troponin from natural actomyosin. Biochim Biophys Acta 175:301-319

Hayden AR (1981) Use of antisera to heat stable antigens of adrenals for species identification in thoroughly cooked beef sausages. J Food Sci 46:810-814

Hitchcock CHS, Bailey FJ, Crimes AA, Dean DAG, Davis PJ (1981) Determination of soya proteins in food using an enzyme-linked immunosorbent assay procedure. J Sci Food Agric 32:157-165

Hofman K (1985) Principal problems in the identification of meat species of slaughter animals using electrophoretic methods. In: Patterson RLS (ed) Biochemical Identification of Meat Species. Elsevier Applied Science Publishers, London and New York, pp 9-31

Hvass A (1985) Species differentiation in minced meat products by immunodiffusion: Patterson RLS (ed) Biochemical Identification of Meat Species. Elsevier Applied Science Publishers, London and New York. pp 53-62

Intori HW, Milgrom F (1968) Thermostable kidney antigen and its excretion in urine. J Immunol 100:1195-1198

Johnston LAY, Tracey-Patte PD, Pearson RD, Hurrell JGR, Aitken DP (1983) Identification of the species origin of meat in Australia by radio immunoassay and enzyme immunoassay. In: Clifford MN, Morris BA (eds) Proceedings of Symposium on Immunoassay in Food Analysis, University of Surrey, Guilford, UK. pp 95-109

Jones SJ (1985) Recent improvements in existing techniques and new approaches to species identification. In: Patterson, RLS (ed) Biochemical Identification of Meat Species. Elsevier Applied Science Publishers, London and New York. pp 193-204

Jones SJ, Mortimer RH (1985) Species Identification of cooked meats by isoelectrofocussing. Preliminary studies to resolve heat stable components isolated from adrenal and muscle tissues. In: Patterson RLS (ed) Biochemical Identification of Meat Species. Elsevier Applied Science Publishers, London and New York. pp 118-128

Jones SJ, Patterson RLS (1985) Double antibody ELISA for detection of trace amount of pig meat in raw meat mixtures. Meat Sci 15:1-13

Jones SJ, Patterson RLS (1986) A modified indirect ELISA procedure for raw meat speciation using crude anti species antisera and stabilized immuno-reagents. J Sci Food Agric 37:767-775

Jones SJ, Patterson RLS (1988) Recent developments in meat speciation immunoassays for veterinary and food analysis. In: Morris BA, Clifford MN, Jackman R (eds) Proceedings of Conference on Immunoassays in Food Analysis, Guilford, Surrey, UK, pp 121-126

Kang'ethe EK, Jones SJ. Patterson RLS (1982) Identification of the species origin of fresh meat using an enzyme-linked immuno sorbent assay procedure. Meat Sci J 12:229-240

Kang'ethe EK, Lindquist KJ, Gathuma JM (1985) Immunological reactions of thermostable muscle antigens and their possible use in speciation of cooked and fresh animal meats. In: Patterson RLS (ed) Biochemical Identification of Meat Species. Elsevier Applied Science Publishers, London and New York, pp 129-144

Kang'ethe EK, Gathuma JM, Lindquist KJ (1986) Identification of the species origin of fresh, cooked and canned meat and meat products using antisera to thermostable muscle antigens by Ouchter Lonys double diffusion test. J Sci Food Agric 37:157-164

Kang'ethe EK, Gathuma JM (1987) Species identification of autoclaved meat samples using antisera to thermostable muscle antigens in an enzyme in immunoassay. Meat Sci 19:265-270

Kang'ethe EK, Lindquist KJ (1987a) Detection of beef and pork in fresh and heat treated meat products using EIA. J Food Sci Tech 24:289-293

Kang'ethe EK, Lindquist KJ (1987b) Themostable muscle antigens suitable for use in EiA for identification of meat from various species. J Sci Food Agric 39:179-184

Kaplan MH, Meyeserian M (1962) Immunological studies of heart tissue. J Immunol 88:450-459

Karkare UD, Sherikar AT, Khot JB, Jayarao BM (1988) Meat speciation in commercial canned meats using species antisera to thermostable antigens of adrenal glands. Ind J Meat Sci Technol 1(2):84-88

King NL, Kurth L (1982) Analysis of raw beef samples for adulterant meat species by enzyme staining of isoelectric focussing gels. J Food Sci 47(50):1608-1612

Kohler G, Milstein C (1975) Continuous cultures of fused cells secreting antibody of pre-defined specificity. Nature 246:495-497

Ljungstorm I, Engvall E, Ruitenberg EJ (1974) ELISA-Enzyme linked immunosorbent assay in serological diagnosis of *Trichinella spiralis* infection. In: Proceedings of Third International Congress of Parasitology, Vols. I, II, III. World Federation of Parasitologists, Congress Centre, Exhibition Grounds, Munich, Facta Publications, Vienna, pp 1204-1205

Mageau RP, Cutrufelli ME, Schwab B, Johnston RW (1984) Development of an overnight bovine identification test (ORBIT) for field use. J Assoc Off Anal Chem 67(5): 949-954

Mandokhot UV (1995) Report of the ad hoc research project speciation of meat for export. Department of Veterinary Public Health and Epidemiology, Haryana Agricultural University, Hisar, pp 1-18

Martin R, Azcona JI, Hernandez PE, Tormo J, Casas C, Sanz B (1986) Detection of chicken meat in unheated meat mixtures by an indirect ELISA. In: Proceedings of 32nd European Meet. Meat Research Workers, pp 429-433

Martin R, Wardale RJ, Jones SJ, Hernandez PE, Patterson RLS (1989) Production and characterization of monoclonal antibodies specific to chicken muscle soluble proteins. Meat Sci 25(1989):199-207

Martin R, Wardale RJ, Jones SJ, Hernandez PE, Patterson RLS (1991) Monoclonal antibody sandwich ELISA for the potential detection of chicken meat in mixture of raw beef and pork. Meat Sci 30:23-31

Martin R, Garcia T, Sanz B, Harnandez PE (1992) Partial purification of horse-specific soluble muscle proteins by immuno-adsorption chromatography. J Sci Food Agric 58:447-449

Milgrom F, Witebsky E (1962a) Immunological studies on adrenal glands. I. Immunization with adrenals of foreign species. Immunol 5:46-66

Milgrom F, Witebsky E (1962b) Immunological studies on adrenal glands. II. Immunization with adenals of the same species. Immunological reaction of thermostable muscle antigens and their possible use in speciation of cooked and fresh animal meats. Immunol 5:67-78

Milgrom F, Tuggac ZM, Witebsky E (1963) Immunological studies on adrenal glands inter-species relationship of thermostable adrenal specific antigens. Immunol 6:105-118

Milgrom F, Tuggac ZM, Campbell WA, Witebsky E (1964a) Thermostable ethanol insoluble antigens of brain. J Immunol 92:82-90

Milgrom F, Tuggac ZM, Witebsky E (1964b) Studies on species specificity. J Immunol 93:902-909

Milgrom F, Tuggac ZM, Witebsky E (1965) Organ specific antigens of liver, testicle and pituitary. J Immunol 94: 157-163

Milgrom F, Tuggac ZM, Campbell WA, Witebsky E (1967) The thermostable brain specific auto antigens. Immunol 98: 459-463

Morales P, Garcia T, Gonzalez I, Martin R, Sanz B, Hernandeg PE (1994) Production of a pig specific monoclonal antibody and detection of pig meat in raw meat mixtures by an indirect ELISA. J Food Prot 57:146-149

Olsman WJ, Dobbelaere S, Hitchcock CHS (1984) Methodology for meat species identification. In: Patterson RLS (ed), Biochemical Identification of Meat Species. Elsevier Applied Science Publishers, London and New York, pp 1-8

Parisi E, Aguiari D (1985) Methods of differentiating meats of different species of animals. In: Patterson RLS (ed), Biochemical Identification of Meat Species. Elsevier Applied Science Publishers, London and New York, pp 40-49

Patterson RLS, Jones SJ (1983) Species identification of meat in raw unheated meat products. In: Clifford MN, Morris BBA (eds) Proceedings of Symposium on Immunoassays in Food Analysis. University of Surrey, Guilford, UK, pp 87-94

Patterson MR, Spencer TL (1983) A rapid on-site test for speciation of meat. Australian Vet J 60(12):381-382

Patterson RM, Whittaker RG, Spencer TL (1984) Improved species identification of raw meat by double sandwich enzyme-linked immunosorbent assay. J Sci Food Agric 35:1018-1023

Porath J (1970) Radio-immunoassays as a means of determining small quantities of specific protein. In: Bendex AE, Kihlberg B, Loefqvuist B, Munck L (eds), Evaluation of Novel Protein Products. Pergamon Press, Oxford, pp 285-286

Radhakrishna K, Rao DV, Sharma TR (1988) Studies on the electrophoretic and immuno-diffusion methods in the differentiation of mutton and beef subjected to serve thermal processing. J Food Sci Technol 25(5):280-284

Radhakrishna K. Rao DV, Sharma TR (1989) Characterization of the major components in thermostable muscle proteins. J Food Sci Technol 26:32-36

Reddy PM (1986) Studies on the identification of cooked meats of cattle, sheep/goat. Ph.D. Thesis. Haryana Agricultural University, Hisar

Reddy PM, Madokhot UV, Chandiramani NK (1990) A comparative study on heated adrenal and IgG antisera for identification of cooked meats of cattle and sheep. J Food Sci Technol 27:221-223

Reddy PM, Reddy MG (1995) Immuno-chemical detection of pork using thermostable muscle antigens. J Food Sci Technol 32(4):326-328

Ruitenberg EJ, Steerenber PA, Brosi BJM, Buys J, Ljungstrom I, Engvall E (1974) Application of ELISA for the serodiagnosis of *T. spiralis* infection in pigs, under slaughter house conditions. In: Proceedings of 3rd International Congress of Parasitology Vols. I, II, III. World Federation of Parasitologists, Congress Centre, Exhibition Grounds, Munich, Facta Publications, Vienna, pp 1204-1205

Saisekhar Y, Reddy PM (1995) Use of troponin for species identification of cattle and buffalo meats. J Food Sci Technol 32(1):68-70

Sawaya WN, Mameesh MS, El-Rayes E, Husain A, Dashti B (1990) Detection of pork in processed meat by an enzyme-linked immunosorbent assay using antisevine antisera. J Food Sci 55(2):293-297

Schaub MC, Perry SV, Hacker W (1972) The regulatory proteins of myofibril: Characterization and biological activity of the calcium sensitizing factors (Troponin A). Biochem J 126:237-241

Scopes RK, Penny IF (1971) Subunit size of muscle proteins as determined by SDS-gel electrophoresis. Biochem Biophys Acta 236:409-415

Sherikar AT, Khot JB, Jayarao BM, Pillai SR (1987) Species differentiation in raw and heat treated meats using serological methods (agar gel precipitation tests). J Food Sci Technol 24:293-299

Sherikar AT, Khot JB, Jayarao BM, Pillai SR (1988a) Use of species specific antisera to adrenal heat stable antigens for the identification of raw and cooked meats by agar gel diffusion and counter immuno-electrophoretic techniques. J Sci Food Agric 44:63-73

Sherikar AT, Khot JB, Jayarao BM, Pillai SR (1988b) Differentiation of organs of meat animals and identification of their flesh in chicken using anti-adrenal BE sera. Ind J Anim Sci 58(5):565-573

Shulman S, Milgrom F, Witebsky E (1964) Immunological studies on adrenal glands. IV. Chemical studies on the bovine thermostable adrenal specific antigen. Immunol 7:605-615

Sinclair AJ, Slattery WJ (1982) Identification of meat according to species by isoelectric focussing. Aus Vet J 58:79-80

Slattery WJ, Sinclair AJ (1983) Differentiation of meat according to species by the electrophoretic separation of muscle lactate deagglutinase and esterase isoenzymes and isoelectric focussing of soluble muscle proteins. Aus Vet J 60(2):47-51

Swart KS, Wilks CK (1982) An immuno diffusion method for the identification of the species of origin of meat sample. Australian Vet J 59:21-22

Tinbergen BJ, and Olsman WJ (1976) Isoelectric focussing as a species identification technique in the inspection of food products.Fleischweirtschaft 56:1500-1506

Van Weeman BK, Schuurs AHWM (1971) Immunoassay using antigen enzyme conjugate. FEBS Letters 15:232-236

Watson K (1990) Microbial stress proteins. Adv Microbial Physiol 31:183-217

Whittaker RG, Spencer TL, Copland JW (1982) Enzyme-linked immunosorbent assay for meat species testing. Aus Vet J 59:125

Whittaker RG, Spencer TL, Copland JW (1983) An enzyme-linked immunosorbent assay for species identification of raw meat. J Sci Food Agric 34:1143-1148

Wijngaards G, Van Biert M (1985) Quantitative estimation of meat species by immunological methods : Problems and improvements. In: Patterson RLS (ed) Biochemical Identification of Meat Species. Elsevier Applied Science Publishers, London and New York, pp 90-96

Zade NN (1995) Differentiation of buffalo and cattle meat using immunological and electrophoretic techniques. Ph.D. Thesis, Haryana Agricultural University, Hisar

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Comparative Appraisal of Quality of Buffalo Milk Mozzarella Cheese Manufactured by Two Different Methods

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Two processes for the manufacture of Mozzarella cheese from buffalo milk, standardized at GAU, Anand and NDRI, Karnal were compared with respect to vat performance, cheese making efficiency, composition, textural, sensory and baking characteristics. It was concluded that either of the two methods could be used for Mozzarella cheese manufacture. The cheeses obtained by the two processes were of low-moisture category. The GAU process was beneficial with regard to reduced manufacturing time, higher cheese yield, desirable meltability and fat leakage on pizza and moderate chewiness in the cheese. On the other hand, NDRI process gave improved recovery of milk solids in cheese and better body and texture characteristics, culminating in ease of shredability.

Keywords : Mozzarella cheese, Stringiness, Fat leakage, Meltability, Texture profile, Sensory quality.

The process of manufacture has a bearing on the final quality of the resultant Mozzarella cheese. The two methods of Mozzarella cheese manufacture from buffalo milk, among others, one standardized at the Dairy Science College, Gujarat Agricultural University, Anand (Upadhyay et al. 1986) and the other at National Dairy Research Institute, Karnal (Ghosh and Singh 1991) differ from each other in some respects. Therefore, the present study was undertaken to evaluate the two processes with respect to the process efficiency, cheese characteristics and suitability of resultant cheese for its end use.

These two processes would be referred to as 'GAU process' and 'NDRI process' throughout the text.

Materials and Methods

Manufacture of cheese : Experimental Mozzarella cheeses were prepared by GAU process (Upadhyay et al. 1986) and NDRI process (Ghosh and Singh 1991) as outlined in Fig 1. The experimental values indicated in the figure are the ones, which were actually arrived at during the experimentation and not of the authors, who had standardized the processes. Two batches of cheeses were prepared at a time from 5 kg standardized, pasteurized buffalo milk each. In all, six replications were undertaken. Totally, twelve batches of Mozzarella cheeses were prepared.

The cheeses, after packaging in polyethylene pouches, were stored overnight at 8±1°C and then subjected to analysis for sensory, chemical, rheological and baking characteristics.

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Analyses : Milk, whey, mould water and Mozzarella cheese were analyzed for fat by Gerber method and for protein by semi-micro-Kjeldahl method (Menefee and Overman 1940). The total solids (TS) of milk, whey, mould water and Mozzarella cheese were determined by the Mojonnier method (Milk Industry Foundation 1959). The titratable acidity (TA) values of milk and whey were determined, using the methods described by the Indian Standards Institution (IS:1479, Part I 1961a). The cheese samples were analyzed for ash, following ISI method (IS: 1479, Part II 1961b) and salt by the modified Volhard method (Kosikowski 1982). The pH and TA of cheeses were determined, using the procedure outlined by Patel et al (1986).

All the analyses were carried out in duplicate and mean values are reported.

Baking characteristics of cheese : Fat leakage test was performed as per the method outlined by Breene et al (1964). The meltability of cheese was assessed using Schreiber test, in which the expansion of a disc of cheese (20 mm dia, 5 mm thick) was measured on a target graph of concentric circles after 5 min. in an oven at 232°C (Park et al. 1984). Schreiber meltability is given as the mean of 6 readings measured along 6 lines marked on a concentric set of circles, on the arbitrary scale of 0-10 units (Kosikowski 1982).

Textural characteristics of cheese : The textural properties were determined using Instron Universal Testing Instrument (model-1000, M/s Instron Ltd., England), using 5 kg load cell at a crosshead speed of 20mm/min. and a chart speed of 100 mm/min. The sample size of 1 cm^3 was subjected to 70% compression. The values for hardness, springiness,

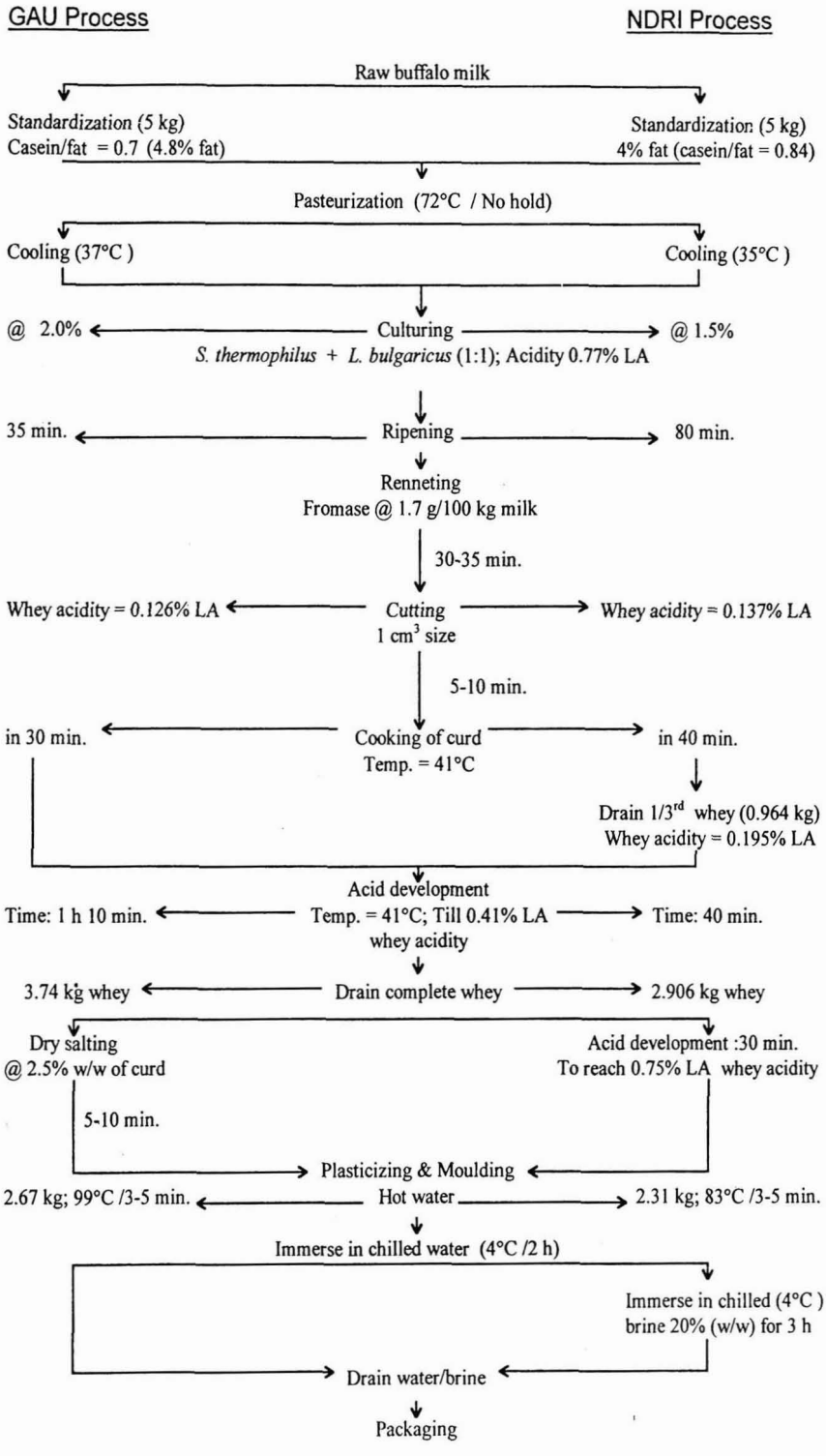

Fig. 1. Methods of Mozzarella cheese making as per GAU and NDRI processes

cohesiveness, gumminess and chewiness were derived from the force-distance compression curve (Larmond 1976).

The stretching property of the cheese curd was assessed subjectively as per Kosikowski (1982), during the plasticizing stage.

Sensory evaluation of cheese : Fresh cheese samples were evaluated for organoleptic characteristics by a panel of 6 judges, using the score-card suggested by Duthie et al (1980).

Baking trials : About 100 g of shredded cheese was topped on each prebaked pizza loaf (~ 20 cm dia, ~1.2 cm height, without any vegetable filling) and transferred to an oven maintained at 150°C and kept for about 15-20 min. to allow the cheese to melt, till the shreds fused uniformly. The pizzas were served hot to 6 judges, who evaluated them subjectively for flavour, springiness, meltability, fat leakage and browning.

Statistical analysis : Statistical analysis of the average of data was carried out, using 'Completely Randomized Design' with equal number of observations per treatment (Steel and Torrie 1980).

Results and Discussion

Vat performance : The cheese making time (culture addition to plasticizing stage) required was on an average 3 h 20 min and 4 h 5 min, respectively for GAU and NDRI processes (Fig. 1). The difference in the stretching acidity (GAU-0.41% LA, NDRI-0.75% LA) led to the observed difference in the manufacturing time. Furthermore, the latter procedure involved 3 h of brining, whereas dry salting in the former process required only 10-15 min, additionally, using less quantity of salt.

During plasticizing, the cheese curd obtained by GAU process exhibited better stretching characteristics (i.e., judged by the length of stretch without breaking from a unit quantity of curd and the spiral winding of the plastic curd to the wooden ladle during plasticizing) than that of curd obtained by NDRI process. This probably resulted from the higher temperature of mould water (99°C vs. 85°C), used in GAU process.

Composition of cheese : Table 1 reveals that GAU cheese contained significantly higher amount of moisture and moisture in-fat-free substances (MFFS), fat and fat-on-dry matter (FDM) and pH, whereas it had significantly lower protein, salt, ash and acidity than the NDRI process. Nevertheless, the protein content, when corrected to the same moisture content, did not vary significantly.

TABLE 1. CHEMICAL COMPOSITION, YIELD, RECOVERY OF MILK CONSTITUENTS AND INSTRON TEXTURAL PROFILE OF MOZZARELLA CHEESE

Parameters	Cheese made by		
	GAU process	NDRI process	C.D. (P<0.05)
Chemical composition, %			
Moisture	50.50	48.18	2.025
MFFS*	68.34	63.88	2.187
Fat	26.10	24.58	1.040
FDM**	52.74	47.46	1.583
Proteins	21.91	23.88	1.556
Proteins (46% moisture)	23.90	24.88	NS
Salt	1.49	2.38	0.088
Ash	2.26	3.16	0.228
pH	5.51	5.41	0.039
Acidity (as lactic acid)	0.50	0.60	0.014
Yield of cheese			
Kg cheese/ 100 kg milk	15.92	14.85	NS
at 46% moisture	14.58	14.22	NS
Recovery of milk constituents, %			
Fat 86.36	91.13	NS	
Proteins	76.92	80.91	NS
Total solids	52.84	54.24	NS
Textural characteristics			
Hardness, kg	3.00	3.67	0.51
Springiness, mm	4.82	4.89	NS
Cohesiveness	0.33	0.36	NS
Gumminess, kg	100.69	131.87	NS
Chewiness, kg-mm	4.82	6.44	NS

* Moisture-in-fat-free substances

** Fat-on-dry matter

The higher moisture content of GAU cheese was due to the reduced cheese making time (i.e., 45 min less than for NDRI), as the curd was stretched at lower whey acidity (i.e., 0.41 vs. 0.75% LA). The higher fat/FDM in former cheese arose due to the higher fat content in cheesemilk itself (4.81 vs. 4.00% in NDRI). The higher pH of GAU cheese was as a result of the lower draining acidity of whey. The higher salt content of NDRI cheese was due to the faster uptake of salt in cheese. This, in turn, led to higher ash content in such cheese. The significantly lower protein content in GU cheese was mainly because of higher moisture and fat contents.

Currently, there is no legal standard prescribed for Mozzarella cheese in India. The values of MFFS and fat observed in both the cheeses complied with the values (i.e., minimum 50% MFFS, 23-25% fat), suggested for our country by

the International Dairy Federation (1984) for buffalo milk Mozzarella cheese. However, NDRI cheese fails to meet the criteria laid down in the Italian decree of 1979 (Anon 1980) for buffalo milk Mozzarella (i.e., minimum 50% FDM, maximum 65% moisture) cheese with regard to the FDM content. According to the classification put forth by Breseman (1973), both GAU and NDRI cheeses can be classified as 'Mozzarella-low moisture'.

Recovery of milk constituents and cheese yield: The yield of cheese in the present study, expressed at constant moisture showed comparable values with those obtained by Ravi Sundar and Upadhyay (1990) (14.58 vs. 14.04%) and by Ghosh and Singh (1996 a,b) (14.22 vs. 13.96%).

The yield of GAU cheese, expressed as kg cheese/100kg milk or at constant moisture, was slightly higher than that of NDRI cheese. However, such difference was statistically non-significant (Table 1). The higher fat/FDM and probably higher lactose and mineral (calcium and phosphorus) contents (due to stretching of curd at lower whey acidity) in the former reflected in the higher percent yield. Ravi Sundar (1986) reported that the lactose and mineral contents of Mozzarella cheese made by stretching the curd at lower whey acidity were higher than those stretched at higher acidities.

The fat, protein and also total solids (TS) recovery of NDRI cheese were high as compared to those of GAU cheese, though the differences were insignificant. This resulted from the lower losses of fat and TS in mould water, probably due to the milder temperature employed during plasticizing. Such effect has been observed by Sharma (1991).

Textural characteristics of cheese : It is evident from Table 2 that all the values of textural parameters studied (viz., hardness, springiness, cohesiveness, gumminess and chewiness) were greater in NDRI cheese than those in GAU cheese. However, significant variation was only noticed in case of hardness.

The greater hardness of NDRI cheese may be attributed to the lower moisture and fat and higher protein contents (Table1). The greater hardness reflected on higher gumminess and chewiness of the cheese. The greater chewiness of NDRI cheese on pizza was not relished by the judges.

Baking characteristics of cheese : As seen from Table 2, both fat leakage and meltability were higher in case of GAU cheese. The difference was significant only with respect to fat leakage. The greater fat leakage was probably due to the higher moisture and FDM and lower protein and salt contents in GAU cheese. Such observation has been made by Patel (1984). According to Kindstedt et al (1992), the amount of fat leakage increases with increase in fat and decrease in salt content. Moreover, the tendency of free-oil separation, when cheese melts, is associated with the melting property of cheese.

The greater meltability of GAU cheese might have resulted from the lower whey acidity at draining and higher moisture and fat contents. Ravi Sundar (1986) found that with increase in the whey acidity at draining, there was decrease in meltability. Meltability is said to be enhanced by increases in the moisture and fat contents (Kindstedt 1993).

In actual baking trials, the fat leakage was found to be slightly lacking in NDRI cheese. Moreover, it was required to be kept for a longer period (~ 15 min.) in the oven in order to have proper melting on pizza loaf. GAU cheese tasted slightly sour, while NDRI cheese was more sour and salty. The chewiness and stringiness were greater in case of NDRI cheese, which were sensorily less desirable. Slight browning of cheese, especially at the periphery of pizza loaf, was only observed in NDRI cheese. Such browning probably occurred as a result of more galactose accumulation in cheese due to longer duration of starter activity. More residual galactose content in Mozzarella cheese has been implicated in browning of cheese (Jana 1992).

Sensory characteristics of cheese : NDRI cheese was allotted slightly higher scores for all the sensory characteristics. Nevertheless, such differences were statisticaly insignificant (Table 2).

Appearance : GAU cheese was whiter, more moist and exhibited wavy strands of spretched curd and it often exuded free whey. On the other hand, NDRI cheese was yellowish-white, but with greater gloss and exhibited uniformity.

Body and texture : GAU cheese was smooth, softer and less chewy and fibrous than its NDRI counterpart. NDRI cheese was more elastic and was referred to as 'Cheddar-like' by some judges.

Flavour : Mild sour taste was prevalent in GAU cheese, whereas NDRI cheese was decidedly sour in taste. NDRI cheese had a drier mouthfeel and had uneven salt distribution. Such variations in a brine-salted cheese are quite natural (Kosikowski 1982).

From the results of the present study, it is obvious that either of the two methods could be used for Mozzarella cheese manufacture from buffalo

TABLE 2. SENSORY SCORES AND BAKING CHARACTERISTICS OF MOZZARELLA CHEESE

Attributes	Cheese made by		
	GAU process	NDRI process	C.D. (P<0.05)
Sensory score			
Appearance (Maximum score-3)	2.76	2.88	NS
Body and texture (Maximum score-5)	4.01	4.39	NS
Flavour (Maximum score-10)	8.30	8.42	NS
Total score (Out of 18)	15.07	15.69	NS
Baking characteristics			
Objective assessment			
Fat leakage (cm^2)	4.09	2.92	0.53
Meltability (Arbitrary units)	0.87	0.71	NS
Subjective assessment			
Shredability	Satisfactory	Excellent	
Flavour	Slight sour	Sour and salty	
Meltability	Excellent	Satisfactory*	
Fat leakage	Moderate to excess	Lacks fat leakage	
Springiness	Satisfactory	More springy	
Chewiness	Moderate	More chewy	
Browning, especially at periphery	Nil	Slight	

* Required about 15 min. longer to melt in oven (at 150°C) than GAU cheese for adequate melting on pizza loaf

milk. The GAU process is advantageous with regard to reduced manufacturing time, higher cheese yield (on weight basis), desirable meltability and fat leakage on pizza and moderate chewiness in the resultant cheese. On the other hand, NDRI process is advantageous with respect to improved recovery of milk constituents in cheese, better body and texture characteristics and shredability of the resultant cheese.

References

Anon (1980) Recognition of the cheese name 'Buffaloes' milk Mozzarella'. Industria del Latte 16:31-32

Breene WM, Price WV, Ernstrom CA (1964) Manufacture of Pizza cheese without starter. J Dairy Sci 47:1173-1180

Breseman AP (1973) Customer preferences for Mozzarella and Provolone as revealed by survey of supermarket chains. Am Dairy Rev 35:42 D,F

Duthie AH, Lemaire JT, Nilson KM, Partridge JA Atherton HV (1980) Score card for Mozzarella cheese. Cult Dairy Prod J 15:5-7

Ghosh BC, Singh S (1991) A new method for vegetarian Mozzarella cheese from buffalo milk. Indian J Dairy Sci 44:140-142

Ghosh BC, Singh S (1996a) A comparison of cow and buffalo milk Mozzarella cheese. Indian J Dairy Sci 49:38-40

Ghosh BC, Singh S (1996b) Quality of Mozzarella cheese from acidic buffalo milk. Indian J Dairy Sci 49:172-175

Indian Standards (1961a): 1479 (Part-I) Methods of test for dairy industry. Rapid examination of milk. Indian Standards Institution, New Delhi

Indian Standards (1961b): 1479 (Part-II) Methods of test for dairy industry. Chemical analysis of milk. Indian Standards Institution, New Delhi

International Dairy Federation Bulletin (1984) Interim cheese market report. IDF Doc. No. 175:10-23

Jana AH (1992) Browning of Mozzarella cheese-A review. Indian Dairyman 44:129-132

Kindstedt PS (1993) Effect of manufacturing factors, composition and proteolysis on the functional characteristics of Mozzarella cheese. Crit Rev Food Sci Nutri 33:167-169

Kindstedt PS, Kiely LJ, Gilmore JA (1992) Variation in composition and functional properties within brine-salted Mozzarella cheese. J Dairy Sci 75:2913-2921

Kosikowski FV (1982) Cheese and Fermented Milk Foods, 3rd edn. Edwards Brothers Inc., Michigan, USA, p 179

Larmond E. (1976) Sensory measurement in food texture. In: DeMan JM, Voisey PW, Rasper VF, Stanley DW (eds), Rheology and Texture of Food Quality, AVI Pub Co Inc., Connecticut, p 546

Menefee SG, Overman OR (1940) A semi micro-Kjeldahl method for the determination of total nitrogen in milk. J Dairy Sci 23: 1177-1185

Milk Industry Foundation (1959) Laboratory Manual. Methods of analysis of milk and its products, 3rd edn., Washington, USA , p 283

Park J, Rosenau JR, Peleg M (1984) Comparison of four procedures of cheese meltability evaluation. J Food Sci 49:1158-1170

Patel GC (1984) Manufacture of Mozzarella cheese from buffalo milk and judging its suitability for pizza and processed cheese making. M.Sc. Thesis, Gujarat Agricultural University, Anand

Patel GC, Vyas SH, Upadhyay KG (1986) Evaluation of Mozzarella cheese made from buffalo milk using direct acidification technique. Indian J Dairy Sci 39:394-403

Ravi Sundar M (1986) Evaluation of the influence of casein/fat ratio and whey acidity at draining on the quality of Mozzarella cheese made from buffalo milk. M.Sc. Thesis, Gujarat Agricultural University, Anand

Ravi Sundar M, Upadhyay KG (1990) Effects of standardization of buffalo milk for casein/fat ratio on Mozzarella cheese composition and cheese making efficiency. Indian J Dairy Sci 43:588-597

Sharma AM (1991) Process standardization for manufacture of Mozzarella cheese from concentrated buffalo milk and characterization of resultant cheese. M.Sc. Thesis, Gujarat Agricultural University, Anand

Steel RGD, Torrie JH (1980) Principles and Procedures of Statistics-A Biometrical Approach, 2nd edn., Mc Graw Hill Kogakusha Ltd., Japan, pp 137-167

Upadhyay KG, Patel GC, Vaghela MN, Sundar MR (1986) Manufacture of Mozzarella cheese from buffalo milk. Indian Dairyman 38:479-486

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Response Surface Analysis of Enzyme Aided Extraction of Soybean

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Response surface analysis of enzymatic hydrolysis of soyflakes was conducted and the parameters optimized to enhance both the extractable oil and the extractability of soyflakes. The optima were at 24.58% wb moisture content, 14.23% v/w enzyme concentration and 13.29 h hydrolysis period. Soyflakes hydrolyzed under optimal conditions had an extractable oil content of 24.93% on moisture-free basis compared to 22.88% in unhydrolyzed flakes and an extractability of 99.83% compared to 82.22% of the extractable oil in 16 h on Soxhlet apparatus. For 99% oil recovery, as practised commercially, enzymatic hydrolysis reduced the Soxhlet extraction time by over 44% in soyflakes.

Keywords : Soybean, Enzymatic hydrolysis, Response surface modelling, Soybean pre-treatment, Oilseeds pre-treatment, Solvent extraction, Oil extraction.

Soybean is one of the important oilseed crops, containing 18-20% oil and 40% proteins. Soybeans are directly treated with hexane to extract oil. Like other oilseeds, soybeans need to be pre-treated to facilitate extraction of oil by breaking the cell walls and releasing the extractable oil. The conventional pre-treatments include such operations as cleaning, cracking, dehulling, conditioning and flaking (Hutchins 1976; Becker 1978).

Enzymatic hydrolysis has recently been shown to be another option for pre-treatment of oilseeds, as it opens up the oil cell walls through biodegeneration. Further, it breaks up the complex lipoprotein and lipopolysaccharide molecules-not extractable for oil so far-into simple molecules, releasing extra oil for extraction. Fullbrook (1983) observed that the crude protein isolate from melon seeds, when enzymatically hydrolyzed, released extra oil. It was further demonstrated that enzymatic hydrolysis was the appropriate step in the processing of ground soybean and rapeseed to obtain better quality of oil and proteins of high nutritive value. Enhanced release of extractable oil was later shown by Bhatnagar and Johri (1987) in crushed soybean, cottonseed and caster hydrolyzed in the presence of hexane by Sosulski et al (1988) in canola flakes and by Smith et al (1993) in soybrokens. Sosulski et al (1988) further reported a reduction in oil extraction time and investigated the enzymatic hydrolysis conditions for canola. Though, Smith et al (1993) optimized the enzymatic hydrolysis parameters along with the mechanical extraction parameters for enhanced oil recovery by mechanical expelling of soybrokens, enzymatic hydrolysis process for solvent extraction of soybeans has not yet been developed.

The objective of this study was to optimize and determine the enzymatic hydrolysis process parameters for enhanced release of extractable oil and solvent extractability of commercial soyflakes.

Materials and Methods

Materials : Commercially prepared soyflakes, from 'PK-262' variety soybeans, having a moisture content of 8.88% wet basis (%wb) and a Soxhlet extractable oil content of 22.88% on moisture-free basis were obtained from a solvent extraction plant.

Aspergillus fumigatus NCIM 902, obtained from National Chemical Laboratory, Pune, was used for preparing the enzyme solution on wheat bran medium (Bhatnagar and Johri 1987). Crude enzyme so prepared was a mixed activity enzyme mainly containing cellulase, hemicellulase, chitinase, xylanase, pectinase and protease. The mixed activity enzyme was more effective than pure enzymes in improving oil yield (Bhatnagar and Johri 1987). Cellulase and protease are the key enzymes in rupturing the cell walls and lipoproteic membrane, leading to higher amount of oil recovery (Santos 1979) and were, therefore, assayed. In addition, lipase activity of the enzyme solution was also measured, as it had a direct bearing on oil quality.

Cellulase activity was assayed by the method of Mandels et al (1976). One international unit (IU) of activity was defined as one micro-mole of glucose released per min per ml under the assay condition. Protease activity was measured, following the procedure used by Myers and Ahearn (1977). One unit of activity was defined as one micro-mole of

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TABLE 1. CODED AND UNCODED PARAMETER LEVELS

Parameters	Code				
	+ 1.682 (Augmented point)	+1 (Factorial point)	0 (Centre point)	-1 (Factorial point)	-1.682 (Augmented point)
Moisture content during hydrolysis, % wb, (X_1)	27.94	25	23	21	18.07
Enzyme concentration, % vol/weight of sample, (X_2)	16.93	14	12	10	7.07
Incubation period, h, (X_3)	21.87	16	12	8	2.14

tyrosine released per min per ml under the defined conditions. Lipase activity was assayed, following the procedure of Breuil and Kushner (1975). A unit of lipase activity was defined as the quantity of enzyme liberating one micro-equivalent fatty acid per min under the assay conditions. The cellulase, protease and lipase activities of the enzyme solution were 0.3014 IU/ml, 0.11 IU/ml and 0.009 U/ml respectively.

Experimental design : The response surface methodology (Biles and Swain 1980) was adopted for experimental design, as it emphasizes the optimization of a process or system. A second order central composite rotatable design in 3 variables at 5 levels was used. The coded and uncoded parameter values are presented in Table 1, wherein 6 replications at the centre point and 2 at the other points were considered. The levels of parameter values were chosen based on the limited literature available on the enzymatic hydrolysis of oilseeds (Kashyap 1990).

Moisture content : Moisture contents at the different stages of the experiment were determined, using hot air oven method (AOCS 1973). A Mettler balance of 120 g capacity with an accuracy of 0.0001g was used for weighing.

Soxhlet extractable oil content : The release of Soxhlet extractable oil in raw and hydrolyzed soyflakes was determined by grinding the flakes to 0.5 mm particle size and extracting in duplicate 2g samples each on a Soxhlet extractor, using hexane at a flow rate of 150 drops per min (AOCS 1973). Hexane was used as solvent in place of petroleum ether for uniformity with the rest of the experiments. The ground soyflakes were also analyzed for moisture content to express the oil content on moisture-free basis.

Hexane extractability of soyflakes : In order to establish the effect of enzymatic hydrolysis parameters on extractability of soyflakes, oil recovery from 10 g sample each in 8 h, sufficient for around 50% extraction, was determined on a Soxhlet extractor, using hexane at a flow rate of 150 drops per min. The number of replications were according to the experimental design. The moisture contents of flakes during extraction were kept at 10% wb to be commensurate with the commercial practice (Becker 1978). The final experiment under the optimal enzymatic hydrolysis conditions determined in this study was continued, till complete extraction of soyflakes.

For each experiment, 60g soyflakes were taken and moisture content was adjusted to the desired level for hydrolysis by adding appropriate amounts of water and enzyme solution commensurate with the desired enzyme concentration (Kashyap 1990). The flakes were equilibrated in a refrigerator for 24 h (Alvarez-Martinez 1987). Five gram samples in duplicate were drawn to check the moisture content and the rest was incubated at 45°C (Smith et al. 1993) for the desired time of hydrolysis. The hydrolyzed soyflakes were dried in an oven at 104°C to inactivate the enzyme as also to readjust the moisture content to the desired level for extraction. Five gram samples in duplicate were drawn to check the moisture content and 2 g samples in duplicate to determine the Soxhlet extractable oil content. Ten grams hydrolyzed flakes, as replications according to the experimental design, were used to determine the hexane extractability. The Soxhlet extractable oil content and the hexane extractability were also determined in unhydrolyzed soyflakes. The data on the extractable oil and the extractability of unhydrolyzed and hydrolyzed soyflakes under different conditions were analyzed, using multiple regression technique to develop the response surface models and thereby determine the optimal combination of parameter values in view of enhanced release of extractable oil content and hexane extractability of commercial soyflakes.

Results and Discussion

The extactable oil (EO) content on moisture-free basis in unhydrolyzed soyflakes was 22.88%, while it ranged from 23.92 to 24.97% in enzymatically hydrolyzed soyflakes, depending on the conditions of hydrolysis. This showed that enzy-

TABLE 2. EXTRACTABLE OIL[A] (EO) AND EXTRACTABILITY[B] (HE) OF SOYFLAKES HYDROLYZED ENZYMATICALLY UNDER DIFFERENT EXPERIMENTAL CONDITIONS

Coded parameters			Extractable oil in soyflakes,	Increase in	Hexane extractability	
X_1	X_2	X_3	%, moisture-free sample	extractable oil due to hydrolysis[C], moisture-free basis	Oil recovery in 8 h extraction, % of moisture-free sample	Oil recovery in flakes[D], %
Unhydrolyzed flakes			22.88±0.10	-	13.64±0.13	59.61
-1	-1	-1	24.34±0.01	1.46	18.69±0.08	76.78
+1	-1	-1	24.56±0.01	1.69	18.64±0.00	75.82
-1	+1	-1	24.61±0.00	1.72	18.88±0.02	76.72
+1	+1	-1	24.70±0.01	1.82	19.35±0.02	78.35
-1	-1	-1	24.62±0.02	1.74	18.94±0.00	76.90
+1	-1	+1	24.68±0.02	1.79	19.22±0.00	77.87
-1	+1	+1	24.66±0.02	1.77	19.15±0.14	77.67
+1	+1	+1	24.71±0.01	1.83	19.60±0.01	79.31
-1.682	0	0	24.12±0.03	1.24	16.81±0.03	69.70
+1.682	0	0	24.70±0.02	1.82	19.50±0.04	78.92
0	-1.682	0	24.32±0.01	1.44	18.32±0.03	75.32
0	+1.682	0	24.87±0.01	1.99	19.90±0.01	80.00
0	0	-1.682	23.92±0.00	1.03	16.72±0.06	69.91
0	0	+1.682	24.31±0.01	1.43	18.08±0.13	74.38
0	0	0	24.84±0.03	1.96	19.60±0.16	78.90

[A] Soxhlet extractable oil present in soyflakes as determined after grinding

[B] Oil recovery from soyflakes (without grinding) in 8 h on a Soxhlet extractor

[C] By subtracting extractable oil in unhydrolyzed flakes from that in hydrolyzed flakes

[D] Column 7 = (column 6/ column 4) x 100

matic hydrolysis led to an enhancement of EO by 1.04-1.99%, depending on the conditions of the enzymatic hydrolysis.

Hexane extractability (HE) from the hydrolyzed soyflakes was 16.72-19.90% (Table 2), as against 13.64% from unhydrolyzed soyflakes. Clearly, hydrolysis had increased HE by 3.08-6.26%, depending upon the conditions of hydrolysis. Obviously, enzyme hydrolysis not only increased the extractable oil EO, but also the rate at which the oil could be extracted.

TABLE 3. ANOVA OF RESPONSE FUNCTIONS 1 AND 2

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value (calculated)
For Response Function 1				
Regression	0.9655	6	0.1609	-
Residual	5.9987×10^{-2}	8	7.4983×10^{-3}	21.46
Total	1.0255			

Standard error of the estimate = 0.0865, R-value = 0.9703
Table F-Value with df (6,8) 0.95= 3.58

For Response Function 2				
Regression	101.656	4	25.414	-
Residual	33.056	10	-	7.69
Total	134.711			

Standard error of the estimate = 1.8181, R-value = 0.8687
Table F-Value with df (4,10) 0.95= 3.48

Increases in EO and HE for treated sample have been attributed to the mixed activity enzyme action mainly carbohydrase and protease, leading to biodegradation of cell walls, lipid bound complexes (lipoprotein, lipopolysaccharide etc) present in oilseeds. The carbohydrases degrade the cell walls and lipopolysaccharide, rendering easy extraction and improvement in oil recovery. Protease acts on lipoprotein molecule, making available more oil for extraction (Fullbrook 1983; Sosulski et al. 1988).

In order to optimize the enzymatic hydrolysis process parameters, response surface model for the increase in EO of flakes and for HE of flakes were developed, employing multiple regression technique. A linear model and second order models with and without parameter interaction terms were tested for their adequacy to describe the response surface, using the Fisher's F-test at 95% confidence level (Kashyap 1990). If the response model was found adequate, the regression coefficients were examined for their significance at 95% confidence level, using the students' t-test. The response functions, ANOVA presented in Table 3, so developed were:

For the increase in EO:

$$Y = -11.6488 + 0.852 * 10^{-2} X_1 + 0.2860 X_2 + 0.1966 X_3 - 1.7528 * 10^{-2} X_1^2 - 1.0048 * 10^{-2} X_2^2 - 7.4559 * 10^{-3} X_3^2 \quad ...(1)$$

TABLE 3. ANOVA OF RESPONSE FUNCTIONS 1 AND 2

Sources of varition	Sum of squares	Degree of freedom	Mean of squares	F-value (calculated)
For Response Function 1				
Regression	0.9655	6	0.1609	
Residual	5.9987×10^{-2}	8	7.4983×10^{-3}	21.46
Total	1.0255			

Standard error of the estimate = 0.0865, R-value = 0.9703
Table F-Value with Df (6,8) 0.95= 3.58

For Response Function 2				
Regression	101.656	4	25.414	
Residual	33.056	10		7.69
Total	134.711			

Standard error of the estimate = 1.8181, R-value = 0.8687
Table F-Value with Df (4,10) 0.95= 3.48

For HE,

$$Y_E = -38.9854 + 8.5620X_1 + 1.7583 X^3 - 0.1724X_1^2 - 6.5412 \cdot 10^{-2}X_3^2 \quad \text{.....(2)}$$

Where

- Y= increase in EO in soyflakes due to hydrolysis, % of moisture-free sample.
- Y_E = HE expressed as oil recovery from soyflakes in 8 h extraction, % of extractable oil in flakes.
- X_1 = moisture content during hydrolysis, %wb.
- X_2 = enzyme concentration, % enzyme vol/sample weight.
- X_3 = incubation period, h

Absence of the interaction terms (X_1X_2, X_1X_3, X_2X_3) in response functions 1 and 2 reflected that the enzymatic hydrolysis parameters did not interact amongst themselves. Absence of interactions was also observed by Smith et al (1993) in mechanical extraction of enzymatically hydrolyzed soybeans. Further, absence of the terms containing X_2 in response function 2 reflected the insignificance of the enzyme concentration over the range of investigation in its effect on the HE of flakes, while it did significantly affect the EO response function 1. The insignificance of both the interactions and the enzyme concentration were based on the student's t-test (Kashyap 1990).

The three dimensional response surfaces as predicted by the response functions 1 and 2 are shown in Fig 1 and 2. Both the EO and HE first increased with the increasing parameter values and then decreased, indicating the existence of an optimum within the experimental range. The optima for the maximum enhancement in EO and in HE were calculated by partially differentiating the

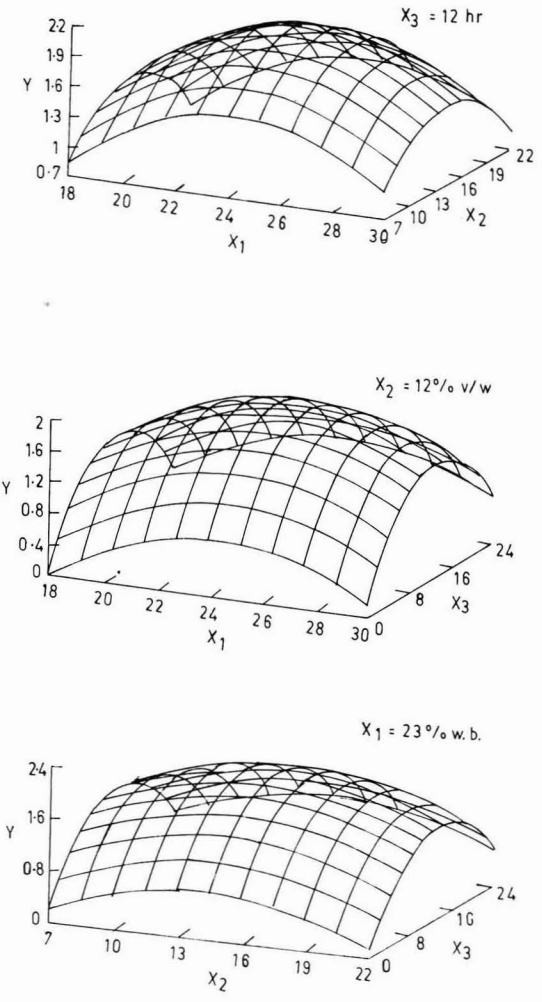

Fig. 1. Response surfaces for extractable oil, X_1 = moisture content during hydrolysis, X_2= enzyme concentration, X_3 = incubation period, Y= increase in extractable oil

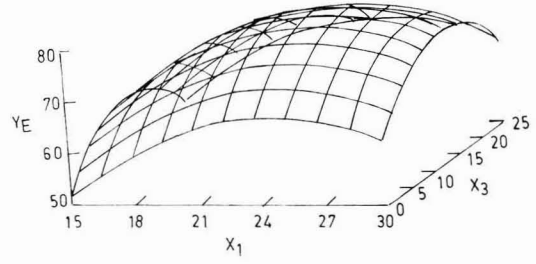

Fig. 2. Response surface for hexane extractability, X_1=moisture content during hydrolysis, X_3=incubation period, Y_E=hexane extractability (oil recovery in 8 h extraction)

response functions 1 and 2 with respect to each parameter and setting these to zero (Kashyap 1990). These optima were:

For enhancement in EO in soyflakes,

X_1 = 24.32%, wb

X_2 = 14.23% enzyme vol/sample weight.... ..(3)

X_3 = 13.14 h

For the maximum enhancement in HE,

X_1 = 24.83%, wb

X_3 = 13.44 h .....(4)

The two sets of optima, equations 3 and 4, were consistent in respect of X_1 and X_3, in that either values could be used without significantly altering the predicted values of the increase in EO, the difference being only 0.02% and of HE, the difference being only 0.05%. Hence, an average could be used. Further, the optimum values of enzyme concentration, X_2, for the maximum enhancement in EO equation 3, could be used, since it did not affect the HE over the investigation range. Thus, the optimal enzymatic hydrolysis conditions recommended for enhancing the EO as well as HE of commercial soyflakes were:

moisture content during hydrolysis, X_1=24.58% wb

enzyme concentration, X_2=14.23% vol/sample weight

incubation period, X_3 = 13.29 h

Commercial soyflakes hydrolyzed enzymatically under the optimal conditions were found to have an extractable oil EO of 24.93% as against 22.88% in unhydrolyzed flakes, showing an enhancement of 2.05% due to hydrolysis. Smith et al (1993) had observed a maximum enhancement of 1.51% in soybean. Further, the optimally hydrolyzed flakes, when fully extracted for 16 h on Soxhlet apparatus, resulted in oil recovery of 99.83% of the extractable oil as against 82.22% from unhydrolyzed flakes. The 99% oil recovery, as practised commercially, was achieved in about 15 h from hydrolyzed flakes, compared to 27 h in case of unhydrolyzed flakes (Kashyap 1990), resulting in a saving of over 44% in Soxhlet extraction time.

Acknowledgement

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References

Alvarez-Martinez LR (1987) Modelling fungal growth on extruded corn by solid substrate fermentation. Ph.D. Thesis, Colorado State University, Fort Collins, Colorado

AOCS (1973) Official and Tentative Methods of Analysis. American Oil Chemists' Society, Champaign, IL

Becker W (1978) Solvent extraction of soybeans. J Am Oil Chem Soc 55: 754-761

Bhatnagar S, Johri BN (1987) Microbial enzymes in the processing of oilseeds. Current Science 56:775-776

Biles WE, Swain JJ (1980) Optimization and Industrial Experimentation, John Wiley and Sons, New York

Breuil C, Kushner DJ (1975) Lipase and esterase formation by psychrophilic and mesophilic acinetobacter species. Can J Microbiol 21:423-433

Fullbrook PD (1983) The use of enzymes in the processing of oilseeds. J Am Oil Chem Soc 60:476-478

Hutchins RP (1976) Continuous solvent extraction of soybeans and cotton seeds. J Am Oil Chem Soc 53:279-282

Kashyap MC (1990) Enzymatic hydrolysis pretreatment to solvent extraction of soybean for enhanced oil availability and extraction rates. M. Tech. Thesis, G.B. Pant University of Agriculture and Technology, Pantnagar

Mandels M, Andreotti R, Charles R (1976) Measurement of saccharifying cellulase. Biotechnol Bioeng Symp 6:21-23

Myers SP, Ahearn DG (1977) Extracellular proteolysis by *Candida lipolytica*. Mycologia 69:646-651

Santos AAF (1979) O uso de auxiliares technologicos enzimaticos na extraccao do azeite. Bo do Inst do azeite e Prod Oleaginosos 5:39-52

Smith DD, Agrawal YC, Sarkar BC, Singh BPN (1993) Enzymatic hydrolysis pretreatment for mechanical expelling of soybeans. J Am Oil Chem Soc 70:885-890

Sosulski K, Sosulski FW, Coxworth E (1988) Carbohydrase hydrolysis of *canola* to enhance oil extraction with hexane. J Am Oil Chem Soc 65:357-361

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Comparative Study of Jellies Prepared from Apple, Pomace and Concentrate

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Methods were standardized for the preparation of apple jellies from fresh fruit, pomace and juice concentrate and their physico-chemical, nutritional and sensory characteristics were compared. Jelly prepared from fruit extract obtained the highest scores for colour, flavour, taste and overall acceptability. The texture of pomace jelly was found significantly better. Magnesium, iron and potassium contents were highest in concentrate jelly, followed by pomace and fruit jellies. All jellies, irrespective of raw materials used obtained mean sensory score of more than 5 (like slightly). The jelly prepared from fruit extract was the most expensive as compared to concentrate and pomace-based jellies.

Keywords : Apple, Pomace, Concentrate, Jellies, Chemical characteristics, Sensory scores, Economics.

Apple (*Malus domestica* Borkh) is a principal horticultural crop of temperate region of the world. In India, it is cultivated in Himachal Pradesh, Jammu and Kashmir and hills of Uttar Pradesh. Apple is primarily used for (a) table purpose and (b) processing. The objective of processing of apple is to produce juice in single strength or concentrated form. An insignificant proportion is also utilized for the production of preserves like jam, jelly, fruit leather and candy. However, Eipeson and Bhowmik (1992) have listed the output and share of jams, jellies and marmalades under FPO licences in 1986 as 18,857 metric tonnes comprising of 12.55%.

Among preserves, fruit jelly is delicious and considered to be a fancy item. It is rarely produced in any of the fruit processing factories, due to inadequate technical knowhow about exact/proper gel forming conditions. Fresh apple and apple pomace (waste) are good sources of pectin (Jain et al. 1984), having important nutritional and technological properties (Westerlund et al. 1991), but are being rarely utilized. Therefore, there is a need to develop a jelly-like product by utilizing the surplus apple and pomace during peak season and regulate its availability from juice concentrate during scarcity. The present investigation was, therefore, undertaken to standardize techniques for the preparation of jellies from fresh apple, pomace and concentrate.

Materials and Methods

Materials: 'Golden Delicious' apples were purchased from the local retail market. Apple pomace and juice concentrate (brand name 7-in-1) were procured from Himachal Pradesh Horticultural Produce Processing and Marketing Corporation, Parwanoo.

Preparation of jelly extracts : Fruits were washed, cut into slices and kept in air for 20 min for enzymatic browning. Jelly extracts were prepared from these slices and pomace by adding the required amount of water according to treatment (Table 1), boiled in stainless steel pan over a single cylinder LPG burner and filtered through a muslin cloth. Apple juice concentrate was diluted by factor method (Table 1). The extracts obtained were evaluated for total soluble solid (TSS) contents. The jelly factor was also calculated (Barwal 1995) for each treatment.

Preparation of standard jellies : Requirement of extract for each treatment was calculated according to FPO specification (Ranganna 1986) to keep fruit contents same in jelly. Sugar was added to the extract from each treatment according to jelly factor (Table 1) and cooked in a stainless steel pan over a single cylinder LPG burner. During cooking, when jelly became semi-viscous, a calculated quantity (after substracting contents in the extracts) of citric acid and pectin (150 grade) were added so that a standard/uniform product could be prepared from all sources. Cooking was stopped, when the jelly reached 65±1°Brix. In all, there were 12 treatments, designated as T_1-T_{12} (Table 1).

Physico-chemical analysis : Specific gravity was determined by water displacement method in fruit and by specific gravity bottle method in pomace and concentrate. TSS was measured in an Abbe type refractometer and expressed as °Brix. Titrable acidity (TA) was determined by volumetric method and expressed as % maleic acid (MA). pH was

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TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF JELLY EXTRACTS PREPARED FROM DIFFERENT SOURCES

Treatment	Ingredients		Boiled for min	Extract yield, g	TSS, °Brix	TA, % MA	pH	Jelly factor
	Raw material, g	Water, ml						
T_1	Apple = 1000	2000	25	1325.0	5.3	0.18	3.8	204.9
T_2	Apple = 1000	1500	25	1008.3	6.9	0.20	3.2	157.4
T_3	Apple = 1000	100	25	811.7	7.7	0.20	3.2	141.1
T_4	Apple = 1000	750	25	616.7	8.3	0.22	3.1	130.9
CD at 1%				52.2	0.4	0.02	0.2	-
T_5	Pomace = 1000	2500	35	1040.0	2.7	0.20	3.0	402.3
T_6	Pomace = 1000	2000	35	783.3	3.2	0.22	2.8	343.7
T_7	Pomace = 1000	1500	35	583.3	3.7	0.25	2.7	291.2
T_8	Pomace = 1000	1000	35	466.7	4.5	0.30	2.5	241.4
CD at 1%				64.5	0.3	0.02	0.2	-
T_9	Conc = 100	196	-	296.0	25.0	0.48	2.9	43.4
T_{10}	Conc = 100	270	-	370.0	20.0	0.38	3.0	54.3
T_{11}	Conc = 100	394	-	494.0	15.0	0.29	3.2	72.4
T_{12}	Conc = 100	640	-	740.0	10.0	0.19	3.4	108.6

Conc = Concentrate MA = Maleic acid

recorded with the help of Systronics pH meter. Reducing and total sugars were determined according to the method of Lane and Eynon (1923) and expressed in percentage. Total solids were determined by drying in an oven at 70°C for 24 h (Ranganna 1986) and expressed in percentage. Minerals were estimated after digestion of samples by wet method (Ranganna 1986) and calculated in accordance with instrument's standardization. Calcium was determined, using Flame Photometer (Eppendorf Geratebau, Germany). Magnesium and iron were estimated, using Atomic Absorption Spectrophotometer, [AA-175 series (varian), Australia]. Sodium and potassium were estimated using Flame Photometer (Evans Electroselenium Ltd, England).

Sensory evaluation : Jelly samples were presented to a panel of 25 judges for sensory evaluation for colour/appearance, flavour/aroma, taste, texture and overall acceptability on a 9-point Hedonic scale. The judges were selected at random and were same for all the evaluations.

Economics : The economics was worked out based on the cost of all the ingredients plus flat cost of processing. To reduce error, the per unit cost was determined on the bulk (10kg) basis.

Statistical analysis : The data were analyzed according to the design and procedure of Panse and Sukhatme (1967).

Results and Discussion

Proximate composition values of juice concentrate were higher (Table 2), as compared to pomace and fruit. The concentrate had been prepared by evaporating moisture to a level where its contents got concentrated, thereby increasing its share as compared to juice (Babsky et al. 1986). Mineral composition of pomace was higher than that of fruit. Pomace (the leftover material after extraction of juice) mainly contained peel, being a rich source of minerals (Carson et al. 1994; Wang and Thomas 1989).

Chemical characteristics of jelly extracts : As the amount of water added to a fixed quantity of fruit and pomace decreased, yield of extract also decreased significantly (Table 1). With the decrease in extract yield, the TSS increased significantly both in fruit as well as pomace extracts (Table 1). An insignificant increase was also noticed in TA between the extracts i.e., T_1 and T_2, T_2 and T_3 and

TABLE 2. PROXIMATE COMPOSITION OF APPLE FRUIT, POMACE AND CONCENTRATE (VALUES PER 100g)

Characteristic	Fruit Mean ±SD	Pomace Mean ±SD	Concentrate Mean ±SD
Specific gravity, wt/vol	0.79±0.03	1.03±0.04	1.44±0.02
TSS, °Brix	12.50±1.25	9.80±0.75	74.00±0.2
Total solids, %	15.50±1.15	28.90±2.87	76.00±0.40
Total sugars, %	9.59±0.71	8.34±0.93	57.50±1.3
Reducing sugars, %	7.46±0.14	6.21±0.41	41.12±0.9
pH	3.28±0.35	2.80±0.25	3..65±1.0
Acidity, %MA	0.28±0.05	0.57±0.13	1.42±0.(
Calcium, mg	3.15±1.50	10.93±3.72	22.10±2..3
Magnesim, mg	4.12±0.85	3.64±0.91	18.37±1.49
Iron, mg	0.32±0.07	0.41±0.10	1.84±0.70
Sodium, mg	2.07±0.05	16.44±0.74	11.02±0,05
Potassium, mg	104.50±3.47	173.00±13.90	684.00±7.50

SD = Standard deviation

TABLE 3. MEAN SCORE OF SENSORY CHARACTERISTICS AND MINERAL COMPOSITION OF JELLIES PREPARED FROM DIFFERENT SOURCES

Treatment	Characteristics					Calcium, mg	Magnesium, mg	Iron, mg	Sodium, mg	Potassium, mg
	Colour/ appearance	Flavour/ aroma	Taste	Texture	Overall acceptability					
T_1	8.20	7.80	7.60	7.40	7.75	2.91	3.09	0.21	1.80	79.50
T_2	8.40	8.20	7.80	7.60	8.00	2.82	3.00	0.18	1.74	70.70
T_3	8.80	8.60	8.40	8.00	8.45	2.77	3.19	0.17	1.70	65.00
T_4	8.60	8.40	8.20	7.80	8.25	2.63	3.27	0.17	1.70	65.50
T_5	6.20	4.20	5.20	6.20	5.45	28.10	6.18	0.65	26.09	296.50
T_6	6.40	4.40	5.60	6.40	5.70	26.58	6.53	0.63	25.89	267.00
T_7	6.60	4.60	6.00	8.60	6.45	24.15	6.82	0.60	25.84	255.00
T_8	6.80	4.80	6.40	8.40	6.60	22.23	7.03	0.57	25.80	252.00
T_9	6.80	5.80	5.20	3.60	5.30	18.87	15.76	1.59	9.04	586.00
T_{10}	7.00	6.20	5.60	4.20	5.75	18.79	15.64	1.57	8.83	587.00
T_{11}	7.20	6.60	5.80	5.20	6.20	18.74	15.61	1.57	8.80	585.00
T_{12}	7.60	6.20	5.40	5.60	6.15	18.74	15.50	1.57	8.80	585.00
CD at 1%	1.06	1.23	1.35	1.20	1.70	-	-	-	-	-

Sensory evaluation on a 9-point scale; 1 = dislike extremely, 9 = like extremely

T_5 and T_6. Concentrate diluted with factor method yielded extract in ascendng order and TSS and TA contents in descending order (Table 1).

Sensory characteristics of jellies : T_3 obtained the highest scores for colour, flavour and taste (Table 3) and remained statistically non-significant with T_1, T_2 and T_4 and significant from rest of the treatments. T_{12}, T_{11}, T_{10} and T_9 obtained intermediate values for colour and flavour and were statistically at par. It showed that jelly prepared from fruit extract had good colour, flavour and acceptability, followed by concentrate and pomace. It might be due to the fact that uncontrolled and undesirable reactions might have taken place in pomace, which lowered the acceptability (Carson et al. 1994), whereas in concentrate, amino acids, reducing sugars and organic acids had been involved in Maillard type reaction, producing pigments (Babsky et al. 1986) and volatile flavouring compounds were lost during evaporation process (Dziezak 1989).

Highest score for texture was obtained for T_7 and lowest for T_9 (Table 3). T_1, T_2, T_3, T_4, T_7 and T_8 were statistically non-significant, but differed significantly from rest of the treatments. The textures of pomace and fruit jellies were significantly better as compared to concentrate jelly. Pectin is a natural polysaccharide found in apple peel and subsequently in pomace to improve the setting of the jelly (Jain et al. 1984). Panelists gave the highest overall acceptability scores for T_3, followed by T_4 and T_2. Apple fruit jellies (T_1, T_2, T_3 and T_4) were statistically non-significant, but differed significantly from pomace and concentrate

TABLE 4. ECONOMICS OF JELLIES PREPARED FROM DIFFERENT SOURCES

Ingredient	Rate,	Apple fruit jelly (10 kg)		Pomace jelly (10kg)		Concentrate jelly (10 kg)	
		Quantity required	Amount, Rs Ps	Quantity required	Amount, Rs Ps	Quantity required	Amount, Rs Ps
Apple fruit	12/kg	10.20 kg	122.40	-	-	-	-
Pomace	0.5/kg	-	-	30.30 kg	15.15	-	-
Concentrate	103/kg	-	-	-	-	0.86 kg	88.58
Sugar	16/kg	5.86 kg	93.84	5.86 kg	93.84	5.86 kg	93.84
Pectin (150 grade)	0.38/g	35.00 g	13.30	22.00 g	8.36	67.00 g	25.46
Citric acid	0.29/g	53.40 g	15.49	29.60 g	8.58	59.80 g	17.34
Sodium benzoate	0.28/g	2.00 g	0.56	2.00 g	0.56	2.00 g	0.56
Glass jar	4.60/jar	20 Number	92.00	20 Number	92.00	20 Number	92.00
Total cost of ingredients			337.59		218.49		317.78
Processing cost flat			58.00		58.00		58.00
Total preparation cost			359.59		276.49		375.78
Total yield (10 kg) produced			20 Jars		20 Jars		20 Jars
Cost/jar or 500 g pack			19.78		13.83		18.79

jellies, whereas, pomace and concentrate ingredient jellies obtained 'like slightly' and 'above overall acceptability' scores i.e., more than 5.

Mineral composition : Calcium and sodium contents were significantly higher in pomace jellies, as compared to fruit jellies (Table 3), whereas, concentrate jellies had intermediate values. High calcium and sodium contents in pomace jellies might be due to the presence of calcium pectate and sodium ions in the peel of the fruit (Bramlage et al. 1990). Magnesium, iron and potassium contents were highest in concentrate jellies, followed by pomace and fruit jellies (Table 3). The possible reasons might be that magnesium, iron and potassium get extracted in the juice and concentrated during evaporation.

Economics : Fruit jelly was the most expensive, costing about Rs 19.78 per 500 g jar, as compared to concentrate and pomace-based jellies (Table 4). The overhead expenses viz., labour, interest on capital, depreciation on equipments and/or building etc. have not been taken into consideration. The per unit costs worked out and reported in this paper for different types of jellies are for the purpose of comparison only.

References

Babsky NE, Toribio JL, Lozano JE (1986) Influence of storage on the composition of clarified apple juice concentrate. J Food Sci 51(3):564-567

Barwal VS (1995) Use of sugar substitutes for the development of dietetic apple preserves. Ph.D. Thesis, Department of Food Science and Nutrition, Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India

Bramlage WT, Weis SA, Greene DW (1990) Observations on the relationships among seed number, calcium and senescent breakdown in apples. Hort Sci 25(3):351-353

Carson KJ, Collins JL, Penfield MP (1994) Unrefined, dried apple pomace as a potential food ingredient. J Food Sci 59(6):1213-1215

Dziezak DJ (1989) New process concentrate juices, preserving 'Fresh notes'. Food Technol 43(3):116-130

Eipeson WE, Bhowmik SR (1992) Indian fruit and vegetable processing industry-Potential and challenges. Indian Food Packer 46(5):7-12

Jain RK, Ghankrokta SS, Agrawal JD (1984) Isolation and characterization of pectin from apple pomace. Indian Food Packer 38(6):65-70

Lane JH, Eynon L (1923) Determination of sugars. J Soc Chem Ind 42:322-327

Panse VS, Sukhatme PV (1967) Statistical Methods for Agricultural Workers. ICAR Publication, New Delhi

Ranganna S (1986) Handbook of Analysis and Quality Control for Fruit and Vegetable Products. Tata McGraw Hill Pub Co Ltd, Delhi

Wang HJ, Thomas RL (1989) Direct use of apple pomace in bakery products. J Food Sci 54(3):618-628

Westerlund PA, Anderson RE, Rahman SMM (1991) Chemical characterization of water soluble pectin in papaya fruit. Carbohyd Res 15:67-78

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Quality of Yoghurt and Probiotic Yoghurt Prepared from Milk Pre-cultured with *Psychrotrophic Pseudomonas* spp

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Aseptically drawn cow's milk samples were inoculated with either proteinase and lipase-positive *Pseudomonas* sp. M26 or proteinase and lipase-negative *Pseudomonas* sp. S15 and stored at 7°C. Pre-inoculated milk samples drawn after specified days of storage showed rapid increase in TV and FFA levels, with milks becoming COB positive on the 10th day in case of former culture. But, in respect of the latter culture, the increases in the levels of TV and FFA were only marginal and the samples became COB positive on the 12th day. Short and long-set yoghurts and probiotic yoghurts, prepared from milks pre-inoculated with *Pseudomonas* sp. M. 26 and sp. S15. In general, the characteristics of these yoghurts prepared from milk pre-inoculated with *Pseudomonas* sp 26 culture were marginally lower compared to yoghurts prepared from milk pre-inoculated with culture *Pseudomonas* sp S15.

Keywords : Yoghurt, Probiotic yoghurt, Psychrotroph, *Pseudomonas*, Proteinase, Lipase.

Use of refrigerated raw or pasteurized milks over fresh milks for the manufacture of fermented milks provides sufficient time to the manufacturer to test the suitability of such milks (Lightbody 1966; Luck 1972). However, during refrigerated storage, psychrotrophic bacteria present in the milk cause compositional changes by elaborating various types of enzymes such as proteinases, lipases, phospholipases etc. (Thomas and Thomas 1973; Fairbairn and Law 1986). The degree of changes in milk characteristics is, however, dependent on the components of psychrotrophic flora growing, their level attained and their capability of producing enzymes at refrigeration temperature. (Kraft and Rey 1979; Kikuchi and Mutsui 1974). These changes may subsequently influence the growth and activity of starters as well as quality of resultant fermented milk product. *Pseudomonas*, being a predominant component of psychrotrophic bacteria, two strains of *Pseudomonas* were individually added to raw milk and such milks after storage were used for the preparation of fermented milks. The influence of added psychrotrophic *Pseudomonas* cultures on the quality of yoghurt and probiotic yoghurts is described in this paper.

Materials and Methods

Cultures : Pseudomonas sp M26 an isolate positive for both proteinase and lipase activities and *Pseudomonas* sp. S15, an isolate negative for both proteinase and lipase activities were added to aseptically drawn low microbial count raw milk at the rate of 10^4 cells/ml and stored at 7°C to monitor the changes in milk.

Streptococcus salivarius ssp. *thermophilus* STW and *Lactobacillus delbruckii* spp. *bulgaricus* LBR 3 were used together as yoghurt starters.

Lactobacillus acidophilus 111 and *Bifidobacterium bifidum* ATCC 11863 were used as probiotic cultures along with yoghurt cultures.

Starters and *Pseudomonas* cultures were maintained in plain skim milk and on yeast glucose agar slants, respectively.

The viable counts of *Pseudomonas* cultures were determined by serial dilution technique, using standard plate count agar and incubating at 30°C for 48h. Similarly, the viable counts of starter counts were determined, using yeast glucose agar at 37°C for 48 h. under anaerobic condition.

Milk samples : Aseptically drawn cow's milk samples, having an initial microbial count of less than 50 cfu/ml, were used for monitoring the changes in pre-inoculated milks and for preparation of yoghurts. Fat and SNF contents of these samples were adjusted to 3.5 and 8.5% respectively.

Preparation of yoghurts : A known amount of pre-inoculated and refrigerated milk was taken and heated to 85°C for 10 min. The milk was cooled to room temperature and inoculated with a mixed culture of (S. *thermophilus* STW and *L. bulgaricus* LBR3) at the rate of 1% each (v/v) and mixed thoroughly. Short-set yoghurt was prepared by incubating at 42°C for 4 h., while long-set yoghurt was prepared by incubating at 30°C for 20h. Similarly, probiotic yoghurts were prepared by

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inoculating yoghurt cultures in association with *L. acidophilus* or *B. bifidum* or both at the rate of 1% each and incubating at 30°C for 20h.

Chemical changes : The following tests were performed on stored milks and yoghurt samples using the standard procedures:

1) Clot on Boiling (COB test); 2) Methylene blue reduction test (MBRT); 3) pH; 4) Titratable acidity (TA); 5) Free fatty acids (FFA) (Fraskel and Tarassuk 1955); 6) Tyrosine value (TV) (Hull 1947) and 7) Diacetyl (Harrigan and MECance 1976).

Overall acceptability scores : Yoghurt samples were given to a panel of judges to award scores on a 10 point scale for the characteristics, such as aroma (2), taste (2), firmness (3), whey separation (1), body and texture (2).

Results and Discussion

Changes in pre-inoculated raw milks : Aseptically drawn milk samples (having a microbial count of less than 50 cfu/ml), containing added *Pseudomonas* culture were stored at 7°C and the changes observed are given in Table 1. Milk samples containing proteinase and lipase-positive *Pseudomonas* sp M26 became COB positive on the 10th day of storage and the MBRT reduced to 1.5 h. It is interesting to note that stored samples became alkaline with a pH of 7.15 and a TA of 0.095%. A 4-fold increase in FFA levels and more than 2-fold increase in TV were observed during the 10 day storage period. The viable count of *Pseudomonas* culture increased from 4.69 to 8.86 $\log_{10}$ cfu/ml at the end of the storage period.

Addition of proteinase and lipase-negative *Pseudomonas* sp S15 culture to raw milk made the samples COB positive on the 12th day of storage (Table 1). A marginal increase in pH from 6.70 to 7.0 and concomitant decrease in TA from 0.14 to 0.115% were noticed. Small increases in FFA and TV were also observed at the end of 12 days storage. However, interestingly, the viable count increased from 5.64 to 9.01 $\log_{10}$ cfu/ml, indicating appreciable growth of the culture in milk sample.

In the present study, it was observed that although both enzyme positive and negative cultures grew well in milk, the enzymes positive culture caused substantial increase in the concentration of proteolytic and lipolytic breakdown products, while enzymes negative culture caused only marginal increase in their concentration. These results, which are in conformity with those of Cousin and Marth (1977), indicate the significance

TABLE 1. CHEMICAL AND BACTERIOLOGICAL CHANGES IN MILK PRE-INOCULATED WITH *PSEUDOMONAS* CULTURES AND STORED AT 7°C

Pre-ref period, days	MBRT, h	pH	TA, %LA	FFA, ml	TV, mg	TPC, $\log_{10}$/ml
			***Pseudomonas* sp. M26 (P^+L^+)**			
0	>5	6.70	0.140	1.20	0.245	4.69
2	>5	6.70	0.140	1.80	0.190	5.30
4	4.5	6.80	0.130	2.10	0.270	6.50
6	4.0	6.90	0.120	3.60	0.320	7.17
8	3.5	7.00	0.110	4.20	0.460	8.62
10	1.5	7.15	0.095	4.90	0.510	8.86
			***Pseudomonas* sp. M26 (P^+L^-)**			
0	>5	6.70	0.140	0.575	0.15	5.04
2	>5	6.70	0.140	0.300	0.15	5.47
4	5.0	6.80	0.135	0.450	0.19	6.44
6	4.5	6.80	0.130	0.600	0.21	7.60
8	3.5	6.90	0.120	0.540	0.23	8.17
10	2.5	7.00	0.120	0.600	0.24	8.90
12	1.0	7.00	0.115	0.600	0.27	9.01

COB was negative except for 10 and 12 days of pre-refrigeration in case of strains M26 and S15, respectively

FFA expressed in terms of ml of 0.025 N alcoholic NaOH

Values reported here are the average of three trials

TPC-Total plate counts

of the enzymes producing psychrotrophs in reducing the keeping quality of refrigerated milks.

Yoghurts and probiotic yoghurts prepared from pre-inoculated milk : Aseptically drawn milk samples containing added *Pseudomonas* sp M26 (positive for proteinase and lipase activity) were held at 7°C and after specified days of storage. These samples were used for preparing yoghurt and probiotic yoghurt, after a heat treatment at 85°C for 10 min. Table 2 shows the characteristics of short-set and long-set yoghurts. It can be seen that as the storage period of pre-inoculated milk increased, the performance of yoghurt cultures in it also increased by producing more TA, higher FFA and TV values and increased starter counts. The overall acceptability scores (OAS), which were 6-8 for yoghurt, prepared from unstored sample, got increased to 9.1 when 8 day refrigerated milk was used to prepare short-set yoghurt. In respect of long-set yoghurts, similar trends were noticed, but both chemical and bacteriological changes were higher, when compared to short-set yoghurt samples. The OAS of long-set yoghurt although increased with the increase in storage period, the total scores were less than those obtained for short-set yoghurt samples. This could be due to higher acidity, contributing to the sourness of the samples.

TABLE 2. CHARACTERISTICS OF YOGHURT PREPARED FROM MILK PRE-INOCULATED WITH *PSEUDOMONAS* M26 (P^+ L^+) AND THEN REFRIGERATED

Pre-ref period, days	pH	TA, %LA	FFA, ml	TV, mg	Starter count, $\log_{10}$/ml	OAS/ 10
Short-set yoghurt (42°C/4h)						
0	4.70	0.70	1.80	0.270	7.64	6.8
2	4.45	0.83	2.40	0.370	8.07	8.8
4	4.40	0.85	2.65	0.420	8.04	7.7
6	4.40	0.85	2.64	0.550	8.10	9.0
8	4.15	0.97	2.70	0.665	8.21	9.1
Long-set yoghurt (30°C/20h)						
0	4.40	0.85	3.60	0.285	8.49	7.5
2	4.10	1.00	4.35	0.525	8.80	7.8
4	3.90	1.15	4.55	0.580	8.85	8.1
6	3.75	1.30	4.55	0.710	8.97	8.8
8	3.70	1.35	4.65	0.865	9.07	8.6

FFA expressed in terms of ml of 0.025 N alcoholic NaOH

Average of three replicates. Diacetyl was negative in all cases

Probiotic yoghurts such as acidophilus-yoghurt, *bifidus*-yoghurt and *acidophilus-bifidus*-yoghurt were also prepared from pre-inoculated milks (Table 3). The results show that *acidophilus*-yoghurt prepared from 0 day milk had a pH of 4.10, TA value of 1.00%, FFA of 4.80 ml, TV of 0.36 mg and starter count of 8.55 $\log_{10}$ cfu/ml and OAS of 7.3. These values increased to 3.75, 1.3%, 5.10 ml, 0.69ml, 9.0 $\log_{10}$ cfu/ml. and 8.5, respectively, when 8 day old stored milks were used. Similar

TABLE 3. CHARACTERISTICS OF PROBIOTIC YOGHURTS PREPARED FROM *PSEUDOMONAS* M26 (P^+ L^+) PRE-INOCULATED AND PRE-REFRIGERATED MILKS

Pre-ref period, days	pH	TA, %LA	FFA, ml	TV, mg	Starter count, $\log_{10}$/ml	OAS/ 10
***Acidophilus*-yoghurt (30°C/20h) (*S.thermophilus* STW + *L. bulgaricus* LBR3 + *L.acidophilus* 111)**						
0	4.10	1.00	4.80	0.36	8.55	7.3
2	3.95	1.10	4.80	0.40	8.60	7.3
4	3.80	1.25	4.75	0.60	8.86	8.5
6	3.80	1.25	5.10	0.65	8.86	8.5
8	3.75	1.30	5.10	0.69	9.00	8.5
***Bifidus*-yoghurt (30°C/20h) (*S.thermophilus* STW + *L. bulgaricus* LBR3 + *B. bifidum* ATCC 11863)**						
0	4.05	1.00	4.20	0.460	8.67	6.0
2	3.75	1.30	4.20	0.500	8.86	8.1
4	3.70	1.35	4.10	0.540	8.88	5.5
6	3.60	1.45	4.35	0.7500	8.95	7.5
8	3.56	1.45	4.40	0.890	8.99	7.1
***Acidophilus*-bifidus-yoghurt (30°C/20h) (*S.thermophilus* STW + *L.bulgaricus* LBR3 + *L.acidophilus* 111 + *B. bifidum* ATCC 11863)**						
0	4.00	1.05	4.20	0.480	8.55	6.2
2	3.95	1.10	4.70	0.500	8.66	7.7
4	3.85	1.20	4.90	0.525	8.89	6.3
6	3.70	1.35	5.30	0.700	8.96	7.6
8	3.65	1.40	5.70	0.910	9.02	7.7

Diacetyl was positive in all cases

FFA expressed in terms of ml of 0.025 N alcoholic NaOH

TABLE 4. CHARACTERSTICS OF YOGHURTS AND PROBIOTIC YOGHURT PREPARED FROM *PSEUDOMONAS* S15 (P^- L^-) PRE-INOCULATED AND PRE-REFRIGERATED MILKS

Pre-ref period, days	pH	TA, %LA	FFA, ml	TV, mg	Starter count, $\log_{10}$/ml	OAS/ 10
Short-set yoghurt (42°C/4h)						
0	4.80	0.68	2.55	0.285	7.67	8.9
2	4.76	0.69	2.25	0.300	7.69	8.3
4	4.70	0.70	2.70	0.325	7.73	7.8
6	4.74	0.68	2.90	0.330	7.74	8.4
8	4.70	0.70	2.95	0.385	7.71	9.0
10	4.70	0.70	2.95	0.385	7.71	9.0
Long-set yoghurt (30°C/20h)						
0	4.30	0.90	3.45	0.450	8.51	6.4
2	4.30	0.90	3.45	0.465	8.51	7.8
4	4.20	0.95	3.00	5.520	8.50	8.1
6	4.19	0.96	3.80	0.580	8.57	8.6
8	4.20	0.94	3.90	0.510	8.57	8.4
10	4.20	0.95	4.05	0.500	8.59	8.6
***Acidophilus*-yoghurt (30°C/20h) (*S.thermophilus* STW + *L. bulgaricus* LBR3 + *L.acidophilus* 111)**						
0	4.05	1.05	3.75	0.445	8.38	7.0
2	3.95	1.10	3.75	0.510	8.43	8.1
4	3.99	1.07	3.00	0.550	8.32	8.1
6	3.96	1.009	3.75	0.600	8.38	8.3
8	3.97	1.09	3.75	0.580	8.43	7.9
10	3.97	1.09	3.82	0.550	8.43	8.2
***Bifidus*-yoghurt (30°C/20h) (*S.thermophilus* STW + *L. bulgaricus* LBR3 + *B. bifidum* ATCC 11863)**						
0	4.00	1.05	3.90	0.460	8.68	6.8
2	4.00	1.05	3.90	0.480	8.67	6.8
4	3.97	1.09	4.50	0.540	8.68	7.3
6	3.96	1.09	3.00	0.560	8.75	7.6
8	3.95	1.09	4.25	0.580	8.77	7.8
10	3.95	1.10	4.25	0.600	8.76	8.0
***Acidophilus*-bifidus-yoghurt (30°C/20h) (*S.thermophilus* STW + *L.bulgaricus* LBR3 + *L.acidophilus* 111 + *B. bifidum* ATCC 11863)**						
0	3.95	1.10	4.00	0.425	8.74	5.8
2	3.95	1.10	4.65	0.525	8.74	5.4
4	3.90	1.14	4.00	0.585	8.76	7.3
6	3.90	1.15	4.10	0.585	8.82	7.5
8	3.90	1.16	4.55	0.560	8.81	8.0
10	3.90	1.15	4.55	0.455	8.76	8.0

FFA expressed in terms of ml of 0.025 N alcoholic NaOH

Diacetyl was not detected in the first two cases and was positive in the rest

trends were observed in respect of *bifidus*-yoghurt, prepared from 0 day old and 8 day old stored samples. All values except pH and OAS in *bifidus*-yoghurt increased with the increase in the storage period of the pre-inoculated milks. In respect of *acidophilus-bifidus*-yoghurt, all the characteristics monitored, except pH, were lowest for 0 day milk, while they were highest for the 8 day stored milk.

The characteristics of yoghurts and probiotic yoghurts prepared from pre-inoculated milks with proteinase and lipase-negative *Pseudomonas* sp. S15 are given in Table 4. It may be seen in Table 4 that in respect of short-set yoghurt, all the parameters, except pH and OAS, were lowest in 0 day milk. These values either increased marginally or remained the same, as the storage period of the milk increased. In respect of OAS values, the lowest score was observed for yoghurt prepared from the 4 day stored milk and highest for yoghurt prepared from the 8 day stored milk.

In respect of long-set yoghurt, the values for all the characteristics were lower for yoghurts prepared from 0-day milks, which gradually increased with increase in storage period of milk used. It is interesting to note that both short and long-set yoghurts could be made from milks which had been stored even upto 10 days. The diacetyl content remained unchanged in fresh and all types of probiotics prepared.

Results of the experiments carried out in respect of probiotic yoghurts prepared from milks pre-inoculatd with *Pseudomonas* sp. S15. are also given in Table 4. In case of *acidophilus*-yoghurts, the values in respect of pH, TA, FFA and starter counts were marginally altered, as the storage period of milk increased. However, both TV and OAS increased initially (upto 6 day milk), but subsequently decreased. The characteristics of the *acidophilus-bifidus*-yoghurt prepared from the 10 day milk were marginally altered compared to those prepared from the 0 day stored milk, except acceptability score, which was 5.8 for the product prepared from the 0 day milk as against 8.0 for the product prepared from the 10 day stored milk.

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References

Cousin MA, Marth EH (1977) Growth of psychrotrophic bacteria alters stability of milk to coagulation by rennet and heat J Dairy Sci 60 (Suppl. I) :34-35

Fairbairn DJ, Law BA (1986) Proteinases of psychrotrophic bacteria. Their production, properties, effects and control. J Dairy Res 53:139-177

Fraskel EN, Tarassuk NP (1955) Detection of lipolytic activity in stored milk by extraction titration method. J Dairy Sci 38:751-753

Harrigan WF, MECance EM (1976) Laboratory Methods in Food and Dairy Microbiology. Academic Press, London

Hull ME (1947) Studies on milk proteins-II. Colorimetric method for determination of the partial hydrolysis of the proteins in milk J Dairy Sci 30:881-884

Kikuchi M, Matsui Y (1974) Changes in the number of bacteria and bacterial flora in bulk collected milk. Jap J Zootechnical Sci 45:592-596

Kraft AA, Rey CR (1979) Psychrotrophic bacteria in foods: An update. Food Technol 33:66-71

Lightbody LG (1966) The effect of low temperature storage of pasteurized milk on bacterial counts using penicillin agar. Aust J Dairy Technol 21:94-99

Luck H (1972) Bacteriological quality tests for bulk-cooled milk. A review, Dairy Sci Abst 34:101-122

Thomas SB, Thomas BR (1973) Psychrotrophic bacteria in refrigerated bulk-collected milk. Part II. Dairy Ind 38:11-15

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Low Oxygen Treatment Before Storage in Normal or Modified Atmosphere Packaging of Mangoes to Extend Shelf Life

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The quality of 'Keitt' mangos was evaluated during storage for 6 days at 20°C under low O_2 (CA. 0.3%) atmosphere before storage in modified atmosphere (MA) packaging in 3 low density polythylene (LDPE) films of different characteristics. After low O_2 (CA) treatment, fruits were individually packaged and stored for 30 days at 10°C and 20°C. Every three days, fruit samples from CA and MA were evaluated and every ten days, treated and untreated fruits were transferred to 20°C to determine fruit quality. Both CA and MA treatments delayed the losses of colour, weight and firmness. Fruits maintained a good appearance with a significant delay of ripening. It was observed that mangos were very tolerant to low O_2 treatment. However, some individual MA-packaged fruits developed a fermented taste after 10 and 20 days at 20°C. Short duration (6 days) storage of mangoes at low O_2 (≈0.3%) did not have any deleterious effect on fruit quality during subsequent storage under MA or normal atmosphere.

Keywords : *Mangifera indica*, Plastic film, Modified atmosphere storage, Low O_2 effect, Quality changes.

Tropical and sub-tropical fruits have a considerable appeal in international markets. Mexico is an important producer of these crops. However, the post-harvest life of such fruits is short due to their perishable nature and the application of the legally required quarantine treatments. The best example is mango, which is host to Caribbean fruit fly (*Anastrepha suspensa, Loew*) (CFF), must be treated with hot water (HW) to kill fly infection, before shipment to certain international markets. However, HW treatment has been shown to accelerate the maturation process and reduce the shelf life of the fruit. Hot water treatment is the only approved treatment against CFF in the main international markets for mangoes (Animal and Plant Health Inspection Service 1986). Due to these problems, alternative quarantine treatments for mangoes are needed. Different treatments tested such as forced hot air and vapour, resulting in internal breakdown in fruits (Mitcham and McDonald 1993; Esquerra and Lizada 1990). Low O_2 concentration (<0.5%) alone or in conjunction with high CO_2 concentrations (>50%) can be used to control insects in fresh horticultural crops (Brandl et al. 1983; Gaunce et al. 1982; Yahia and Vasquez 1993). Insecticidal atmospheres, however, can induce a shift from aerobic to anaerobic respiration, leading to fermentation with the accumulation of ethanol and acetaldehyde and the increased activity of anaerobic enzymes (Yahia 1993). In our facility, some studies have been carried out with mango, avocado and papaya fruits to determine the tolerance level to very low O_2 (<0.5%) and/or high CO_2 (>50% for 3 to 6 days, as an alternative quarantine treatment of fruits intended to be exported (Yahia 1993; Yahia and Carrillo 1993). Mangoes can tolerate this low O_2 treatment for 6 days at 20°C. In view of the above findings, the present investigation was aimed to study the effect of low O_2CA, treatment on fruit quality changes before packaging the mangoes in MA, using three LDPE films and storage at 10°C and 20°C for 30 days.

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Materials and Methods

'Keitt' mangoes (*Mangifera indica* L.) at the mature green stage, were obtained on the day of harvest in Mazatlan, Sinaloa, Mexico and immediately transported to the laboratory. Fruits were sorted, cleaned and selected by skin colour, weight and size. Respiration rate (ml CO_2/kg. h) was measured in 20 fruits at 20°C and initial quality was evaluated in 40 fruits for skin colour, weight, firmness, total soluble solids (TSS), pH, titrable acidity (TA) and decay. Fruits were placed in jars and CA was performed by passing a humidified N_2 atmosphere at a rate of 300 ml/min for 1h. The jars were, then, closed to maintain a stable atmosphere and ventilated with the appropriate humidified gas mixture.

Modified atmospheres were built up, by using three LDPE bags (15 x 21 cm). Thickness, O_2, CO_2 and H_2O permeability rates for LDPE1, LDPE2 and LDPE3 were 0.028, 0.045 and 0.072 mm; 0.062,

0.042 and 0.026 ml/m². h. atm; 0.225, 0.128 and 0.110 ml/m². h. atm; 265, 223 and 185 g/m². h. atm, respectively. Materials were provided by Industrias Plasticas del Noroeste, Sonora, Mexico. Ninety fruits were individually packaged in each bag and then 30 fruits were stored at 10°C and 60 at 20°C with 75 and 67% relative humidity (RH), respectively. Bags were sealed with an electric heat sealer. Other fruits were placed in thirty litre jars (24 fruits/jar) and connected to a constant flow of compressed gases (O_2≈0.3% with N_2 balance), controlled with capillary tubing and stored at 20°C under CA. Gas samples were taken at 2 h intervals to verify the atmosphere composition (O_2 and CO_2) in the jars. At 3 day intervals, fruits from CA and control were sampled and evaluated immediately for quality attributes. After 6 days, 2 lots of 60 fruits each were removed from CA and individually packaged in LDPE1 and LDPE2, and transferred to 10°C and 20°C for 30 days.

The following 7 treatments were used: a) Absolute control, fruits stored at normal atmosphere (control); b) CA control, fruits stored for 6 days in CA (CA); after 6 days in CA, fruits were packed in c) LDPE1 (CA-LDPE1) and d) LDPE2 (CA-LDPE2); fruits without previous CA storage and packed directly to obtain a MA with e) LDPE1 (MA-LDPE1); f) LDPE2 (MA-LDPE2) and LDPE3 (MA-LDPE3).

The O_2 and CO_2 concentrations in the packages were determined every 3 days by sampling air surrounding the fruit using a 5 and 1 ml hypodermic syringes, respectively. Every 10 days, 10 fruits from each treatment were randomly sampled and analyzed for quality attributes. Changes in skin colour were evaluated according to the % of skin green area. Changes in pulp colour were evaluated according to the Munsell chart of colours (Munsell Book of Colour, Baltimore, Maryland 21218, USA) using the following scale.

Munsell value	Score
7.5 Y 9/4	1.0
5.0 Y 9/6	1.5
5.0 Y 8.5/8	2.0
2.5 Y 8/10	2.5
2.5 Y 8.5/10	3.0
2.5 Y 8/12	3.5
2.5 Y 8.5/12	4.0
1.25 Y 8/12	4.5
1.25 Y 8/14	5.0
10 YR 8/12	5.5
10 YR 7/14	6.0

Decay was expressed as % fraction of fruit with fungal lesions after removal from the package. Fruit weight loss was evaluated by weighing the fruit before and after storage. Flesh firmness was measured as penetration force on 6 points of each fruit, using a firmness tester (Chatillon Model DFG 50, John Chatillon and Sons, Inc. New York, USA.) with an 8 mm tip and skin removed. Sub-samples were taken to determine the TSS with an ABBE temperature, adjusted refractometer (Model 40482,-Reichert Scientific Instruments, Buffalo, N.Y.), and TA by titrating pressed juice from 20 g tissue to pH 8.2 with 0.1 mol/l NaOH and expressed as g/100g citric acid and pH using a Corning pH meter (Model 140, Corning Medical and Scientific, Medfield, MA, USA.) according to the AOAC(1984) methods.

Measurement of film characteristics : A digital micrometer (Model DDT, E.J. Cady and Co. Wheeling, IL) with a range of 0 to 1.25 mm was used to measure film thickness. Water vapour permeability was determined according to the ASTM methodology (ASTM 1978). Oxygen permeability was measured by an oxygen analyzer, OXTRAN 100 A (Mocon, Modern Controls, Inc., Minneapolis MN. USA.) and CO_2 permeability, using a permetran CO_2 analyzer.

Atmosphere composition analysis : Respiration rate (CO_2 ml/kg.h) was determined on single fruit sample exposed to a continuous flow of ethylene-free air at 200 ml/min. Carbon dioxide was measured by taking 1 ml of air, either from the outlet tube or directly from the package and injecting it into an infrazred CO_2 analyzer (Horiba Model PIR 2000, Horiba Instruments Inc., Irvine, California, USA.). Oxygen content in the package was measured by injecting 5 mol of in-package atmosphere into a portable O_2 analyzer (Mocon Model LC 700F Toray Engineering Ca., Ltd, Japan).

Statistical analysis : Data were subjected to analysis of variance and mean comparison was done by Tukey test at 5% level, using the SAS Software, version 6.03 (SAS 1990).

Results and Discussion

The CO_2 production of mangoes ranged from 40 to 55 ml/kg. h during the first 6 days (data not shown). The fruits reached the climacteric peak on the 8th day and declined thereafter, following a typical climacteric respiration pattern.

No significant changes were found in O_2 and CO_2 concentrations in the CA jars during the initial 6 day treatment (Fig 1). It was possible to maintain

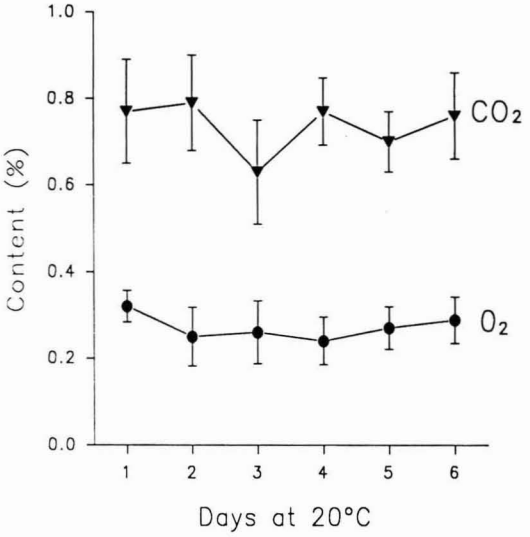

Fig. 1. Changes in O_2 (●) CO_2 (▼) concentration during controlled atmosphere storage. Vertical bars indicate standard deviation of the mean.

the O_2 levels between 0.25 and - 0.35% with N_2 balance. CO_2 concentration was kept between 0.65 and 0.8%. These concentrations did not affect the respiratory behaviour of the fruit, but delayed fruit ripening as indicated by after storage changes in skin and pulp colour, chemical characteristics, firmness, weight loss and decay (Tables 1,2 and 3).

Fig 2 shows the in-package O_2 and CO_2 concentrations of mangoes packed in LDPE1, LDPE2 and LDPE3 films, at 10°C and 20°C. The in-package O_2 concentration declined very quickly after 3 days with levels of ≈1% and <1% at 10°C and 20°C, respectively, in the three packages used. These decreases could be attributed to the low O_2, at which the fruits were exposed before packaging. The O_2 concentration then increased and stabilized after 3 weeks at 10°C, reaching 5.5, 4.0 and 3.5% in LDPE3, LDPE2 and LDPE1, respectively (Fig 2C). The O_2 depletion was more severe at 20°C with the lowest levels (≈2%), occurring in the LDPE3 film and similar levels (3-4%) in LDPE1 and LDPE2 after 3 weeks (Fig 2D). CO_2 accumulation increased after 3 days at 10°C to 25, 24 and 18% in LDPE1, LDPE2 and LDPE3, respectively. These increases were higher at 20°C (Fig 2B) than at 10°C (Fig. 2A), following the same behaviour in the 3 films and were correlated with the reduction of O_2 in the package. The CO_2 content in the package decreased after two weeks, remaining stable at 21, 15 and 13% in LDPE1, LDPE2 and LDPE3, respectively (Fig2A). The differences in O_2 and CO_2 contents in the packages were due to the differences in physical properties of the 3 films used and were correlated

TABLE 1. CHANGES IN MANGO SKIN AND PULP COLOUR AFTER REMOVAL FROM THE DIFFERENT STORAGE TREATMENTS FOR 20 AND 30 DAYS AT 10°C AND 20°C

Treatment	Storage period, days: 20 — Storage temperature, °C: 10	20	30 — 10	20
	Changes in skin colour			
Control	13.6±4.3	100.0±0.0	45.8±3.4	100.0±0.0[1]
CA	11.1±5.7	91.3±7.3	17.7±5.4	97.3±2.5
CA-LDPE1	7.7±3.4	11.3±4.0	18.1±1.1	18.3±11.6
CA-LDPE2	18.6±5.8	8.8±0.6	22.5±6.8	13.9±2.2
MA-LDPE1	10.1±4.9	22.2±10.4	15.7±5.1	57.45±5.4
MA-DLPE2	10.2±3.4	10.9±5.6	14.4±4.2	9.7±12.3
MA-LDPE3	22.8±10.4	25.8±7.6	28.9±9.5	53.5±9.9
	Changes in pulp colour,%			
Control	3.5±0.5	5.5±0.1	3.7±0.3	ND
CA	3.0±0.5	3.7±0.3	4.0±0.9	4.0±0.8
CA-LDPE1	3.2±0.8	2.8±0.3	3.7±1.2	4.2±0.8
CA-LDPE2	3.7±1.2	2.7±0.3	4.5±0.4	1.5±0.8
MA-LDPE1	1.8±0.6	4.0±0.5	1.9±0.5	4.2±0.3
MA-DLPE2	1.3±0.6	4.3±1.2	1.5±0.3	2.5±0.9
MA-LDPE3	3.5±0.5	4.0±0.9	3.3±0.3	5.0±0.1

[1]Each value is the mean of 10 replicates ± standard deviation

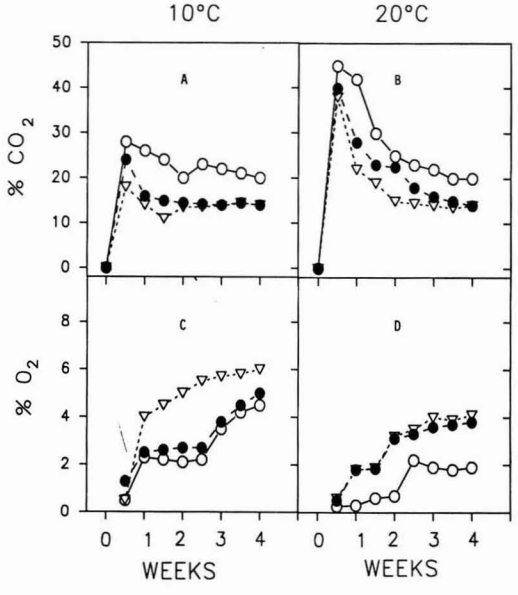

Fig. 2. Influence of plastic films on the composition (O_2 and CO_2) of the atmosphere surrounding mangoes stored at 10°C and 20°C for 30 days. LDPE1 (▽) LDPE2 (●); LDPE3 (○). Each value is the mean of 10 replicates

with their thickness and permeability. Films with the lowest O_2 and CO_2 permeabilities (LDPE3) maintained higher levels of these gases in the package. Water condensation in the package was higher at 20°C than 10°C in the films with the lowest premeability to water vapour.

Table 1 shows skin and pulp colour changes after 20 and 30 days at 10°C and 20°C. No significant changes were found after 10 days in the different treatments. No appreciable changes were found in skin colour of mangoes after 20 days at 10°C. However, changes were observed at 20°C under the same treatments. The greatest loss of skin colour occurred in control fruits (100%), followed by that of CA-fruits (91.3%), MA-LDPE3 and MA-LDPE1 (25.7 and 22.2%, respectively), and similar losses in MA-LDPE2, CA-LDPE2 and CA-LDPE1, treated fruits. After 30 days, increases in skin colour were observed in all treatments except in control and CA-treated fruits at 10°C. The changes in skin colour were more noticeable at 20°C, with greatest losses in control (100%) and CA-fruits (97%). Less colour loss was observed in MA-LDPE1, MA-LDPE3, CA-LDPE1 fruits (close 57,53 and 18% colour loss, respectively) and the lowest loss in MA-LDPE2 and CA-LDPE2 with 9.71 and 13.9%, respectively.

Slight changes in pulp colour were found in fruits in MA-LDPE1 and MA-LDPE2 after twenty days at 20°C. Pulp colour loss increased significantly at 20°C with less change in fruits in CA-LDPE1 and CA-LDPE2 (Table 1). Less change was found in fruits stored for 30 days at 10°C. The lowest changes in pulp colour were found in fruits in CA-LDPE2 and MA-LDPE2. The variation observed after 20 and 30 days at 20°C could be due to the fact that different fruits were sampled.

Table 2 shows the changes in firmness, weight loss and decay of mangoes after storage under different treatments at 10°C and 20°C. Firmness decreased during the marketing condition in all treatments. Fruits at 10°C treated only with CA maintained their firmness better than those treated with CA plus MA conditions. Fruits on CA and MA-LDPE3 conditions were firmer after 10 days at 10°C, compared with the other treatments. After 10 days at 10°C, significant differences in firmness were found in fruits stored in CA. However, control and CA fruits showed the lowest firmness at 20°C. Weight loss was higher in control fruits than those in other treatments.

The plastic covering plays an important role in preventing dehydration, creating a saturated micro-atmosphere around the fruit. Mangoes stored in open air (10°C, 75% RH) suffered a pronounced loss of weight, this being 1.6% after 10 days and 4.8% after 30 days (Table 2). These losses were more severe at 20°C and 65% RH, being 3.8% after 10 days and 17.3% after 30 days. Fruits stored in plastic bags lost less than 1.5% at 10°C and 1.7% at 20°C after thirty days, and less than 2.6% at 10°C and 4.7% at 20°C after 30 days, with significant differences among those fruits packed in the different bags and control and CA-treated (without package) fruits (Table 2). Firmness decreased sharply during the storage period, ranging from 74N at zero time to 19.5 N in control fruits at 10°C, with significant changes with respect to fruits stored in CA-LDPE2 and MA-LDPE3 after 30 days (Table 2). Differences in firmness were more pronounced at 20°C in CA-treated (without package) than packaged fruits after 30 days. Delay of the maturity process was observed in fruits stored in plastic bags, compared with control and correlated with the changes in skin and pulp colour observed during storage at 10°C and 20°C (Table1).

The mango samples in the different treatments showed lower symptoms of decay than control fruits, after 10 days of storage at 10°C (Table 2). Fruits stored at 20°C in MA presented higher

TABLE 2. FIRMNESS, WEIGHT LOSS AND DECAY OF MANGOES STORED AT 10 AND 20°C FOR 10 AND 30 DAYS UNDER DIFFERENT CA AND MA TREATMENTS

	Firmness[1],N		Weight loss[2],%		Decay[3],%	
	Storage period, days					
Treatment	10	30	10	30	10	30
	Storage temperature, 10°C					
Control	58.0^{b,A}	19.5^{de}	1.6^{bc}	4.8^{d}	20	75
CA	73.7^{a}	48.6^{c}	0.9^{b}	3.4^{d}	17	38
CA-LDPE1	55.4^{a}	46.0^{c}	0.9^{b}	2.6^{cd}	0	6
CA-LDPE2	65.4^{a}	60.2^{a}	1.5^{bc}	2.5^{cd}	5	25
MA-LDPE1	50.4^{b}	8.5^{efg}	0.5^{a}	1.2^{b}	10	22
MA-DLPE2	58.8^{b}	25.5^{d}	0.8^{b}	1.9^{bc}	3	10
MA-LDPE3	72.1^{a}	54.6^{b}	1.9^{bc}	2.0^{bc}	20	45
	Storage temperature, 20°C					
Control	3.5^{g}	ND	3.8^{d}	17.3^{ef}	25	95
CA	5.7^{fg}	1.2^{gh}	3.0^{cd}	15.5^{ef}	21	75
CA-LDPE1	18.3^{de}	11.8^{ef}	0.5^{a}	4.7^{d}	4	17
CA-LDPE2	16.5^{de}	4.6^{fg}	1.5^{bc}	2.1^{c}	30	80
MA-LDPE1	43.7^{bc}	2.3^{gh}	1.7^{c}	2.7^{bcd}	24	65
MA-DLPE2	19.0^{de}	16.0^{de}	0.7^{a}	4.2^{cd}	8	24
MA-LDPE3	12.1^{ef}	2.5^{gh}	3.5^{cd}	3.6^{cd}	31	54

ND= not determined

[1] Each value is the mean of 6 replicates

[2] Each value is the mean of 10 replicates

[3] Each value is the mean of 30 replicates

Values of each variable (within columns) followed by the same letter are not significantly different ($p<0.05$)

symptoms of decay than fruits stored at 10°C, even in the same package. After 30 days, incidence of decay was more pronounced and control fruits and few packaged in polyethylene bags were discarded because of a high incidence of fungal lesions (Table 2).

Changes in mango quality after storage in the different treatments are shown in Table 3. No significant changes were observed in pH and TA in fruits under the different treatments. The results indicated that the storage treatments had no effect on the changes in pH and TA in mango flesh during conservation. However, TSS of control fruits were higher than those stored in the CA and/or MA treatments, especially at 20°C.

The results presented above agree with those reported for other fruits, in which low oxygen treatments delayed the maturation process and prolonged shelf life (Ke and Kader 1992; Yahia 1993). Off-odours were detected after 10 days in 30% of fruits, stored under LDPE1 and LDPE2 films at 20°C. These off-odours were correlated with the low oxygen and high CO_2 contents of the atmosphere surrounding these fruits. Other researchers have studied the optimal atmosphere for mango, and found no significant favourable quality changes due to the use of plastic films for this fruit (Hatton and Reeder 1965; Spalding 1977; Lakshminarayana and Subramanyam 1970). McCollum et al. (1992) wrapped 'Tommy Atkins' mangoes in plastic films and stored them at 12°C and 21°C for 2 weeks without finding any beneficial results on quality in comparison to unwrapped fruits. In a previous study, it was found that MA packaging preserved the quality of mango and avocado fruits, but off-odours resulted in mangoes stored at 20°C (Gonzalez et al. 1990). Noomhorm and Tiasuwan (1995) found that 'Rad' mangoes could be stored well under a 4% CO_2/6%O_2 atmosphere at 13°C and 94% RH for more than 25 days without CO_2 injury. The present results show that the appropriate use of the packages used in mangoes, seems suitable. The initial conditioning in CA for 6 days alone or with MA, was effective in delaying the maturation process in terms of preventing weight and firmness losses, decay and the avoidance of pulp colour changes. However, it is necessary to establish the optimal packaging system for prolonging post-harvest life for different mango varieties. For practical purposes, it is necessary to control the conditions, used in this experiment to obtain the best results.

It may be concluded that the storage of 'Keitt' mangoes using the combination of CA and MA, could improve the shelf-life to the same extent as continuous MA packaging, and that the low O_2 (for six days) used in CA storage can be considered as safe for the fruit.

TABLE 3. pH, TITRATABLE ACIDITY (TA) AND TOTAL SOLUBLE SOLIDS (TSS) CHANGES OF MANGOES STORED AT 10 AND 20°C FOR 10 AND 30 DAYS UNDER DIFFERENT CA AND MA TREATMENTS

	pH		TA		TSS	
	Storage period, days					
Treatment	10	30	10	30	10	30
	Storage temperature, °C					
Control	3.37^{b,1}	1.49^{b}	0.6^{h}	0.70^{h}	4.8^{de}	5.5^{de}
CA	3.45^{ab}	2.55^{ab}	0.84^{h}	0.67^{h}	3.3^{fgi}	4.1^{efg}
CA-LDPE1	3.71^{ab}	3.04^{ab}	0.52^{h}	0.48^{h}	30^{fgi}	3.4^{fgi}
CA-LDPE2	3.75^{ab}	3.21^{ab}	0.73^{h}	0.30^{h}	3.0^{fgi}	3.4^{fgi}
MA-LDPE1	3.40^{ab}	2.93^{ab}	0.79^{h}	0.48^{h}	2.9^{fgi}	3.5^{fgi}
MA-DLPE2	3.38^{ab}	2.72^{ab}	0.79^{h}	0.77^{h}	2.4^{ii}	2.6^{ii}
MA-LDPE3	3.77^{ab}	3.22^{ab}	0.47^{h}	0.52^{h}	3.4^{efgi}	3.3^{fgi}
	Changes temperature, 20°C					
Control	4.72^{a}	*ND*	0.19^{h}	*ND*	6.6^{c}	*ND*
CA	4.01^{a}	5.2^{a}	0.50^{h}	0.12^{h}	5.5^{cd}	5.4^{cd}
CA-LDPE1	3.32^{ab}	3.38^{ab}	0.78^{h}	0.35^{h}	3.0^{gi}	2.9^{gi}
CA-LDPE2	3.38^{ab}	4.32^{ab}	0.76^{h}	0.37^{h}	2.7^{gi}	3.4^{gi}
MA-LDPE1	4.01^{ab}	4.72^{ab}	0.34^{h}	0.16^{h}	4.1^{ef}	4.7^{ef}
MA-DLPE2	4.01^{ab}	4.55^{ab}	0.53^{h}	0.37^{h}	3.3^{fgi}	3.3^{efg}
MA-LDPE3	3.99^{a}	4.60^{a}	0.37^{h}	0.23^{h}	4.5^{def}	4.5^{def}

ND= not determined

[1] Each value is the mean of 6 replicates

[2] Values of each variable (within columns) followed by the same letter are not significantly different ($p<0.05$)

References

AOAC (1984) Official Methods of Analysis. 14th edn, Association of Official Analytical Chemists, USA

ASTM (1978) WVTR of materials in sheet form. American National Standard. E 96-22. American Society for Testing Materials, Philadelphia, Pa, USA

American Animal and Plant Health Inspection Service (1986) Plant protection and quarantine treatment manual, M319.56-2-A. Section VI-T102

Brandl DG, Soderstom M, Shreiber FE (1983) Effects of low-oxygen atmospheres containing different concentrations of carbon dioxide on mortality of the navel orange worm, Amyelosis transitella Walker (*Lepidoptera:Pyralidae*). J. Econ Entomol 76:828-830

Esquerra EB, Lizada C (1990) The post-harvest behaviour and quality of "Carabao" mangoes subjected to vapour heat treatment. ASEAN Food J 5:611-613

Gaunce AP, Morgan CVG, Meheruik M (1982) Control of tree fruit insect with modified atmosphere. In: Richardson DG, and Meheruik M (eds). Controlled Atmosphere for Storage and Trnasport of Perishable Agricultural Commodities. Oregon State University School of Agriculture Symposium Series No.1 Timber Press. Beaverton, Oregon, pp 383-385

Gonzalez G, Yahia E, Higuera I (1990) Modified atmosphere packaging of mango and avocado fruits. Acta Horticulturae 269:334-344

Hatton Jr TT, Reeder WF (1965) Controlled atmosphere storage of 'Keitt' mangoes. Proc Am Soc Hortic Sci 10:114-119

Ke D, Kader AA (1992) Potential of controlled atmospheres for post-harvest insect disinfection of fruits and vegetables. Postharvest News and Information 3(2):3IN-37N

Lakshminarayana S, Subramanyam H (1970) Carbon dioxide injury and fermentative decarboxylation in mango fruit at low temperature storage. J Food Sci Technol 8:121-126

McCollum TG, d'Aquino S, Miller WR, McDonald R (1992) Individual shrink film wrapping of mangoes. Proc Fla State Hortic Sci 105:103-105

Mitcham EJ, McDonald RE (1993) Respiration rate, internal atmosphere and ethanol and acetaldehyde accumulation in heat-treated mango fruit. Post Biol Technol 3:77-86

Noomhrorm A, Tiasuwan N (1995) Controlled atmosphere storage of mango fruit, *Mangifera indica* L. cv. 'Rad'. J Food Proc Preservation 19:271-281

SAS Institute (1990) SAS User's Guide: Statistic Version 6.03. SAS Institute, Inc. Carey N.C. USA

Spalding DH, (1977) Current Recommendations for transport and storage of tropical fruit. In: Dewey DH (ed). Controlled Atmospheres for the Storage and Transport of Perishable Agricultural Commodities. Horticultural Report No. 28. Department of Horticultural, Michigan State University. East Lansing, MI USA. pp 242-249

Yahia EM, Carrillo A (1993) Responses of avocado fruit to insecticidal O_2 and CO_2 Atmospheres. Food Sci Technol 26:307-311

Yahia EM, Vasquez L (1993) Responses of mango to insecticidal oxygen and carbon dioxide atmospheres. Food Sci Technol 26:42-48

Yahia EM (1993) Responses of some tropical fruit to insecticidal atmospheres. Acta Horticulturae 343:371-376

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Nutritional Evaluation of Groundnut Cake Detoxified by *In Situ* Generated Ammonia in Albino Rats

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Market samples of groundnut cake contaminated upto 700 ppb alfatoxin B_1 was mixed with urea, urease source and formaldehyde and heated to 50°C for 3h followed by 121°C for 30 min. The process was effective for detoxification of aflatoxin B_1 to more than 90% efficiency. The resulting product showed no toxicity, typical of aflatoxins in albino rats.

Keywords: Groundnut cake, Aflatoxin B_1, Urea, Urease, Detoxification, Formaldehyde.

Aflatoxins are known for their toxicity and carcinogenic properties both in higher and lower animals (Newberne and Butler 1969; Gopalan et al. 1972; Krishnamachari et al. 1975). The export markets in tropical countries for groundnut products often face a blockade due to high level of aflatoxin contamination. Hence, detoxifying contaminated groundnut cake has become inevitable in order to bring down this toxin levels to permissible limits of 30 ppb as per Indian Standards Institution and 20 ppb as per Food Drug Administration, USA. It is equally important to assess the nutritional quality of the treated material. This communication describes the method of detoxification of groundnut cake and also the safety aspect of detoxified groundnut cake.

Samples collected from local market were milled to pass through British Standard Mesh 20. Fifteen kg of groundnut cake flour was mixed with soybean flour (4% w/w), 200 ml of formaldehyde (37%) and water (3 l) in a planetary mixer. Urea (1.05 kg) dissolved in water (1 l) was, then, added slowly and mixed. The material at this stage contained 27-30% moisture, 7% urea, 4% soybean powder and 0.5% formaldehyde (dry weight basis). It was tightly covered in a stainless steel bin and heated at 50°C for 3 h in a steam-heated autoclave. Subsequently, the temperature was raised to 121°C and maintained for 30 min. The treated material was spread on aluminium trays and dried in hot air oven at 60°C. In another experiment, the milled groundnut cake was treated with formaldehyde alone as per the above methodology.

For the estimation of aflatoxin, the bulk of the treated material was probed at different points to collect about 2 kg sample, which was mixed thoroughly and milled. Quantitative estimation of aflatoxin B_1 in treated and untreated cake was carried out in triplicates, according to the method of Pons et al (1966) with slight modification. To 50 g samples, 75ml water was added and the pH was adjusted to 6.0 The material was transferred quantitatively to a conical flask and 175 ml acetone was added. The rest of the procedure was as described in the method.

For animal feeding trials. 21 days old male albino rats weighing 40 g on average were segregated into 3 groups of 10 animals and housed individually in aluminium-painted wire cages. Synthetic diet (corn starch 64, groundnut carbohydrate 12, fat 10, vitamin oil 1.0, groundnut protein 10.0 and salt mixture 2.0 (% w/w) was given *ad libitum* in the form of hot water slurry.

Treated and untreated groundnut cakes were used as the sole source of protein. The groundnut cake also contributed to a portion of the carbohydrate (12%) in the diet. The control group was fed untreated solvent-extracted groundnut cake, while experimental groups 2 and 3 were fed with the diets, containing the cake treated with urea-formaldehyde and formaldehyde alone, respectively for 3 months. The body weights were monitored every week. The urine output was recorded and its pH monitored. At the end of three months, blood was collected by cardiac puncture for haematology, serum albumin (Bartholoweu and Deloney 1966) and blood urea nitrogen (Wybenga et al. 1971) analysis. The liver, kidney and heart were weighed and processed for histopathological examination.

The data on the treatment of groundnut cake by urea, soybean flour and formaldehyde as per the methodology developed, show 99.6, 98.0 and 90.0% to 99.6% destruction of aflatoxin, when the initial aflatoxin levels were in the range of 0.17 to 0.70 ppm, respectively. The percent destruction of

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aflatoxin is similar to that achieved by other workers with ammonia (Mann et al. 1970; Brekke et al. 1978). The detoxification trials with urea were known to give inconsistent results probably due to the insufficient release of ammonia during the treatment. In the present study, the process of the release of nascent ammonia from urea was achieved by the action of urease present in soybean flour. Though detoxification of groundnuts by urea under sunlight was possible (Shantha et al. 1986), the method was not successful with groundnut cake, since it was not convenient to raise the moisture content of the cake to facilitate the reaction for destroying bound aflatoxin in groundnut cake (Shantha and Sreenivasa Murthy 1981). The addition of water to milled groundnut cake resulted in a sticky mass that posed problems during processing. The problem appeared to be overcome by including formaldehyde in the reaction mixture, as it prevented agglomeration of the milled cake, even when the moisture was as high as 27%. The use of formaldehyde in detoxification of groundnut cake was strongly defended by Codifer et al (1976). Eventhough the treatment of the proteins or protein containing dietary supplements with formaldehyde prevented microbial degradation of amino acids in the rumen of cattle, Codifer et al (1976) stressed that the absorption of essential amino acids could take place in the abomosum or the lower gut of animals.

Results of the metabolic studies indicated that there was no significant change in urine output in 24 h among the groups: the average volumes of urine of 10 rats per group were 23.3, 24.0 and 19.4 ml in the control and experimental groups, respectively. Final body weights of group 1 (control), 2 and 3 were 154, 117, 124g, respectively. Slight reduction of gain in body weight in experimental rats in initial stages of feeding trials (Fig. 1) (responsible for the vast difference even in final stages) was rectified by the supplementation of shark liver oil, lysine and methionine to the diets of these animals. The haematological picture was normal for all the groups. The results tabulated in Table 1 illustrate that no abnormality was found in the weights of liver and hearts even after 3 months feeding on experimental diet. Norred (1979) also reported liver to be normal after feeding on ammoniated corn for 72 h period. In the present studies, kidney weights of experimental rats were significantly ($p<0.05$) more than those of the control rats (Table 1). Although blood urea nitrogen did not significantly vary among the three groups, a

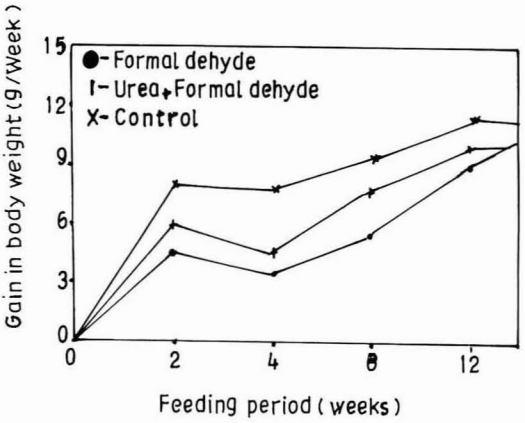

Fig. 1. Weekly gain in body weight of rats fed with formaldehyde urea + formaldehyde-treated and untreated control groundnut cake

slightly enhanced values were noted in the experimental groups, but this values were within the acceptable range. There was no decrease in the albumin value nor was it detected in the urine, thus showing that the ammonia-treated cake did not elicit any abnormality in the kidney. The feeding trials for a prolonged period of three months, using *in situ* generated ammonia and formaldehyde-treated groundnut cake showed no histopathological abnormalities in liver, kidney and heart. Though the kidney weight indicated statistically significant increment, no histopathological changes were observed in the kidney tissue.

The method of detoxification of groundnut cake by using urea as a source of ammonia in the

TABLE 1. EFFECT OF FEEDING OF GROUNDNUT CAKE TREATED WITH FORMALDEHYDE ALONE OR UREA + FORMALDEHYDE ON ORGAN WEIGHTS, ALBUMIN AND BLOOD UREA NITROGEN OF ALBINO RATS AFTER THREE MONTHS PERIOD

	Organ weights, g			Albumin, mg/100 ml	BUN, mg/100 ml
	Liver	Kidney	Heart	serum	serum
Diet with commercial groundnut cake	3.492[a]	0.590[a]	0.346[a]	2.00[a]	12.18[a]
Diet containing formaldehyde-treated commercial GN cake	3.877[a]	0.686[b]	0.363[a]	2.70[b]	12.69[b]
Diet containing Urea+formaldehyde-treated commercial GN cake	3.642[a]	0.670[b]	0.383[a]	2.55[b]	13.45[b]
Standard error of mean	+0.111 (27df)	+0.016 (27df)	+0.011 (27df)	+0.18 (27df)	+0.969 (27df)

Mean of the same column followed by different letters differ significantly according to Duncan's New Multiple Range Test ($p<0.05$), GN = Groundnut cake, BUN = Blood urea nitrogen

presence of soy flour (as source of urease enzyme) and the formaldehyde, as described in the present studies has the same advantages and disadvantages as that of direct ammonia treatment in imparting brown colour to the treated material. But, in this process, a cheaper chemical like urea effectively destroyed aflatoxin in groundnut cake. The process is economical and advantageous as large investment on ammoniation plant can be eliminated. The feeding trials for a prolonged period of 3 months on *in situ* generated ammonia-treated groundnut cake revealed no abnormality in terms of haematological and histopathological changes in liver, kidney and heart. The novel process for detoxification of groundnut cake is an economical process and the product is safe for consumption as evaluated in feeding trials with albino rats.

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References

Bartholoweuw RJ, Deloney AM (1966) Dye binding method. Proc Aust Assoc Clin Biochem 1: 214–215

Brekke DL, Stringfellow AC, Peplinski AJ (1978) Aflatoxin inactivation in corn by ammonia gas: Laboratory trials. J Agric Food Chem 26: 1383–1349

Codifer Jr LP, Mann GE, Dollear FG (1976) Aflatoxin inactivation: Treatment of peanut meal with formaldehyde and calcium hydroxide. J Am Oil Chem Soc 53: 204–206

Gopalan C, Tulpule PG, Krishnamachari D (1972) Induction of hepatic carcinomas with aflatoxin in the rhesus monkey. Food Cosmet Toxicol 10: 519–521

Krishnamachari D, Bhat RV, Nagarajan V, Tilak TBG (1975) Hepatitis due to aflatoxicosis. An outbreak in Western India. Lancet 1: 1061–1063

Mann GE, Codifer Jr LP, Gardner Jr HK, Kaltun SP, Dollear EG (1970) Chemical inactivation of aflatoxins in peanut and cottonsed meals. J Am Oil Chem Soc 47: 173–176

Newberne PM, Butler WH (1969) Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals. A review. Cancer Res 29: 236–250

Norred WP (1979) Effect of ammoniation on the toxicity of corn artificially contaminated with aflatoxin B_1. Toxicol Appl Pharmacol 51: 411–416

Pons Jr WA, Cucullu AF, Lee LS, Robertson JA, Franz AD, Goldblatt LA (1966) Determination of aflatoxins in agricultural products: Use of aqueous acetone for extraction. J Assoc Off Agric Chem 49: 554–562

Shantha T, Sreenivasa Murthy V (1981) Use of sunlight to partially detoxify groundnut (peanut) cake flour and casein contaminated with aflatoxin B_1. J Assoc Off Anal Chem 64: 291–293

Shantha T, Sreenivasa Murthy V, Rati ER, Prema V (1986) Detoxification of groundnut seeds by urea and sunlight. J Food Safety 7: 225–231

Wybenga D, Di Giorgio, Pileggirj J (1971) Direct manual determination of blood urea nitrogen. Clin Chem 17: 891–892

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Effect of Gamma-Irradiation on Cooking and Eating Qualities of Scented Rice Varieties

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Studies on gamma-irradiation of export quality scented rices of the trade names 'Lal Qilla', 'Pari' and the variety 'Pusa Basmati-1' showed that irradiation at doses of 0.25-1.25 kGy did not reduce significantly the length of the cooked rice grain. Water uptake during cooking was also not affected by irradiation upto a dose of 1.0 kGy in case of 'Pari' and 'Pusa Basmati-1', but it was slightly affected in case of 'Lal Qilla'. Volume expansion and gel consistency, however, decreased on irradiation. The changes in colour on irradiation of cooked or uncooked rices of 'Lal Qilla' and 'Pusa Basmati-1' were reflected in the decrease of colour-scores according to Hedonic scale, by 7-10 panelists. The decreases in aroma scores also suggested some loss of aroma in these two rice qualities. Irradiation had very little effect on texture and after-cooking hardening on cooling of cooked rice. All these scores (above 5.0) suggested that the rices irradiated with these doses (0.25-1.25 kGy) were of acceptable quality in spite of the stated effects.

Keywords: Gamma-irradiation, Eating and cooking qualities, Scented rices.

In an earlier study on the grains of 'Pusa-33', a non-export quality rice variety, containing 12.0% moisture, the acceptibility limit of irradiation, keeping in view the changes in eating and cooking quality parameters, was determined as 3.0 kGy (Roy et al. 1991). This dose was also quite effective in reducing mould population in the irradiated grains. A subsequent study (Prasad and Roy 1995) for insect-disinfestation of export quality rices, 'Pari' and 'Lal Qilla', showed that a dose of 1.0 kGy was the best treatment for the control of two important storage pests, namely *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (Linnaeus). Similar results were obtained in the insect disinfestation of pulses (Roy and Prasad 1993). The target for export of rice from India has been fixed at 1.8 million tonnes. Infestation of rice in packages by the named pests is of common occurrence during storage.

The current practice for the export of rice is to treat the grains with methylbromide (MB) at an interval of about 45 days. About 3 such treatments are required before shipment and subsequent marketing. The treatments are given in bulk before packing. But, irradiation can be given in pre-packaged condition and an application at one stretch is good enough to take care of the infestations. Little or no information is available about the effects of pest-killing doses of radiation on eating and cooking characteristics of scented rices, including grain length and colour, which are important determinants of market price of rice and hence, the present study was undertaken.

The rice grains (dehusked paddy) of export quality, marketed by trade names 'Lal Qilla' (Dehra Dun No 1), 'Pari' and the variety of Indian Agricultural Research Institute, 'Pusa-Basmati-1', with 12% moisture were irradiated (^{60} Co) in 20 g lots in polythene bags (400 gauge) with 0.25, 0.5, 1.0 and 1.25 kGy at a dose rate of 0.033 kGy/min. The water uptake was determined by cooking in an autoclave (25 min. at 100°C) for 20 g of rice of each quality or variety, placed in 100 ml water in 250 ml beakers. The water uptake was calculated, as studied earlier, after necessary processing (Roy et al. 1991). Expansion per unit volume of uncooked rice was determined on the basis of difference in volume of water displaced by uncooked and cooked grains. The gel consistencies of irradiated and unirradiated rice samples were determined by using 100 mg of 100-mesh rice flour, wetted with 0.2 ml of 95% ethanol, containing 0.025% thymol blue (Cagampang et al. 1973). The lengths of 10 grains from each treatment and control were determined and averaged out. The sensory scores of cooked, uncooked and both irradiated and unirradiated 'Lal-Qilla' and 'Pari' rices were recorded by 7-10 panelists, according to a 9-point Hedonic scale. The scores of 5.0 or more were considered acceptable. The textures of treated and cooked rices and the hardening of such rices after cooling were judged by pressing between fingers and by crushing with a glass rod (Wootton et al. 1986). All the experiments were done in duplicates from each treatment.

Water uptake upon cooking of rice was not affected upto 1.0 kGy with rice qualities 'Pari' and 'Pusa Basmati-1' but with 'Lal Qilla', it was marginal (Table 1). The volume expansion on cooking decreased only slightly with increase in dose levels of irradiation. These results are in good

TABLE 1. EFFECT OF GAMMA IRRADIATION ON WATER UPTAKE, VOLUME EXPANSION, GRAIN LENGTH AFTER COOKING AND GEL CONSISTENCY OF THREE RICE QUALITIES

Dose, kGy	Water uptake#			Volume expansion/unit volume of rice			Gel consistency, cm			Grain length, cm		
	'Pari'	Pb*	Lq**	'Pari'	Pb*	Lq**	'Pari'	Pb*	Lq**	'Pari'	Pb*	Lq**
0.00	4.00	3.01	2.77	3.15	3.92	3.86	8.08	7.57	7.87	1.70	1.44	1.43
0.25	3.94	2.98	2.72	2.94	3.71	3.76	7.44	7.27	7.60	1.62	1.43	1.41
0.50	3.89	-	-	3.05	-	-	7.11	-	-	1.67	-	-
1.00	3.95	2.97	2.67	3.05	3.66	3.57	7.54	7.17	7.40	1.65	1.44	1.41
1.25	-	2.91	2.64	-	3.58	3.52	-	7.10	7.34	-	1.42	1.40
CD at 5%	NS	0.09	0.09	NS	0.15	0.14	0.17	0.13	0.27	NS	NS	NS

* 'Pusa Basmati' ** 'Lal Qilla' # wt. after cooking/wt. before cooking

TABLE 2. EFFECT OF IRRADIATIONON SENSORY ATTRIBUTE OF THREE RICE VARIETIES AFTER COOKING

Dose, kGy	Aroma		Texture		Colour (Cooked)		Colour (Uncooked)		Hardening after cooling	
	Pb*	Lq**	Pb	Lq	Pb	Lq	Pb	Lq	Pb	Lq
0.00	7.3	7.9	6.6	7.0	7.6	7.9	6.7	7.7	8.0	7.9
0.25	7.0	7.0	7.0	7.4	7.5	7.5	6.7	7.9	7.9	7.6
1.00	6.4	6.4	6.9	6.7	6.8	7.0	6.3	6.5	8.1	7.6
1.25	6.5	6.6	7.0	6.9	7.0	6.9	5.3	6.7	8.3	7.6
CD at 5%	0.8	1.1	1.1	0.9	0.9	0.6	1.3	1.4	0.1	1.2

* 'Pusa Basmati' ** 'Lal Qilla'

agreement with other studies, which reported 1.0 kGy as the limit of acceptability for brown rice and 2.5 kGy for milled rice (Ismail et al. 1978). Irradiation at 1.0 or 1.25 kGy reduced gel consistency in the rice grain, but the grain length of cooked rice was not affected by the treatment (Table 2). A reduction in viscosity in brown rice irradiated with 1–3 kGy has been reported (Sabularse et al. 1992).

The scores of sensory evaluation (Table 3) for colour and aroma upon cooking of both 'Pusa Basmati–1' and 'Lal· Qilla' rice varieties decreased with increments in doses of irradiation. Increments in irradiation levels also lowered the scores for textures of the two cooked rices. The sensory scores for colour of uncooked rice of these two qualities were lower for the irradiated samples. All the lowered values were, however, above the acceptable scores. Effect of irradiation on hardening of rice upon cooking and subsequent cooling was insignificant. The sensory evaluation scores are in good agreement with other studies (Wang et al. 1983).

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References

Cagampang GB, Perez M. Juliano BO (1973) A gel consistency test for eating quality of rice. J Food Sci Agric 24: 1589–1594

Ismail FA, El-Wakeil FA, El-Dash SM (1978) Tolerance, quality and stability of gamma-irradiated Egyptian rice. Food Preservation by irradiation, IAEA, pp 501–515

Prasad HH, Roy MK (1995) Efficacy of gamma-irradiation against important insects of export quality rice. Indian Food Packer 49(2): 25–30

Roy MK, Ghosh SK, Chatterjee SR (1991) Gamma-irradiation of rice grains. J Food Sci Technol 28: 337–340

Roy MK, Prasad HH (1993) Gamma-irradiation in the control of important states pests of three grain legumes. J Food Sci Technol 30: 275–278

Sabularse VC, Liuzzo JA, Rao RM, Grodner RM (1992) Physico-chemical characteristics of brown rice as influenced by gamma-irradiation. J Food Sci 57(1): 143–145

Wang UP, Lee CY, Chang JY, Yen CL (1983) Gamma-irradiation effects on Taiwan-produced rice grains. Agric Biol Chem 47(3): 461–472

Wootton M, Dio Jonegoro H, Driscoll R (1986) The effect of irradiation on the quality of Australian rice. J Cereal Sci 7: 309–315

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Dehydration of Turnip and Radish Slices

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A study was conducted to develop dehydrated turnip and radish slices, using a tray drier at 55-60°C. The dehydration kinetics revealed that the whole drying process took place in the falling rate period for both the root crops, while the turnip slices showed the higher drying rate, as compared to radish slices. The sorption isotherms were also conducted for the storage of the dehydrated root crop slices.

Keywords: Dehydration, Turnip, Radish, Drying constant, Shape factor, Rehydration ratio, Sorption isotherm.

Turnip (*Brassica napus* L.) and radish (*Ramphanus sativus* L.) are considered as root crops. Both these root crops belong to 'Cruciferae' family. Early maturing radishes are consumed raw in salads, whereas mature radishes and turnips are consumed after cooking. These vegetables are seasonal and available in plenty during the glut season. Heavy losses occur due to non-availability of sufficient storage, transport and proper processing facilities at the production point. The preservation of vegetables by dehydration offers a unique challenge (Singh and Heldman 1993) and it may be considered as an alternative low cost preservation process (Uddin et al. 1990).

In this study, the potential of preservation of root vegetables viz., turnip and radish by means of air-drying has been investigated.

Both vegetables were procured from the local market. These were sorted and washed under running water. The peeled vegetables were sliced into 3 mm thickness. Both these samples were blanched in boiling water for 3 min, cooled and immersed in 0.5% KMS solution at room temperature, followed by drying in a tray drier (Narang Corporation, New Delhi) at 55-60°C with a tray load of 4.8 kg/sq m. The drying in tray drier was with air of relative humidity of 55-60%. The drying was carried out to a moisture level of 9-10% from initial moisture content of 91-92% in fresh samples. The dried samples were packed and vacuum-sealed (Sevana Electrical Appliances, Kizakkabalam) in 200 gauge high density polyethylene bags for storage.

The moisture contents of samples were determined by standard oven-drying method, till the product attained a constant weight (AOAC 1975). Residual moisture contents of the samples were plotted against corresponding drying period. Drying rate was calculated by taking the weight of sample at regular intervals during drying and plotted as a function of time and average moisture content.

Sorption studies of dehydrated root crop slices: The relationship between total moisture content and the corresponding water activity of the dehydrated root crop slices at 25°C was studied, by using appropriate sulphuric acid solution of varying concentrations in water (Ruegg 1980).

Kinetic studies : The values of moisture ratio (MR) were plotted on semi-log graph paper with time along abscissa. The drying constant (k) and the shape factor (B) were calculated by using the following equation (Chakravorty and De (1988).

$$\frac{M-M_e}{M_o-M_e} = MR = B \exp(-kt) \quad \ldots\ldots\ldots (1)$$

Where M, moisture content of sample (db) at any time; M_o and M_e, initial and final (equilibrium) moisture contents of samples, respectively; t = time (h); k = drying constant (1/h) and B, shape factor.

The whole experiments were repeated thrice and the average values were taken for calculation.

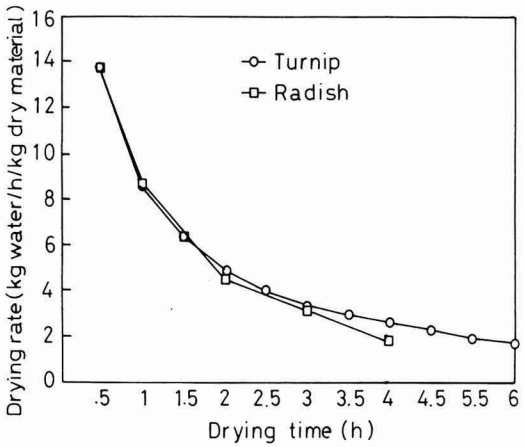

Fig. 1. Effect of time on drying rate of turnip and radish

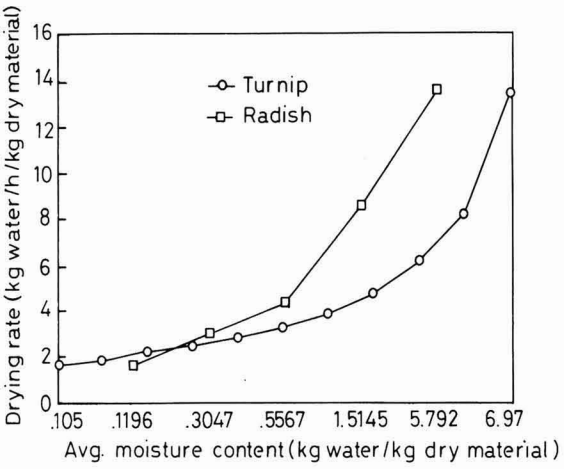

Fig. 2. Effect of drying rate on average moisture content of radish and turnip

The effect of time on drying rate of turnip and radish is presented in Fig. 1. The drying rate of root crop slices decreased very rapidly during the first 30 min of drying. It is believed that in the early stage of drying, the food material behaves as though the surface is saturated with water (Brennan et al. 1990). The drying rates of the root crop slices were almost parallel during the entire drying period. The smaller change in the drying rate is due to the variations of initial moisture content and different cell structure of the vegetables (Mandhyan et al. 1988).

Fig.2 shows the effect of average moisture content of turnip and radish on drying rate. It was noted that turnip or radish did not have any constant rate drying period and complete drying took place in falling rate period. Similar observation was reported by Chirife (1971) for tapioca root.

Moisture adsorption isotherms of turnip and

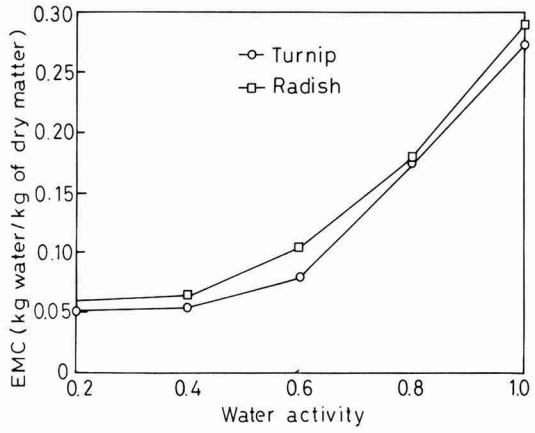

Fig. 3. Moisture sorption isotherm of dried turnip and radish

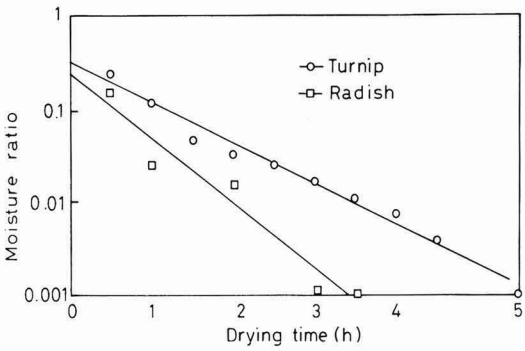

Fig. 4. Effect of time on moisture ratio

radish are presented in Fig. 3 and have been found to show type III behaviour (Rizvi 1986). Initially, the water adsorption was very low for both the vegetables, but later on, it increased rapidly, showing a steep rise in moisture content. The turnip and radish had 8.69% and 10.61% (wb) initial moisture contents, respectively, which equilibrated at 60% RH. As a result, the product became stable with respect to moisture loss or gain, when stored at this RH. It is evident that the dried samples absorb moisture rapidly above 60% RH and behave hygroscopic in nature.

Fig. 4 illustrates the effect of drying time on moisture ratio (MR) in a semi-logarithmic plot for both the vegetables. The values of B and k for each vegetable were determined from the least square linear regression. The drying equation for turnip and radish could be expressed as :

$$MR = 0.4029 \exp(-0.7645\ t) \quad \text{..... (2)}$$

$$MR = 0.4083 \exp(-1.4043\ t) \quad \text{..... (3)}$$

The corresponding values of correlation coefficients (r) were 0.98 and 0.93, respectively. It is clear that the shape factor (B) does not change so much from turnip to radish, whereas the drying constant is markedly changed from one another. The above data are very useful for the design and to improve the performance of the dryer.

References

AOAC (1985) Official Methods of Analysis, 12th edn. Association of Official Analytical Chemists, Washington, DC

Brennan JG, Butters JR, Cowell ND, Lilley AE (1990) Food Engineering Operations. Elsevier Applied Sciences, London

Chakravorty A, De DS (1988) Post-harvest Technology of Cereals and Pulses. Oxford and IBH Pub Co., Calcutta

Chrife J (1971) Diffusional process in the drying of tapioca root. J Food Sci 36: 327-330

Mandhyan BL, Abroal CM, Tyagi, HR (1988) Dehydration characteristics of winter vegetables. J Food Sci Technol 25(1) 20-22

Rizvi SSH (1986) Thermodyanamic properties of foods, In: Rao MA, Rizvi SSH (eds), Engineering Properties of Foods. Marcel Dekker, Inc. New York

Ruegg M (1980) Calculation of the activity of water in sulphuric acid solution at various temperatures. Lebensm Wiss Technol 13: 22-24

Singh RP, Heldman DR (1993) Introduction to Food Engineering. Academic Press, INC., California

Uddin MS, Hawlader MN, Rahman MS (1990) Evaluation of drying characteristics of pineapple in the production of pineapple powder. J Food Preserv 14: 375-391

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Radio-sensitivity of Different Developmental Stages of Pulse Beetle *(Callosobruchus maculatus)*

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The radio-sensitivity of egg, larva, pupa and adult of *Callosobruchus maculatus* was investigated by exposing them to different doses of gamma radiation. All the stages exhibited age and dose dependent response. In early egg stage (0 to 24h), hatching was prevented at the dose of 10 Gy, whereas in older eggs (3 days old), even though hatching was normal at this dose, their further development was significantly affected. The dose of 20 Gy was found to be sufficient to arrest the development of early to late larval stages. The pupae required comparatively higher dose of radiation and even at the dose of 150 Gy, more than 50% adults emerged. Longevity of adults was significantly affected at the dose of 500 Gy and all the adults died within the span of 3 days, when they were exposed to the dose of 1500 Gy.

Keywords: Radiation, Disinfestation, Pulse beetle, *Cllosobruchus maculatus.*

Radiation disinfestation has distinct advantages over commercial methods of pest control in storage. Unlike chemical methods, it does not leave toxic residues and it is highly penetrative, non-hazardous to operators, least energy consuming and economically feasible. Therefore, food irradiation has a great potential for extension of storage of several food items and the quarantine of exportable fruits and vegetables from India, where food irradiation has now been commercially allowed by amendment of Prevention of Food Adulteration Act in 1994. Government of India has accorded clearance for the trade of irradiated onions, seafoods and spices for export purpose. Henceforth, more and more items of food are likely to be added to the list for clearance. Among these, whole pulses form an important commodity as the insect damage, but the economic losses in pulses are alarming.

Beetles of the family Bruchidae are the most important pests of whole stored pulses. Among these, *Callosobruchus maculatus* F. and *Callosobruchus chinensis* F. are the most common bruchid species found in India. Practically, very little work has been carried out on radiation disinfestation of whole pulses. Only a few reports are available on this aspect and these are either inadequate or impractical. These authors have either recommended too high a dose (Roy and Prasad 1993; Gill and Pajni 1990) or emphasized the need for release of sterile males in large numbers (Pajni and Gill 1989), which is not practical. Hence, the need was felt to undertake systematic studies on various aspects of radiation disinfestation of whole pulses. In this communication, results of studies on radiation sensitivity of different stages of *C. maculatus* are presented.

Insects used in these experiments were drawn from laboratory colony of *C. maculatus*, maintained on *mung*bean *(Vigna radiata)* seeds at 29±1°C and 60-70% RH. For each set of experiments, 50 pairs of adults of *C. maculatus* were released over 300 g of fresh mungbean seeds and allowed them to oviposit for 2 h. Adults were removed after 2 h and seeds with single egg were isolated. Such egg-holding seeds were irradiated in groups of 25 at different stages of egg, larval and pupal stage with doses, ranging from 5 to 150 Gy of gamma-rays. Freshly emerged adults were also irradiated with doses, ranging from 100 to 1500 Gy to determine the mortality pattern. For each treatment, 5 replicates of 25 insects each were kept with appropriate controls.

After irradiation, the seeds (with insect stages) were held in plastic containers (5.5 cm dia x 2 cm ht) and stored in temperature controlled incubator set at 29°C. The observations were recorded daily and data were collected on egg hatchability, total developmental period, adult emergence and adult longevity. The ability of irradiated immature stages to reach adult stage was considered as the main criterion for assessing the degree of radiation sensitivity.

The early egg stages were found to be highly susceptible to radiation doses of 10 Gy and above (Table 1). None of the eggs hatched, when fresh or 24h old eggs were exposed to the dose of 10 Gy and above. In case of 48 and 72h old eggs, hatchability was comparable with control. However,

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TABLE 1. RADIATION SENSITIVITY OF EGG AND LARVAL STAGES OF *CALLOSOBRUCHUS MACULATUS*

Age at irradiation, Days post-laying	Percent adult emergence at radiation doses, Gy				
	Control	5	10	15	20
		Egg stage			
Fresh	78 (85%)	0 (4%)	0	0	0
1	81 (86%)	0 (41%)	0	0	0
2	83 (88%)	5 (85%)	0 (88%)	0 (87%)	0 (91%)
3	76 (84%)	63 (85%)	7 (90%)	0 (80%)	0 (68%)
		Larval stage			
5	90.0	90.0	51.3	0.6	0
8	92.6	92.0	58.0	0	0
11	89.3	91.3	56.0	0	0
14	96.6	91.3	54.7	2.0	0
17	93.3	92.0	87.7	83.3	81.0

Numerals in parenthesis are % eggs hatched

the developments of larvae hatched from these irradiated eggs were adversely affected. Even at 5 Gy dose, only 63% larvae reached adult stage, whereas at the dose of 10 Gy and above, adult emergence was almost negligible. Thus, egg stage response to radiation was manifested in 2 ways. In early egg stage, hatching was prevented even at low dose, while in older eggs, their further development was considerably affected. As far as the radio-sensitivity of the eggs of *C. maculatus* was concerned, similar results were reported by Ghogomu (1990). However, Olaifa et al (1990) have recommended a dose of 1Gy to arrest the development of eggs of the same insect. In the present study, even at the dose of 20 Gy, 68% eggs hatched, when they were irradiated at the age of 74h.

All the stages of larvae appeared to be very sensitive to radiation dose of 20 Gy and above. Upto the age of 14 days post-laying, representing early, middle and late larval stages, none of the larvae could complete development, when they were irradiated with 20 Gy and above (Table 1). In an earlier study, Olaifa et al (1990) have reported that the development of the younger larvae of *C. maculatus* could be arrested by irradiating them with the dose of 5 Gy. The present study, however, showed that the developments of all the larval stages were as normal as control, when they were irradiated with the dose of 5 Gy. Only at the dose of 10 Gy and above, the developments of all the larval stages were found to be affected. Ghogomu (1990) and Gill and Pajni (1990) have applied comparatively higher radiation doses in the range of 250 to 300 Gy to find out the radiation sensitivity of the larvae of *C. maculatus*. In the present studies, when insects were irradiated at the stage of 17 days post-laying, the response was found to be dose-dependent and even upto 20 Gy, 81.0 larvae could reach the adult stage. In another set of experiments, the developmental stages from 15 days post-laying to 20 days post-laying were irradiated with dose of 50 Gy and above (Table 2). As expected, the stage of 15 days post-laying appeared to be a late larval stage, since with 50 Gy only 2% larvae could reach the adult stage.

TABLE 2. RADIATION SENSITIVITY OF LATE LARVAL AND PUPAL STAGES OF PULSE BEETLE *(CALLOSOBRUCHUS MACULATUS)*

Stage irradiated, Days post-laying	% adult emergence at radiation doses, Gy			
	50	80	100	150
15	2.0	1.0	0.0	0.0
16	28.0	14.0	4.0	2.0
17	62.4	57.0	25.0	12.0
18	70.0	59.0	33.6	10.4
19	78.0	66.6	47.6	20.3
20	89.0	79.3	78.0	50.6

Common control in which 96.7% adults emerged

Pupal stage appears to be more tolerant to radiation doses and exhibits dose-dependent response to radiation treatment. At any dose level, there was an increase in the percent adult emergence, as the age of the pupae advanced and within the same age group, there was decrease in percent pupae reaching to adult stage, as the dose increased (Table 2). This type of typical radiation

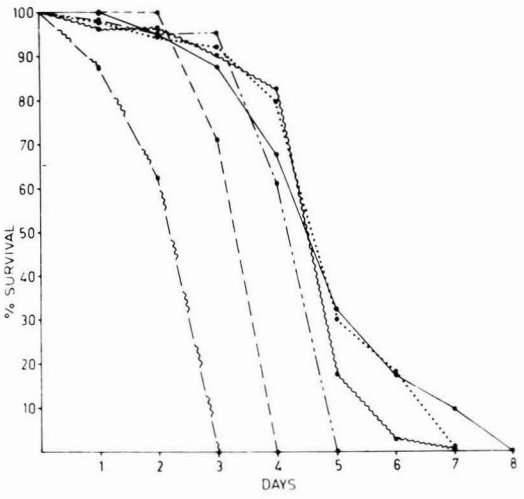

Fig 1. Radiation sensitivity of adults of pulse beetle, *Callosobruchus maculatus*, —●— control, ···●···100 Gy, ∿∿●∿∿ 500 Gy, — - - - 750 Gy, - - - - 1000 Gy, ∿∿——∿∿ 1500 Gy

response was also observed in the case of other stored grain pests (Brower and Tilton 1973; Tilton et al. 1966; Cornwell 1966). The adult emergence could not be completely stopped by irradiating pupae with the dose of 150 Gy. Ghogomu (1990) has also reported that the adults emerged, when pupae of *C. maculatus* were irradiated with the dose of 300 Gy. Gill and Pajni (1990) recommended a dose of 350 Gy to kill the pupae of *C. maculatus*.

Survival pattern of adults exposed to irradiation doses ranging from 50 to 1500 Gy has been illustrated in Fig. 1. From the figure, it could be seen that survival of adults was dose-dependent. Control adults survived for 8 days, whereas all the adults died within the period of 3 days, when they were irradiated with the dose of 1500 Gy. Similarly, Olaifa et al (1990) have reported that the survival of *C. maculatus* adults was not affected at the radiation dose of 100 Gy and Ahmed et al (1981) have found that a dose of 400 Gy shortened the life span of the same insect. The lethal radiation doses required for other stored grain pests were found to be much higher and they ranged from 1600 Gy for *Siltophilus oryzae* to 3000 Gy for *Tribolium confusum* (Padwal-Desai et al. 1987).

References

Ahmed MYY, Abu-Donia SA, Ahmad ZA, Salem AA (1981) Studies on sterilization of cowpea weevil, *Callosobruchus maculatus* F. by gamma radiation. Bulletin de la Societe Entomologique d Egypte 63: 7-12

Brower JH, Tilton EW (1973) Comparative gamma radiation sensitivity of *Tribolium madens* (Carpentier) and *Tribolium castaneum* (Herbst). J Stored Product Res 9: 93-100

Cornwell PB (1966) Susceptibility of the grain and rice weevils, *Sitophilus granarium* (L.) and *Sitophilus zeamais* (Mots) to gamma radiation. In: The Entomology of Radiation Disinfestation of Grain, Permagon Press, NY, pp 1-18

Ghogomu TR (1990) Gamma radiation effects on the development of cowpea weevil, *Callosobruchus maculatus*. Mededelingen Van de Faculteit Londbowwetenschappen Rijksuniversiteit Gent 55: 549-555

Gill M, Pajni HR (1990) Disinfestation of stored pulses with gamma radiations. In: Proceedings of Symposium on Entomology for Defense Services, DRDE Gwalior, pp 239-243

Olaifa JJ, Adebayo TA, Adesanmi CA, Salu AM, (1990) Sensitivity of the different developmental stages of *Callosobruchus maculatus* to gamma radiation. Int J Radiation Application Instrumentation. Part A - Applied Radiation and Isotopes 41: 785-786

Padwal-Desai SR, Sharma A, Amonkar SV (1987) Disinfestation of whole and ground spices by gamma irradiation. J Food Sci Technol 24: 321-322

Pajni HR, Gill M (1989) Suppression of laboratory population of *Callosobruchus chinensis* (Linn.) (Bruchdae: coleoptera) by the release of gamma radiation sterilized males and females. J Nucl Agri Biol 18: 89-91

Roy MK, Prasad HH (1993) Gamma radiation in the control of important storage pests of three grain legumes. J Food Sci Technol 30: 275-278

Tilton EW, Burkholder WE, Coghurn RR (1966) Effect of gamma radiation on *Rhizopertha dominica, Sitophilus oryzae, Tribolium confusum* and *Lasioderma serricorne* J Econ Entomol 59: 1363-1368

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Malting Characteristics and Biochemical Changes of Foxtail Millet

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The foxtail millet grains were steeped in water for 8, 16 and 24 h and then germinated for 24, 48 and 72 h. for the preparation of malt. One lot of grains was dehulled and effect of these processings on the malting characteristics and biochemical changes of foxtail millet were studied. The steeping of grains in water for 8 to 16 h and germination at 30°C upto 72 h significantly decreased the dry matter, starch, protein, crude fibre, phytate and polyphenols, while the soluble proteins, free amino acids, reducing sugars, *In vitro* protein digestibility, *in vitro* starch digestibility, diastatic and proteolytic activities and *in vitro* availability of iron significantly increased. Steeping of grains in water for 16 h and germinating for 48 h were found to be optimum for maximum malt yield.

Keywords: Foxtail Millet, Malting, Polyphenol, Phytate proteolytic and diastatic activities, *In vitro* protein digestibility, *In vitro* starch digestibility.

Foxtail millet *(Setaria italica)* is one of the minor millets, containing high amounts of proteins and minerals. However, the nutritional quality of this millet products is poor due to deficiency of certain amino acids, low protein and starch digestibilities and presence of certain anti-nutritional factors. The sensory properties are also poor due to coarse nature of grain. Like other coarse grains, this millet is rich in phytate and polyphenols, which interfere with minerals (Rao and Prabhavathi 1982; Rao and Deosthale 1988) and protein availability (Ramachandra et al. 1977). Polyphenols also influence the colour and flavour of millet.

To overcome all these problems and to increase the palatability of this millet, one of the feasible methods of processing is malting. Malleshi and Desikachar (1985) reported that soaking for 12 to 16 h and germinating for 24 h were optimum for maximum malt yield from foxtail millet. The objective of the present study was to investigate the effect of malting conditions on the biochemical changes in foxtail millet and remove phytate and polyphenols and study their influence on the *in vitro* availability of iron.

Seeds of foxtail millet (variety 'Arjuna') were purchased from the local market, cleaned, moistened, dehulled manually and hulls were removed by winnowing. One lot was steeped in water for 8, 16, 24 h and then germinated for 24, 48, 72 h and the malt was prepared. The proximate composition of whole, dehulled and malted samples was determined as per AOAC (1980) methods. Starch, reducing sugars and free amino acids were determined by the procedure of McCready et al (1950), Nelson (1944) and Rosen (1957), respectively and soluble proteins by Lowry et al (1951). Total phosphorus and iron were determined as per the procedure described by Raghuramulu et al (1987). Polyphenols were estimated as per the modified vanillin - HCl method of Price et al (1978) and phytate as per the method described by Chauhan (1982). *In vitro* availability of iron was assayed by the method of Rao and Prabhavathi (1978), *In vitro* protein digestibility (IVPD) and starch digestibility (IVSD) were determined by using pepsin (AOAC 1980) and bacterial α-amylase (Singh et al. 1982), respectively. The diastatic and proteolytic activities were assayed by the methods of Singh et al (1982) and Ayre and Anderson (1939), respectively. The malting losses were estimated by difference in the grain weight before steeping and after drying of germinated grains and malt yield was estimated by difference. The cold- and hot-water extracts were determined according to the method described by Jayatissa et al (1980). The data obtained in triplicate for various parameters were statistically analyzed for variance to find out the level of significance, as per the method of Panse and Sukhatme (1967).

The data on all the constituents presented in Table 1 indicate that there was not much reduction in protein and fat contents in dehulled samples. However, significant reductions were observed in crude fibre and ash contents, possibly because of loss of hulls. The effect of malting on protein and fat contents was significant without much affecting the contents of fibre and ash. The significant reduction in crude fibre during dehulling and ash content during malting was possibly because of

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TABLE 1. EFFECT OF STEEPING AND MALTING ON PROXIMATE COMPOSITION, SOLUBLE PROTEINS, FREE AMINO ACIDS, STARCH, REDUCING SUGARS, IVPD, IVSD, DIASTATIC ACTIVITY, PROTEOLYTIC ACTIVITY, MALTING LOSS, MALT YIELD, COLD-WATER EXTRACT AND HOT-WATER EXTRACT OF FOXTAIL MILLET

Parameter	Control	Dehulled	Steeping period, h	Malting period, h 24	48	72	P≤0.05
Crude protein, %	14.1	14.0	8	14.0	13.6	13.4	0.28[a]
			16	13.0	12.8	12.5	0.28[b]
			24	12.6	12.5	12.3	NS[c]
Crude fat, %	4.8	4.7	8	4.7	4.6	4.5	0.05[a]
			16	4.4	4.3	4.3	0.05[b]
			24	4.2	4.1	4.1	NS[c]
Crude fibre, %	9.0	3.6	8	9.2	9.0	8.8	NS[a]
			16	9.1	9.0	8.9	NS[b]
			24	9.0	8.8	8.7	NS[c]
Crude ash, %	3.9	1.0	8	3.2	3.1	3.1	0.12[a]
			16	3.2	3.1	3.1	NS[b]
			24	3.4	3.2	3.2	NS[c]
Soluble proteins, %	1.2	1.5	8	1.4	1.8	2.1	0.05[a]
			16	1.7	3.0	2.6	0.05[b]
			24	2.3	2.4	2.5	0.09[c]
Free amino acids, mg/g	2.5	2.8	8	3.5	6.0	7.0	0.38[a]
			16	3.8	10.4	8.1	0.38[b]
			24	6.0	6.4	8.0	0.68[c]
Starch, %	57.5	64.7	8	56.0	48.5	48.5	0.49[a]
			16	52.2	37.0	43.4	0.49[b]
			24	50.0	45.5	38.5	0.85[c]
Reducing sugars, %	0.2	0.8	8	1.2	2.5	4.5	0.59[a]
			16	1.5	2.5	4.5	0.59[b]
			24	2.4	3.4	4.4	1.02[c]
IVPD, %	82.5	88.0	8	84.6	87.2	85.5	0.32[a]
			16	81.5	92.0	82.8	0.32[b]
			24	80.5	85.0	81.4	0.56[c]
IVSD, mg maltose/ g/2h	122	140	8	120	114	88	2.69[a]
			16	110	121	66	2.69[b]
			24	90	68	50	4.70[c]
Diastatic activity, mg maltose/ g/30 min.	80	ND	8	112	118	124	0.98[a]
			16	134	170	150	0.98[b]
			24	142	160	164	1.70[c]
Proteolytic activity, mgN/100g	70.0	ND	8	75.8	98.8	110.3	NS[a]
			16	85.1	131.8	119.4	19.16[b]
			24	94.2	111.8	112.7	NS[c]
Malting loss, %	-	-	8	10.2	14.9	9.5	0.17[a]
			16	6.2	4.6	17.3	0.17[b]
			24	7.4	12.8	14.1	0.31[c]
Malt yield, %	-	-	8	89.8	85.1	90.5	0.17[a]
			16	93.8	95.4	82.7	0.17[b]
			24	92.6	87.2	85.9	0.29[c]
Cold-water extract, %	-	-	8	15.8	17.9	20.7	0.29[a]
			16	20.5	24.6	21.0	0.29[b]
			24	21.3	23.6	24.0	0.53[c]
Hot-water extract, %	-	-	8	33.6	51.5	67.2	0.29[a]
			16	59.7	80.4	73.7	0.29[b]
			24	63.0	65.5	70.7	0.50[c]

[a, b, c] – CD at p≤ 0.05 for steeping period, malting period and interaction, respectively. Means in the same column bearing different superscripts differ significantly (P≤0.05) ND – Not detected

their association with the hulls and leaching losses in steeped water, respectively. The soluble proteins increased during dehulling and also during malting at all periods, due to dry matter loss and hydrolysis of native proteins to low molecular weight soluble proteins and increase in the enzymic activity during malting, which could further increase the free amino acid contents also. The starch content was decreasing continuously and significantly with concomittant increase in reducing sugars on progressive period of malting. This could be attributed to starch hydrolysis by endogenous amylases. The increases in starch, reducing sugars, free amino acids and soluble proteins in dehulled sample were, however, due to loss of fibre.

The IVPD was found to increase during dehulling because of removal of polyphenols along with hulls. IVSD was also found to significantly increase during dehulling, but decreased during malting. The IVPD increased to its fullest extent in grain steeped for 16 h and malted for 48 h. However, IVSD was found to decrease significantly, when steeping and malting periods increased beyond 16 h and 48 h, respectively. The diastatic and proteolytic activities increased significantly at all periods of steeping and malting. However, the most significant increase was observed with 16 h steeping and 48 h malting periods. The data on malt yield of foxtail millet showed an exact reverse trend against that of malting losses. The malt yield was maximum in 16 h steeped and 48 h germinated grains, the yield being fairly comparable to that reported by Malleshi and Desikachar (1979). The values of cold- and hot-water extracts increased during steeping and malting periods. The results were significant and comparable to those of Pathirana et al (1983) on sorghum.

The data on the effect of dehulling and malting on polyphenols, iron, phosphorus and phytate depicted in Table 2 show significant deductions in all these constituents on dehulling. The same trend was observed during malting at all periods in case of polyphenols, total iron, total phosphorus and phytate with the only exception of ionisable iron. It can be seen that only 16.7% of the total iron in whole grain was ionisable, while on malting, it got increased by about 50 to 94%, suggesting that the availability of iron in malted samples was significantly higher. The significant reductions in polyphenols, total iron, phosphorus and phytate could be partly because of their association with the hulls and increased activity of enzymes during malting. Further, the data in Table 2 show reverse

TABLE 2. EFFECT OF STEEPING AND MALTING ON POLYPHENOLS, TOTAL IRON, IONISABLE IRON, TOTAL PHOSPHORUS AND PHYTATE PHOSPHORUS

Parameter	Whole	Dehulled	Steeping period, h	Malting period, h 24	48	72	P≤0.05
Polyphenols,%	2.1	0.63	8	1.15	0.9	0.75	0.06[a]
			16	1.00	0.8	0.65	0.06[b]
			24	0.95	0.7	0.55	N.S[c]
Total iron, mg/100 g	3.0	1.9	8	2.4	2.3	2.2	0.1[a]
			16	2.2	2.1	2.1	0.1[b]
			24	2.0	1.9	1.8	NS[c]
Ionisable iron, mg/ 100 g	0.5	0.9	8	1.20	1.40	1.60	0.3[a]
			16	1.26	0.99	1.67	0.3[b]
			24	1.28	1.50	1.50	NS[c]
Total phosphorus, mg/100g	230	112	8	196	190	184	1.3[a]
			16	192	186	180	1.3[b]
			24	182	180	176	2.2[c]
Phytate phosphorus, mg/100g	110	40	8	92	74	52	1.8[a]
			16	82	66	44	1.8[b]
			24	71	54	32	NS[c]

[a, b, c] – CD at P≤0.05 for steeping period, malting period and interaction, respectively, Means in the same column bearing different superscripts differ significantly (P≤0.05)

relationship between ionisable iron and the level of polyphenol and phytate contents.

The present studies have shown that availability of iron in foxtail millet was poor. The removal or reduction of polyphenols and phytate either by extraction or grain malting or dehulling enhances iron availability.

It can be concluded that maximum acceptability and availability of nutrients from the foxtail millet could be obtained by steeping it for 16 h and malting for 48 h.

References

AOAC (1980) Official Methods of Analysis, 13th edn, Association of Official Analytical Chemists, Washington DC

Ayre CA, Anderson JA (1939) Determination of proteolytic activity in cereals. Canadian J Res 17: 239-241

Chauhan GS (1982) Studies on the processing of rice legume blends using extrusion technology. Ph.D. Thesis, Punjab Agricultural University, Ludhiana, India

Jayatissa PM, Pathirana RA, Sivayogasundarum K (1980) Malting quality of Sri Lankan varieties of sorghum, J Inst Brew 86: 18-21

Lowry OM, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin-phenol reagent. J Biol Chem 193: 265-275

Malleshi NG, Desikachar HSR (1979) Malting quality of new varieties of ragi *(Eleusine coracana)*. J Food Sci Technol 116: 149-150

Malleshi NG, Desikachar HSR (1985) Milling, popping and malting characteristics of some minor millets. J Food Sci. Technol 22: 400-403

McCready RM, Gugolz J, Silviera V, Owens RS (1950) Determination of starch and amylases in vegetables. Anal Chem 22: 1156-1158

Nelson N (1944) A photometric adoptation of Somogyi method for determination of glucose. J Biol Chem 153: 375-378

Panse VG, Sukhatme PV (1967) Statistical Methods for Agricultural Workers. 2nd edn. Indian Council of Agricultural Research, New Delhi

Pathirana RA, Sivayogasundaram K, Jayatissa PM (1983) Optimization of conditions for malting of sorghum. J Food Sci Technol 20: 108-111

Price ML, Van Scoyoc S, Butler LG (1978) A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J Agric Food Chem 26: 1214-1218

Raghuramulu N, Madhavan K, Kalyanasundaram S (1987) Manual of Laboratory Techniques, National Institute of Nutrition, Hyderabad

Ramachandra G, Virupaksha TK, Sadaksharaswami M (1977) Relcationship between tannin levels and *in vitro* protein digestibility in finger millet. J Agric Food Chem 25: 1101-1104

Rao BSN, Prabhavathi T (1978) An *in vitro* method for predicting the bioavailability of iron from foods. Am J Clin Nutr 31: 169-175

Rao BSN, Prabhavathi T (1982) Tannin content of foods commonly consumed in India and its influence on ionisable iron. J Sci Food Agric 33: 89-96

Rao UP, Deosthale YG (1988) *In vitro* availability of iron and zinc in white and coloured ragi *(Eleusine coracana)* : Role of tannin and phytate. Qual Plant Food Hum Nutr 38: 35-41

Rosen R (1957) A modified ninhydrin colorimetric analysis for amino acids. Biochim Biophys Acta 61: 10-15

Singh U, Kherdekar MS, Jambunathan R (1982) Studies on 'Desi' and 'Kabuli' chickpea (*Cicer arietinum* L) cultivars: The level of amylase inhibitor, level of oligosaccharides and *in vitro* starch digestibility. J Food Sci 47: 510-512

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Levels of Crude Proteins and Some Inorganic Elements in Selected Green Vegetables of Dar es Salaam

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Eight green vegetables commonly grown in Dar es Salaam were analyzed for their contents of crude proteins, 4 macro minerals (Mg, K, Ca and Na) and 7 heavy metals (Pb, Cd, Zn, Cu, Mn, Cr and Fe). The values of crude proteins ranged between 1.03–5.23% on fresh weight. These vegetables were found to be rich in macro minerals. The levels of Pb and Cd in some vegetables were above the permissible limits recommended by FAO/WHO. Overall data have, however, indicated that consumption of these vegetables is unlikely to pose any health hazard to the consumer, as these vegetables are good sources of some other essential elements.

Keywords: Crude proteins, Minerals, Green vegetables, Atomic absorption spectrophotometer, Heavy metals, Dry ashing.

Vegetables are eaten by human beings either raw or cooked. These plants essential as well as toxic elements and other constituents with a wide range of concentrations. While some elements are essential for good health of humans, others such as Pb, Cd and Hg are exceptionally toxic and can be tolerated only at very low concentrations and therefore, have been recognised as dangerous environmental pollutants. The toxic effects of Pb and Cd to human body through food consumption are well documented (Bonner and Bridges 1983; Reilly 1991). Evidence indicates that humans get exposed to these toxic metals through the consumption of polluted food material (Shrikanth and Raja 1991).

Rivers are being polluted by heavy metals through industrial effluents and by indiscriminate disposal of domestic wastes and sewage into the rivers, either untreated or partially treated (Paulsson 1990). Such waters, when used for agricultural purposes, can lead to a significant increase in pollutants. Some studies carried out on heavy metal pollution in Tanzania have revealed the presence of heavy metals in fishes (Mashauri and Mayo 1990) and in some species of algae plants (Wekwe 1990). Protein contents of cassava leaves *(Manihot dulcis)*, sweet potato leaves *(Ipomea babatus)* (Maeda 1977), cereals (Mtenga and Sugiyama 1977) and legume seeds (Sugiyama and Mtebe 1978) of Tanzania have also been determined.

Green vegetables form a substantial proportion of the diet for most of the low income groups of population, but there is little information on the elements and other constituents present in the vegetables grown in Tanzania. The scarcity of reliable data on the nutritional values of local foods is one of the drawbacks in solving the malnutrition in developing countries (Rand and Young 1983). This study was, therefore, aimed at determining the levels of some metals and crude protein contents in selected green vegetables grown in Dar es Salaam, Tanzania.

Sample collection : A total of 192 samples of cowpea leaves *(Vigna unguiculata)*, amaranth *Amaranthus* sp., leafy cabbage *(Brassica chinensis* L.) lettuce *(Lactuca sativa)*, pumpkin leaves *(Curcubita moschata)*, okra *(Hibiscus esculantus)*, green pepper *(Capsicum grossum)* and Chinese cabbage *(Brassica chinensis)* were obtained from Kariakoo market and from peasant farms along the banks of Sinza river during a dry season (June – November). Individual samples from these areas were bulked (2–3 kg) and transported in plastic bags to the laboratory for preparation.

Sample preparation : Stem ends and stalks were removed and the leaves were washed with distilled water to eliminate dust and dirt. They were, then, placed on sheets of paper and left to drain dry at room temperature to remove excess moisture. The samples were weighed and then dried in an oven 'Memmert' equipped with fresh air facilities at 60°C to a constant weight. Each dried sample was ground in a mortar to pass through a 60 mesh sieve. The powder was packed and stored in clean, dry, stoppered glass containers for further studies.

Determination of metals : Destruction of organic matter of the sample was done by dry ashing method (Perkin-Elmer 1982). The ashed sample

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was digested with 5 ml of 20% (v/v) AnalaR hydrochloric acid. Concentrations of metals in the ash solution were measured, using a Perkin Elmer Model 2380 atomic absorption spectrophotometer (AAS). The set up and standardization procedures for AAS and determination of metals were done as stipulated in AAS manual for analytical methods (Perkin–Elmer 1982).

Determination of crude proteins : The nitrogen content of the samples was estimated by Kjeldahl method (Ranganna 1977). The percent nitrogen, thus, obtained was multiplied by the factor 6.25 to get the percentage of proteins in the samples.

The crude protein contents of the samples (Table 1) ranged between 1.03 and 5.23% on fresh weight basis. The highest value for crude proteins was observed in cowpea leaves, whereas lettuce contained the lowest. The ranges of the crude protein contents of the vegetables analyzed were in agreement with the reported values (Ndiokwere 1984; Gross et al. 1989; Maikhuri 1991). The present results and those reported by Ifon and Bassir (1980) reveal that the crude protein contents of various green vegetables of Nigeria and Tanzania are comparable. Nevertheless, the protein contents of cowpea leaves in this study are significantly higher. As compared to the FAO report (1954) on proteins, these vegetables contained higher protein contents.

The contents of magnesium, potassium, calcium, sodium, lead, cadmium, zinc, copper, manganese, chromium and iron are also summarised in Table 1. Potassium was the predominant metal present in all vegetables. Cowpea leaves collected from Kariakoo had the highest potassium level (4.94 g/100 g), while green peppers both from

TABLE 1. CRUDE PROTEINS AND METAL CONTENTS IN VEGETABLES

Attribute	Location	Vegetables							
		Chinese cabbage	Cowpea leaves	Amaranth	Pumpkin leaves	Leafy cabbage	Green pepper	Lettuce	Okra
Crude proteins, % fresh weight	A	1.51	5.23	4.94	4.25	2.59	2.44	1.03	1.94
	B	1.58	4.89	4.81	4.33	3.83	2.63	1.07	2.09
FAO reference proteins, %		-	4.70	4.60	4.00	-	2.00	1.20	2.10
Heavy metals, mg/100g dry weight									
Lead	A	0.32	0.25	0.30	0.34	0.19	0.25	0.38	0.22
	B	0.61	0.66	0.59	0.39	0.31	0.42	0.36	0.16
Cadmium	A	0.02	0.06	0.06	0.03	0.01	0.02	0.03	0.01
	B	0.02	0.03	0.03	0.02	0.01	0.04	0.04	0.01
Zinc	A	2.38	4.36	4.08	2.77	4.18	1.90	1.49	2.43
	B	4.93	3.46	4.81	3.67	3.76	1.94	1.56	3.02
Copper	A	0.75	0.91	1.37	1.60	0.56	0.58	0.25	0.94
	B	0.49	0.85	0.72	0.94	0.50	0.84	0.58	1.39
Manganese	A	7.76	12.92	7.88	5.82	11.80	1.44	3.22	7.38
	B	8.27	6.00	15.26	12.27	11.78	1.29	6.39	6.67
Chromium	A	0.24	0.17	0.13	0.42	0.26	0.49	0.61	0.11
	B	0.08	0.12	0.07	0.48	0.24	0.74	0.34	0.13
Iron	A	12.50	16.23	13.43	9.72	24.59	22.06	13.89	19.25
	B	12.05	15.87	12.20	11.56	23.98	22.99	15.73	17.40
Macro minerals, g/100g dry weight									
Magnesium	A	0.64	2.88	3.38	1.75	1.25	0.24	0.22	0.24
	B	2.58	1.82	3.11	3.04	1.33	0.27	0.24	0.18
Potassium	A	4.34	4.93	4.03	3.80	4.78	2.46	3.86	4.47
	B	4.75	4.68	4.77	3.42	4.32	2.56	3.69	3.65
Calcium	A	4.37	4.68	4.72	2.23	4.80	0.11	0.98	4.05
	B	4.55	3.43	3.76	3.10	3.56	0.11	2.34	2.18
Sodium	A	0.43	4.79	4.74	0.15	0.45	0.17	0.15	2.72
	B	3.38	3.13	4.68	3.32	2.44	0.92	0.18	2.18

A = Samples from Kariakoo, B = Samples from Sinza.. Values represent mean of three independent determinations.
S.D. values ranged between ± 0.001 and ± 0.29 of the mean

Kariakoo and Sinza had the lowest (2.46 and 2.56 g/100 g), respectively. Calcium was the second predominant element in all vegetables. Amaranth from Kariakoo contained the highest calcium (4.72 g/100 g), while green pepper had the lowest (0.11 g/100 g). The sodium level varied from 0.15 g/100 g found in lettuce to 4.79 g/ 100 g found in cowpea leaves. Magnesium level was 0.18 g/100 g in okra and 3.38/100 g in amaranth. Magnesium, sodium, potassium and calcium were present in more than adequate levels, when compared to the Recommended Dietary Allowance (NRC/NAS 1980). Generally, the contents of potassium and calcium were comparable to those from Nigeria (Ifon and Bassir 1979) and Libya (Voegborlo 1993), while magnesium contents were slightly higher than those reported from Nigeria. However, the levels of all macro-minerals reported in this study were lower than the levels of respective metals found in similar green vegetables grown in the western part of Nigeria (Faboya 1983).

All vegetables contained low levels of manganese (1.29 - 15.26 mg/100 g), copper (0.25 - 1.60 mg/ 100 g), zinc (1.49 - 4.93 mg/ 100 g) and iron 9.72 - 24.59 mg/100 g). Among the micro-minerals, iron was predominant and exhibited greater variation among vegetables. High content of iron observed in these vegetables could be attributed to the fact that these vegetables are grown in areas surrounded by metallurgical activities, which might have significantly increased the levels of iron in the soil and the water around the plants (Qamara 1995). The iron contents in these vegetables were in the range of 3.09–53.04 mg/100 g, similar to those reported for raw leafy vegetables from Libya (Voegborlo 1993). While contents of copper and zinc obtained in this study were lower than those reported for similar vegetables by Faboya (1983), they were generally higher than those reported for Nigerian leafy vegetables (Ifon and Bassir 1979) and raw leafy vegetables of Libya (Voegborlo 1993). The contents of manganese were very much higher than those reported by Voegborlo (1993), Ifon and Bassir (1979) and Olaofe and Sanni (1988), but were lower than those reported by Faboya (1983).

The levels of chromium in these vegetables (0.08 to 0.74 mg/100 g) have been satisfactory as regards to human health, since the minimum requirement per day for adults is 0.2 mg and the tolerated amount is 10 mg per day (Schroeder 1976). The levels of lead (0.16 - 0.66 mg/100 g) and cadmium (0.01 - 0.06 mg/100 g) were mostly lower than those reported for vegetable crops grown in Metropolitan Boston and Washington (Preer et al. 1980), while the levels of lead were less than those reported for vegetables grown in New York city (Furr et al. 1976) and in southern California (Page et al. 1971).

Levels of lead in Chinese cabbage, cowpea leaves, amaranth and those of cadmium in cowpea leaves, amaranth, green pepper and lettuce were above the permitted levels in foods (FAO/WHO 1987). Due to known toxicity of these metals to human beings and animals, the World Health Organisation (WHO) had established a provisional tolerable weekly intake of 0.4 to 0.5 mg cadmium and 3 mg lead for adults (WHO 1972). It can be concluded from the above data that the concentrations of lead and cadmium in vegetables tested in this study are unlikely to pose any significant health hazards to humans and animals and the vegetables examined in this study can be consumed as beneficial dietary source of essential elements.

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References

Bonner FW, Bridges JW (1983) Toxicological properties of trace elements. In: Rose J (ed), Trace Elements in Health, Butterworth, London, pp 1-20

Faboya OOP (1983) The mineral contents of some green leafy vegetables commonly found in the western part of Nigeria. Food Chem 12: 213-216

FAO (1954) Food composition table for international use. Food and Agriculture Organisation, Nutritional Studies No. 11. Rome, Italy, pp 74-75

FAO/WHO (1987) Codex Alimentarius Commission, Report of the 17th Session, 29 June to 10 July, Rome, Italy

Furr AK, Kelly WC, Backe CA, Gutenmann WH, Lish DJ (1976) Multi-element absorption by crops grown in pots on municipal sludge-amended soil. J Agric Food Chem 24: 889-892

Gross R, Koch F, Malaga I, Miranda AF, Schoenerberger H, Trugo LC (1989) Chemical composition and protein quality of some local Andean food sources. Food Chem 34: 25-34

Ifon ET, Bassir O (1979) The nutritive value of some Nigerian leafy green vegetables - Part I: Vitamin and mineral contents. Food Chem 4: 263-267

Ifon ET, Bassir O (1980) The nutritive value of some Nigerian leafy green vegetables - Part 2: The distribution of protein, carbohydrates (including Ethanol-soluble simple sugar), crude fat, fibre and ash. Food Chem 5: 231-235

Maeda EE (1977) Solar drying of indigenous vegetables using enclosed conventional solar drier. M.Sc. Thesis, University of Dar es Salaam, Tanzania, pp 44-58

Maikhuri RK (1991) Nutritional value of some lesser known wild food plants and their role in tribal nutrition: A case study in north east India. Tropical Sci 31: 397–405

Mashauri DA, Mayo A (1990) The environmental impact of industrial and domestic waste water in Dar es Salaam, Tanzania. In: Khan MR, Gijzen HJ (eds). Environmental Pollution and Its Management of Eastern Africa, Faculty of Science, University of Dar es Salaam, Tanzania, pp 23–32

Mtenga LA, Sugiyama T (1977) A note on the amino acid composition of three selected cereals in Tanzania. University Science J Dar es Salaam University 3: 13–18

Ndiowere CL (1984) Analysis of various Nigerian foodstuffs for crude protein and mineral contents by neutron activation. Food Chem 14: 93–102

NRC/NAS (1980) National Research Council Committee on Dietary Allowances. Recommended Dietary Allowances, 9th edn. National Academy of Science Press, Washington DC pp 13–15

Olaofe O, Sanni CO (1988) Mineral contents of agricultural products. Food Chem 30: 73–77

Page AL, Ganje TJ, Joshi MS (1971) Pb quantities in plants, soil and air near some major highway in southern California. Hilgardia 41: 1–31

Paulsson BLP (1990) Developments of pollution control in Tanzania. In: Khan MR, Gijzen HJ (eds) Environmental Pollution ant Its Management in Estern Africa. Faculty of Science, University of Dar es Salaam, Tanzania, pp 3–15

Perkin-Elmer (1982) Analytical methods for atomic absorption spectrophotometry. Perkin-Elmer Corporation, Norwalk, USA

Preer JR, Sekhon HS, Stephens BR, Collins MS (1980) Factors affecting heavy metals content of garden vegetables. Environmental Pollution (Series B) 1: 95–104

Qamara J, (1995) Studies of metals as water pollutants in some rivers passing through Dar es Salaam city. M.Sc. Dissertation, University of Dar es Salaam, Tanzania, pp 35–46

Rand WM, Young VR (1983) International network of food data systems (INFOODS): Report of small international planning conference. Food Nutrition Bulletin 5: 15–23

Ranganna S (1977) Manual of analysis of fruits and vegetables products. Tata MacGraw Hill Co Ltd, New Delhi, pp 17–18

Reilly C (1991) Metal Contamination of Foods, 2nd edn. Elsevier Applied Science, London

Schroeder HA (1976) The Trace Elements and Nutrition. Faber and Faber, 3 Queen Square, London

Shrikanth R, Raja S (1991) Lead, cadmium and chromium levels in vegetables grown in urban sewage sludge in Hyderabad, India. Food Chem 40: 229–234

Sugiyama T, Mtebe K (1978) Chemical evaluation of legume seed protein in Tanzania, University Science J Dar es Salaam University 4: 11–18

Voegborlo RB (1993) Elements in raw leafy vegetables grown in Libya. Food Chem 48: 317–319

Wekwe WW (1990) A study of East African marine and freshwater algae as indicators of heavy metals pollution. M.Sc. Dissertation, University of Dar es Salaam, Tanzania pp 36–48

WHO (1972) Evaluation of certain food additives and the contaminats Hg, Pb and Cd. WHO Technical Report Series No. 505. Geneva, pp 28–35

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Studies on the Effect of Hydrocolloids on the Consistency of Tomato Ketchup

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Effect of incorporating different hydrocolloids viz., carboxy methyl cellulose, sodium alginate and gum acacia at 0.75%, 1.50% and 2.25% levels on the consistency of tomato ketchup was studied. The product gave better acceptability at 0.75% level of addition as compared to higher levels due to the product being highly viscous at higher levels of incorporation. The consistency coefficients determined from a log-log plot of shear-stress v/s shear rate data decreased with the addition of hydrocolloids as compared to control.

Keywords: Hydrocolloids, Gum acacia, Sodium alginate, Carboxy methyl cellulose, Tomato ketchup.

Tomato ketchup derives its viscosity and consistency from naturally occurring pectins in the fruits. Tomato varieties with less pectins may result in reduced consistency. The consistency can be maintained by adding polysaccharides such as starch, gum etc. In such products, galactomannans can increase the viscosity and eliminate syneresis. However, temperature fluctuations during storage may promote gelation. Gums are best employed as thickeners. Hydrocolloids are used, in special cases, to contribute towards smoother and more blend flavour in liquid, semi-solid and solid foods (Glicksman and Forkas 1967). Gums act as bodying agent as well as modify the consistency of tomato ketchup. Carboxy methyl cellulose (CMC) has a wide use in reinforcing gel strength in fruit gels (Kozmina et al. 1976). Gum acacia has been used as cloud stabilizer in orange squash and beverages (Ranganna 1977) and it inhibits the starch gelation in the manufacture of instant products (Kodet 1970). Gums are useful as non-caloric bulking agent in diabetic foods as well as low carbohydrate baked goods (Bayer 1974). Sodium alginate prevents sedimentation in fruit drinks (Krigsman 1957). Use of CMC at 0.19% and guargum at 0.09% stabilized the soy protein-based sandwich spread (Pader et al. 1959). In view of these findings, the present investigation was undertaken to study the effect of selected hydrocolloids on the consistency of tomato ketchup.

Sound and ripe tomatoes were produced from the local market, graded on the basis of colour, size and freedom from bruises/green spots. The graded fruits were thoroughly rinsed in water and the juice was extracted, using a laboratory pulper. The yield of the juice was 70% with pH 4.4 and TSS 4.55°Brix. Tomato ketchup was prepared as per the formulation using 2.5 kg tomato juice, 125 g sugar, 30 g salt and 10 ml acetic acid in an open pan and concentrated to TSS of 16.55°Brix. Acetic acid was added near finishing and the final product had a pH of 3.5. The hydrocolloids (carboxy methyl cellulose, sodium alginate, gum acacia) were added to samples of ketchup at different levels (0.75%, 1.5%, 2.25%) and mixed in a Waring blender. The samples were analyzed for consistency, shear stress, shear rate and torque at speeds, varying from 0.5 to 20 rpm at 25°C, using Brookfield Viscometer DV-II and TC 500 water bath (Brookfield Engineering Inc.). The consistency was measured using Helipath Spindle type A, recommended for viscous fluids.

The log-log plots of shear-stress v/s shear rate were drawn and consistency coefficients were calculated using intercept values. The consistency coefficients determined from log-log plots (Figs 1 and 2) of shear-stress v/s shear rate data decreased with the addition of hydrocolloids as compared to control. However, the magnitude as well as reduction in the values was highest for CMC and lowest in case of gum acacia. The values were highest at 0.75% incorporation, as compared to 1.5% levels in case of CMC and gum Acacia. A reverse effect was observed for sodium alginate. The torque values also showed similar trend. The consistency decreased with an increase in shear-rate at 0.75% and 1.5% levels of addition as compared to control, while at 2.25%, it again increased. The product gave better acceptability at 0.75% level of addition as compared to higher levels, due to the product being highly viscous at higher levels of incorporation. Among these hydrocolloids, carboxy methyl cellulose can be best employed at 0.75% level to monitor viscosity of tomato ketchup and this saves

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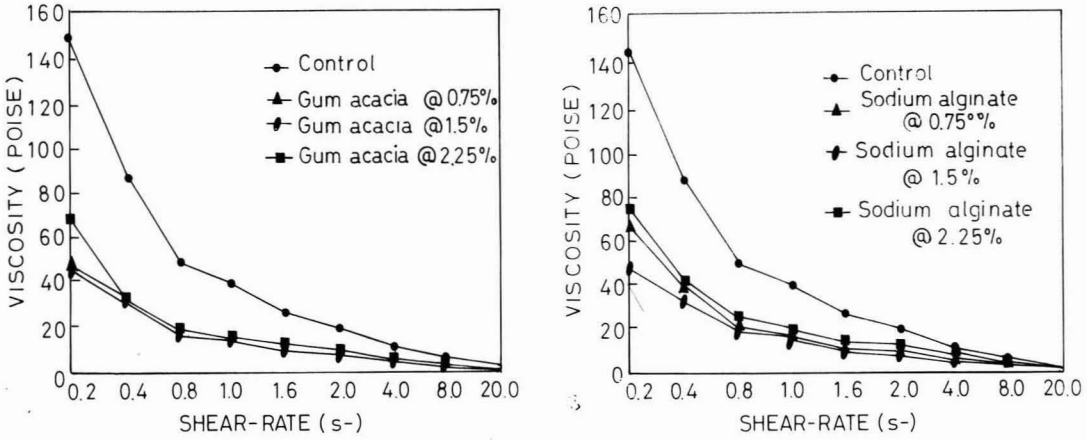

Fig. 1. Relationship between viscosity and shear rate of gum acacia and sodium alginate incorporation in tomato ketchup at different levels

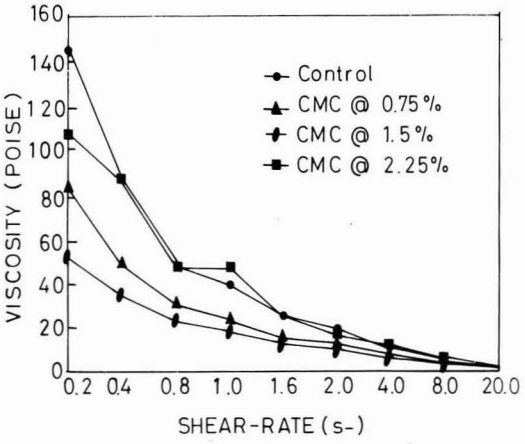

Fig. 2. Relationship between viscosity and shear rate of carboxy methyl cellulose incorporation in tomato ketchup at different levels

considerable energy and time, required for making an acceptable final product.

The use of hydrocolloids is approved by FDA as stabilizer, emulsifier, thickener, suspender, bodying agent or foam enhancer in foods (Glicksman 1969). However, these hydrocolloids are not permitted in tomato ketchup, at present, in India under the provisions of Prevention of Food Adulteration Act. It is vitally important to reconsider this matter for allowing its use in foods in India.

References

Bayer AG (1974) Low carbohydrates pastry products for diabetics. Netherlands Patent, 7509-437

Glicksman M, Forkas EH (1967) Gums in artificially sweetened foods. Food Technol 20: 58-62

Glicksman M (1969) Gum Technology in Food Industry, Academic Press, New York, pp 97-111

Kodet J (1970) Method of producing instant starch products. Czech Patent, 137, 652

Kozmina NP, Potaxina VS, Luganskaya A (1976) Change in enzyme activity during germination. Kubbope Karyana Konditerskaya Pramyshlannost. No. 10: 20. FSTA 12M 3126

Krigsman JG (1957) Alginic acid and the alginates applied to the food industry. Food Technol Australia 9 : 253-261

Pader M, Hamilton HD, Miles JJ, McCrimlisk GJ (1959) US Patent, 2, 558, 012

Ranganna S (1977) Manual of Analysis of Fruit and Vegetable Products. McGraw Hill Publ. New Delhi

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Proteose-Peptone Contents of *Mishti Doi* and Their Correlation with Browning Index

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Proteose-peptone contents were determined in samples collected at different stages during the manufacture of *Mishti Doi*. Intensity of brown colour of *Mishti Doi* and proteose-peptone contents showed a positive correlation coefficient of 0.93. Proteose-peptone contents decreased in the final product, indicating the consumption of proteose-peptone by starter culture microorganisms.

Keywords: Proteose-peptone fraction, *Mishti Doi*, Browning correlation coefficient, Concentrated milk.

Proteose-peptone, though a minor protein fraction of milk, plays very important role as an emulsifying agent, index of proteolysis, inhibitor for lipolysis and enhancement of brown colour in various dairy products. In the present investigation, changes in proteose-peptone content during the preparation of *Mishti Doi* (a fermented milk product of eastern region characterized by light brown colour, caramelized flavour and firm body) and their correlation with intensity of brown colour were studied.

Mishti Doi was prepared on laboratory scale by concentrating the buffalo milk in an open-heated pan to 20% total solids. Cane sugar was added at levels of 16.0 to 20%. The heated milk was, then, cooled and inoculated with *Streptococcus salivarius* ssp. *thermophilus* (Hansen) + *Lactobacillus acidophilus* (Russian) + *Lactobacillus delbrueckii* ssp. *bulgaricus* in 1:1:1 ratio. The inoculated milk was filled in 100 ml earthen pots, covered with aluminium foil, incubated at 42°C for 6 to 8 h and stored in refrigerator. Five trials on *Mishti Doi* were conducted and from each trial, 7 samples were collected at different stages of processing, viz., raw milk, boiled milk, concentrated milk and *Mishti Doi* (final product). These samples were analyzed for their proteose peptone contents by the turbidimetric method of Joshi and Ganguli (1968). To see the correlation between proteose-peptone contents and browning index, the different levels of proteose-peptone (0.02 to 0.70%) isolated from buffalo milk were added to raw milk before concentration so that the proteose peptone contents ranged in concentrated milk from 0.2 to 1.0%. Then, *Mishti Doi* was prepared from these concentrated milk samples. Intensity of brown colour in the final product was measured after hydrolysis of the protein with trypsin as described by Gupta (1982). Graph showing correlation between proteose-peptone contents and browning index were plotted and correlation coefficients were calculated.

Proteose-peptone content changed significantly during the preparation of *Mishti Doi* (Table 1). The proteose-peptone content for raw milk was 112.24 (5.18) mg/100g of milk. A decrease in the level of proteose-peptone content 102.16 (6.13) mg/100g of milk was observed in the boiled milk, when milk was boiled to concentrate in an open heating pan. Such change in proteose peptone contents was associated with browning reaction. Gothwal and Bhavadasan (1992) observed that added proteose-peptone enhanced browning in both cow and buffalo milks. The increase in browning by proteose-peptone can be explained in the light of information that proteose-peptone is a derivative of ß-casein and is a significant lysine residue (Ganguli et al. 1968). It was also reported that level of proteose-peptone decreased on sterilization because sterilization caused browning (Melachouris and Tuckey 1966) and browning was due to proteose-peptone fraction, interacting with reducing sugars to a greater extent (Gopalan et al. 1993). On further heating due to concentration, there was an

TABLE 1. PROTEOSE-PEPTONE CONTENTS (Mg/100 g) AT DIFFERENT STAGES OF MANUFACTURE OF *MISHTI DOI*

Sample	• Mean	SEM
Raw milk	112.24	5.18
After initial boiling	102.16	6.13
Boiled for 10 min	125.62	5.31
Boiled for 20 min	168.49	7.10
Boiled for 30 min	290.71	11.25
Boiled for 40 min or concentrated milk	365.01	12.13
Mishti Doi	200.90	9.30

• values are mean of 5 trials

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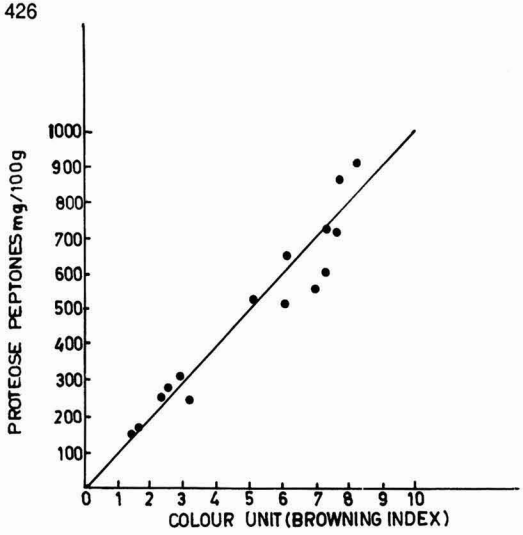

Fig. 1. Correlation between browning index and proteose-peptone contents (correlation coefficient 0.93)
1 colour unit = 1mg iodine/50ml of 10% KI solution

appreciable increase in proteose peptone content (Table 1). The decrease in proteose-peptone content on heating may be compensated by concentration of milk and at the final stage of concentration, it reached to 365.01 (12.13) mg/100 g of milk. But, a decrease in the level of proteose-peptone content to 200.90 (9.30) mg/100 g in the final product was noticed. This clearly indicated that the proteose-peptone fraction was well consumed by starter culture organisms.

A positive correlation was observed between proteose-peptone content of fresh concentrated milk, i.e., before culturing and intensity of brown colour in *Mishti Doi* (Fig. 1) with correlation coefficient being 0.93 (significant at $p < 0.01\%$ level).

It may be concluded that proteose-peptone contents determine the intensity of brown colour in the final product. It is also suggested that in place of caramel, the proteose-peptone, which is a natural ingredient of milk can be added to milk to get the desirable brown colour in *Mishti Doi*.

References

Ganguli NC, Joshi VK, Bhalerao VR (1968) Heat-induced changes in proteose-peptone level in milk. Milchwissenschaft 23: 754-756

Gopal S, Ramabadran S, Srinivasan A (1993) Non-enzymic browning studies of milk proteins. Paper presented at 3rd International Food Convention. Association of Food Scientists and Technologists (India), Mysore, p 91

Gothwal PP, Bhavadasan MK (1992) Studies on browning characteristics in dairy products. Indian J Dairy Sci 45: 146-150

Gupta KS (1982) Studies on the effects of different degrees of browning on the chemical composition of sweetened *khoa* - Some aspects. M.Sc. Dissertation, Kurukshetra University, Kurukshetra

Joshi VK, Ganguli NC (1968) A simple and rapid turbidimetric method for estimation of proteose-peptone, proteose and peptone in milk. Indian J Dairy Sci 21: 15-20

Melachouris NP, Tuckey SL (1966) Denaturation of whey protein in milk heated at high temperature for short time. J. Dairy Sci 49: 1155-1158

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Effect of Using Iron Utensils *vis-a-vis* Teflon-coated Non-stick Wares on Ionisable Iron Content of Traditional Vegetarian Foods

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Use of iron utensils was assessed for bioavailability of contaminated iron during cooking processes with teflon-coated wares as controls. Experiments were undertaken, using 34 commonly used recipies for which iron utensils are used traditionly. There was significant increase in total ionisable iron as well as total iron content due to use of iron utensils ($p<0.05$). 'Bioavailable contaminated iron' calculated as % increase in ionisable iron to increase in total iron, was found to be highest in foods prepared by roasting (8-12%), followed by shallow pan frying (6-8%) and baking (4-6%). Further, when 'Bioavailable iron content' was computed in composite diets for moderately active men and women with use of iron utensils, it was found to be increased by 1.8-2.2 mg/day.

Keywords: Iron utensils, Contaminated iron, Ionisable iron, Common vegetarian foods, Teflon-coated non-stick wares.

Iron deficiency anaemia still remains an important health problem in spite of the worldwide efforts through fortification and prophylaxis programs. Poor iron bioavailability has been extensively reported as the prime factor in considering the aetiology of iron deficiency anaemia in developing countries (Mehta 1982; Kalpana et al. 1986; Hallberg et al. 1983; Shukla 1982). This has been attributed to the fact that a major share of energy comes from plant foods, which contain non-heme iron, known to have low bioavailability (Lyrisse 1975). Vegetarianism in countries like India has been adopted mainly due to socio-economic and cultural factors rather than the health attributes of vegetarian diets. Habitual intakes of trace metals like iron in Indians are 20-30 mg in men and 10-15 mg in women, indicating sub-normal levels (Chiplonkar et al. 1993). It is not practicable to substitute part of the plant foods by animal foods due to their higher cost and therefore, alternative approaches of increasing the total bioavailable iron intakes are necessary as strategies for combating iron deficiency anaemia.

Use of iron pots increases the total iron content of food to a variable extent, depending on the type of preparation of the food (Hallberg et al. 1983; Mistry et al. 1988). However, there are only limited studies reported on the bioavailability of contaminated iron and these too appear to report contradictory results (Guiro and Hersberg 1988; Hallberg and Rossander 1981; Narsinga Rao and Prabhavati 1981). This may be due to the fact that apparently, % bioavailability of cotaminated foods is lower than the uncontaminated foods. Besides, most of these studies are based on raw foods and changes in bioavailability, due to cooking in iron utensils, have not been systematically investigated. The present study aims at assessing the changes in ionisable iron due to cooking in iron utensils for a variety of traditional food preparations and precisely compares ionisable iron contents of foods cooked in iron *vis a vis* teflon coated non-stick wares and establishes superiority of the former over the latter, as a strategy to improve the net ionisable iron of vegetarian diets.

Selection of foods and their preparations : Thirty four common recipies, which are cooked traditionally using iron utensils, were selected. According to the type of cooking process : 9 of these required baking, 14 used shallow pan-frying, 7 involved deep-pan frying and one fell under condensation category. Each of the food item was cooked in iron utensil or teflon coated non-stick ware in 5 replicate sets. Use of glass distilled water and avoidance of any other source of iron contamination was strictly observed. All the cooking was done in metabolic ward kitchen of the Institute.

Analysis of samples : For each of the 34 foods with 10 samples per food preparation, 340 food samples were generated. Samples were analyzed for % moisture, dried in an oven set at 60°C, until constant weight, powdered and stored in cold cabinet, until further analysis.

Estimation of ionisable iron was done as per the method of Narsinga Rao and Prabhavati (1978). Briefly, the technique involved digestion of food sample under simulated gastric conditions viz.,

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pepsin–HCl buffer (pH 1.35), gradually changing the pH to 7.5 and estimating iron in the supernatant after centrifugation.

Total iron in the food samples was estimated as per the AOAC (1990) procedure, which involves dry ashing of sample in muffle furnace at 550°C, preparation of acid extract using concentrated HCl and spectro-photometric determination of iron, using dipyridyl reagent at 510 nm.

Statistical methods : Mean and standard deviation between replicates for each of the sample was calculated. Paired 't' test was used to test significance of differences between test and control values separately for each sample. Differences were considered statistically significant ($P < 0.05$).

Based on the total ionisable iron as mg per 100 g food, % ionisable iron values were calculated as 100 x (Total ionisable iron in 100 g food/total iron in 100 g food). Bioavailable contaminated iron (BCI) was calculated as 100 x (Increase in total ionisable iron/increase in total iron).

Table 1 gives the values of the estimates of ionisable iron, total iron and bioavailable contaminated iron, classified according to the type of product.

Baked products included a variety of routine Indian pancakes, using cereal flours and some special products (viz., items No. 7, 8, and 9). Percent values of ionisable iron of samples cooked in teflon-coated wares were in the range 6.23–22.29. Total ionisable iron also showed a wide range of 0.35–1.15 mg/100 g food in these samples. When these values were compared with values obtained for the samples cooked in iron utensils, there was a variable, but significant increase in total ionisable iron as well as total iron content.

Shallow pan-fried products included different kinds of vegetable and legume preparations. These products also exhibited results similar to baked products in terms of increase in ionisable and total iron as also for variability of the values. Values of BCI were the highest for roasted and condensed products (8–12%), followed by shallow pan-fried products (6–8%), and baked products (4–6%) (Fig. 1). Deep-fried products showed the least values for BCI. Changes between samples cooked in iron utensils and non-stick wares were not statistically significant for this category.

In order to assess the practical significance of these results, an exercise of computing the total ionisable iron with or without the use of iron utensils for composite diets was done, based on the ingredients of typical vegetarian diets for adult man and woman under moderately active category and thus representing the diet of socio-economic class, engaged in manual work. It was seen that use of iron utensils could be helpful in providing 2.15 mg of extra ionisable iron for men and 1.84 mg for women, promising its use as simple and traditional method of moderate iron fortification.

Iron deficiency anaemia has turned out to be one of the most persistent problems of serious concern (FAO 1992). Strategies to be evolved for improving iron status should be simple, traditionally acceptable and affordable in order to have long term and sustainable effects. Iron fortification and its supplementation at prophylactic or therapeutic level has its focus on specific groups. Uncontrolled use of these iron supplements may lead to iron over-load, since bioavailability of iron salts is much high as compared to habitual cereal-based diets. Practice of cooking food using iron utensils is vanishing even among lower socio-economic classes, due to increasing use of aluminium in place of iron and brass utensils. Systematic investigations are of value to examine its usefulness.

In vitro techniques for bioavailability testing are very useful tools at initial stages of investigations on bioavailability, particularly when a large number of samples are needed to be handled. The technique used for the present study of measuring ionisable iron has been shown to have high correlation ($r = 0.94$) with human absorption values as well as *in vitro* method, using radio isotopes (Hallberg et al. 1983).

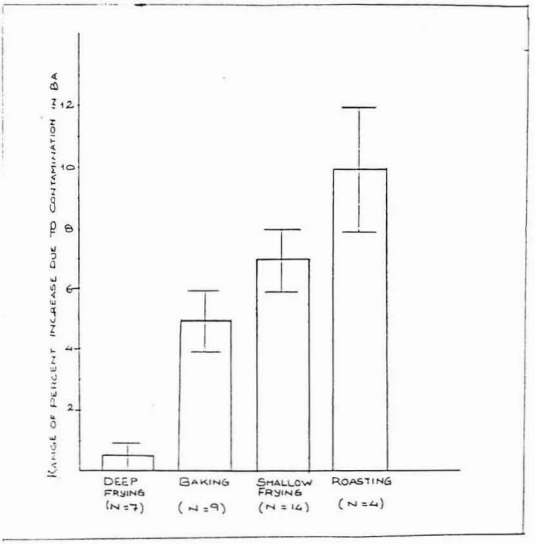

Fig. 1. Increase in bioavailability (BA) due to contamination

TABLE 1. IONISABLE AND TOTAL IRON IN FOODS COOKED IN IRON AND NON-STICK WARES

Food sample	Dry matter, g/100g	Iron utensil		Non-stick ware		% bioavailability of contaminated iron BCI 1-3/ 2-4 x 100	% increase in ionisable iron 1-3/3 x 100
		Ionisable iron, mg %	Total iron, mg %	Ionisable iron, mg %	Total iron, mg %		
Baked products		(1)	(2)	(3)	(4)		
Chapati	70.2	0.70	8.92	0.53	5.15	4.51	32.1
Phulka	74.7	0.47	9.30	0.35	5.60	3.31	31.4
Jowar *roti*	63.5	1.11	7.50	0.94	4.60	5.72	17.0
Bajra *roti*	58.3	0.55	7.70	0.36	3.50	4.43	52.8
Rice *roti*	61.1	0.48	4.60	0.39	2.80	4.74	23.1
Ragi *roti*	64.4	0.60	6.25	0.44	3.70	6.12	36.4
Dosa	54.8	1.27	7.40	1.15	5.15	5.32	11.3
Thalipeeth	69.6	1.07	9.63	1.02	8.05	4.35	3.9
Puran poli	69.8	1.08	11.70	0.89	7.50	4.25	20.2
Shallow pan-fried products							
Toast	77.5	1.07	10.25	0.82	6.80	7.45	31.7
Cutlet	40.9	0.84	9.18	0.52	5.00	7.72	61.5
Pithale	27.6	1.24	11.30	0.71	5.15	8.56	74.6
Greengram *dhal*	58.0	1.82	21.75	1.33	16.00	8.47	36.8
Moth beans	25.5	0.65	8.20	0.31	4.10	7.85	103.2
Whole *Bengal*gram	33.2	1.69	14.50	0.92	6.65	9.27	83.7
Spinach	30.5	0.90	12.40	0.43	6.62	8.12	109.3
Fenugreek	44.6	0.99	15.80	0.50	9.10	7.26	98.0
Snake gourd	29.8	0.75	8.10	0.14	1.10	9.72	435.7*
Bottle gourd	31.4	0.39	5.80	0.01	0.90	6.71	330.0*
Brinjal	19.7	0.35	7.83	0.01	0.73	4.89	350.0*
Cluster beans	34.9	0.32	7.10	0.10	2.00	4.31	320.0*
Potato+ onion	41.7	0.16	5.88	0.004	1.85	3.55	375.0*
Rice flakes	59.5	1.52	13.98	1.48	12.47	2.01	2.03
Deep-fried products							
Puri	73.7	0.89	5.81	0.89	5.80	1.00	0.012
Bhajia	61.5	0.84	5.98	0.84	5.85	0.89	0.14
Chakali	81.7	1.10	9.40	1.09	8.80	1.51	8.35
Gulegule	64.8	0.89	6.90	0.89	6.90	-	0.03
Bundi laddoo	84.6	0.71	5.10	0.69	5.00	0.40	0.06
Shankarpali	81.1	0.29	3.07	0.29	3.04	0.40	-
Karanji	83.6	0.20	2.45	0.20	2.45	0.05	0.10
Roasted products							
Salted groundnut	99.5	0.90	7.30	0.49	3.30	10.22	83.70
Rawa laddoo	87.3	0.22	3.14	0.03	1.03	8.81	633.30*
Til *chikki*	94.6	1.96	14.30	1.65	11.30	10.15	18.20
Condensed products							
Khoa	73.7	0.47	4.07	0.002	0.27	12.32	235.00*

* Values greater than 200

Controverssy has arisen among the reported results about contaminated iron (Hallberg and Rossander 1981; Guiro and Hercberg 1988). One of the reasons to report the negative effect of iron contamination on iron bioavailability may be that % ionisable iron values in contaminated food are lower than the uncontaminated counterparts. The present results also have shown similar trend, when % ionisable iron is taken into account as the only parameter. A closer look at the data, however, reflects the fact that the values of total ionisable iron were higher in iron ware samples than the non-stick ware samples. However, simultaneous increase in total iron was more in magnitute in iron ware samples than samples cooked in non-stick wares. This resulted in reduction of the estimate of % ionisable iron.

Present results being based on estimate of *in*

vitro assay, it is difficult to predict what should be the level of iron, if iron utensils are used. But, these results indicate that there will be 46–51% rise in the bioavailable iron, if iron utensils are used. Considering the wide prevalance of mild anaemia particularly in women, this strategy may prove helpful. However, the deleterious effect of iron utensil on lipid oxidation needs to be examined before its implementation. These results also indicate large variability within and between type of cooking, which involved different types of iron utensils for bioavailable iron as well as its percent bioavailability.

It is now well accepted that bioavailable intakes rather than gross intakes of trace metals are important in trace metal nutrition and present results are of promise in terms of increasing the bioavailable iron intakes through use of iron utensils from individual foods as well as composite diets.

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References

AOAC (1990) Official Methods of Analysis, 15th edn. Association of Official Analytical Chemists, Arlington, Virginia

Chiplonkar SA, Agte VV, Gokhale MK (1993) Copper and zinc intakes in young men and women. Indian J Med Res 98 (B) : 283-289

FAO (1992) Expert Committee Report, Meeting During International Conference on Nutrition, Rome

Guiro A, Hercberg S (1988) Iron exchangeability from pearl millet and Senegalese pearl millet meals. Nutr Rep International, 38(2): 231-238

Hallberg L, Rossander L (1981) Absorption of iron from western lunch and dinner meals. Am J Clin Nutr 35(3): 502-509

Hallberg L, Rasmussen EB, Rossander L, Suwanik R (1983) Iron absorption from Asian meal containing contaminated iron. Am J Clin Nutr 37: 2722-277

Kalpana A, Pooramma K, Saraswathi G (1986) Bioavailability of dietary iron - A Review. Arogya - A Journal of Health Science. XIII, 11-16

Lyrisse M (1975) Bioavailability of Iron in Foods. In : Taylor (ed), Proceedings of 13th International Congress of Nutrition. pp 519-523

Mehta BC (1982) Widespread iron deficiency anaemia in India - Its causes. Ind J Med Sci 36(5/6): 108-112

Mistry AN, Brittin HC, Stoecker BJ (1988) Availability of iron from food cooked in iron utensil determined by *in vitro* method. J Food Sci 53(5) : 1546-1548

Narsinga Rao BS, Prabhavati TC (1978) An *in vitro* method for predicting the bioavailability of iron from foods. Am J Clin Nute 31: 169-175

Narsinga Rao BS, Prabhavati TC (1981) Contaminated iron in foods and its bioavailability predicted by *in vitro* method. Ind J Med Res 74: 37-42

Shukla PK (1982) Nutritional Problems of India. Prentice Hall of India, New Delhi, pp 180-193

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Influence of Pickling Formulation and Storage on the Quality of Chicken Cut-up Parts

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Cooked chicken cut-up parts were pickled in three types of formulations and evaluated for quality on storage. Chicken meat pickled in solution III recorded ($P<0.01$) lower pH, TBA and aerobic mesophilic counts (log/g) and higher ERV values, followed by chicken meat pickled in solution I. Storage ($P<0.01$) reduced the pH, ERV and increased the TBA values and aerobic mesophilic counts of pickled meat. Scores for flavour tenderness and overall acceptance were ($P<0.01$) higher in chicken meat pickled in solution III, All through, the organoleptic scores decreased ($P<0.01$) during storage, but were superior ($P<0.01$) to the scores of fresh cooked chicken meat.

Keywords: Chicken cut-up parts, Pickling formulation, Chemical composition, Storage, Organoleptic characters.

The spent chicken meat, which is considered as tougher than broiler meat, has to be utilized in an appropriate way to suit the taste of consumers. Pickling is one of the most popular methods of food preservation, known for centuries. However, information on pickling of poultry meat is scanty. Therefore, the present investigation was undertaken to evaluate the quality of spent chicken meat with different formulations at different storage periods.

Four samples, each consisting of 39 chicken cut-up parts of equal proportions of breat, thigh and drumsticks were utilized for this experiment. All the parts were steam-cooked at 3.41 kg/cm^2 for 15 min. before pickling them. Initially, three cooked parts, each one of breast thigh and drumstick were utilized for evaluating the qualitative characteristics on fresh basis. From the remaining 36 cut-up parts, 12 parts for each treatment (formulation) were allotted at random, comprising equal number of breast, thigh and drumsticks. For each treatment, four labelled wide mouthed sterilized glass bottles of 500 ml. capacity were used and for each bottle, each one of breast, thigh and drumstick were allotted. After the pickling process, the bottles were stored at 32±3°C and evaluated for quality parameters at 20, 40, 60 and 80 days.

Three types of pickling solutions were prepared. Solution I consisted of 400 ml mustard oil and 250 ml synthetic vinegar. Solution II consisted of 400 ml groundnut oil and 250 ml synthetic vinegar. Solution III consisted of 400 ml groundnut oil, 159 ml synthetic vinegar and 4% citric acid (100 ml). Each formulation was added to 1000 g of chicken cut-up parts and spice mix, consisting of 80 g salt, 50 g chilli powder, 12 g black pepper, 7 g cumin, 1.5 g cloves, 10 g anise, 10 g caraway, 10 g garlic, 15 g dry ginger and 0.2 g sodium nitrate.

Pickling : The ground ingredients were added to the respective mixture of oil and acids and the mixture was boiled for 2 min and simmered. The respective pickling solutions were transferred into labelled bottles, containing all the three cut-up parts. The bottles were screw-capped and stored at ambient temperature (32 ± 3°C). At the end of the respective storage periods, the pickled part as well as the solution was used for qualitative evaluation.

pH and ERV of pickled meat were estimated as per the methods described by Jay (1964). Estimations of moisture, crude protein and ether extractives were done as per AOAC (1980) methods. Thiobarbituric acid values of meat were determined according to the procedure of Wittee et al (1980). Total aerobic mesophilic counts of meat were estimated as per the technique of Chestnut et al (1977) and of pickling solutions as per the method of Indian Standards Institution (1962). Organoleptic evaluation of colour, flavour, juiciness, tenderness and overall acceptability was made on a 9-point Hedonic scale by a 5 member habitual meat consumers. The data obtained were subjected to statistical analysis as per the methods, described by Snedecor and Cochran (1968).

Physico-chemical characteristics: Chicken meat pickled in solutions I and III had lower ($P<0.01$) pH values. Presence of mustard oil, vinegar, citric acid in the solutions might be responsible for lowering the pH (Table 1). The pH of pickled meat decreased significantly ($P<0.01$) from 20th day. These were in agreement with the findings of Singh et al (1982) and Singh and Pads (1984) in quail pickle. Penetration of acetic acid into the musculature may be responsible for such decrease

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TABLE 1. EFFECT OF PICKLING FORMULATION AND STORAGE (32±3°C) ON THE pH, ERV, TBA AND AEROBIC MESOPHILIC COUNTS OF CHICKEN MEAT AND SOLUTIONS

Character	Storage periods, days	Pickling formulations		
		Sol I	Sol II	Sol III
pH	0	5.81^{aC} ±0.12*	5.81^{aC} ±0.12	5.81^{aC} ±0.12
	20	4.87^{bB} ±0.04	5.03^{aB} ±0.03	4.79^{bB} ±0.03
	80	4.66^{aA} ±0.02	4.76^{aA} ±0.02	4.59^{bA} ±0.04
ERV, ml	0	32.00^{aC} ±0.70	32.00^{aC} ±0.70	32.00^{aC} ±0.70
	20	25.90^{bB} ±0.53	25.25^{bB} ±0.49	27.22^{aB} ±0.25
	80	11.97^{bA} ±0.02	11.02^{bA} ±0.17	12.12^{aA} ±0.12
TBA values	0	0.25^{aB} ±0.03	0.25^{aC} ±0.03	0.25^{aB} ±0.03
	20	0.31^{bB} ±0.01	0.43^{aB} ±0.05	0.39^{abB} ±0.03
	80	2.12^{cA} ±0.02	3.49^{aA} ±0.04	3.06^{bA} ±0.05
Aerobic	0	2.74^{aC} ±0.07	2.74^{aB} ±0.07	2.74^{aC} ±0.04
mesophilic	20	2.34^{bB} ±0.13	2.61^{aB} ±0.08	1.69^{cB} ±0.37
counts, log/g	80	3.50^{aA} ±0.01	3.55^{aA} ±0.01	3.47^{aA} ±0.02
pH of pickling	0	3.59^{aB} ±0.01	3.64^{aB} ±0.01	3.57^{aB} ±0.01
solutions	20	4.14^{bA} ±0.05	4.33^{bA} ±0.06	4.03^{cA} ±0.02
	80	4.24^{bA} ±0.04	4.36^{aA} ±0.07	4.13^{cA} ±0.01
Aerobic meso-	0	1.43^{aC} ±0.04	1.51^{aC} ±0.04	1.39^{bC} ±0.05
philic counts	20	2.01^{aB} ±0.03	2.07^{aB} ±0.07	1.92^{bB} ±0.06
of solutions, log/g	80	3.26±0.01	3.34±0.01	3.23±0.01

Means bearing the same small letter row-wise and capital letter column-wise within each criterion do not differ significantly (P<0.01).

* Standard error

(Singh and Panda 1984). Similar to the findings of Singh et al (1982), the pH of solutions increased significantly (P<0.01) during 20 and 40 days of storage and remained almost constant upto 80 days. Chicken meat pickled in solution III yielded significantly (P<0.01) higher ERV, which may be due to the lower microbial growth in the solution and meat (Table 2). Storage significantly decreased the ERV values of pickled meat, which may be due to the microbial growth (Table 1) and consequent breakdown of muscle proteins. Chicken meat pickled in solution I recorded significantly (P<0.01) lower TBA values, which may be due to the presence of mustard oil, which is less prone to rancidity. While the TBA values of pickled chicken meat had not significantly increased at 40 days of storage, further storage upto 80 days had significantly (P<0.01) increased these values. This is in agreement with the finding of Charoenpong and Chen (1980).

Aerobic mesophile : Significantly (P<0.01) lower mesophilic counts were recorded in chicken meat pickled in solutions I and III (Table 2) and as well as in solutions of I and II. The preservative action of mustard oil in solution I and citric acid and vinegar in solution III, was responsible for maintaining lower pH values of pickled meat throughout the storage period of 80 days and suppressed the growth of micro-organisms, significantly (P<0.01). The counts decreased significantly (P<0.01) on 20 days of storage and increased further upto 60 days and became almost static upto 80 days. Fairly low counts (3.5 log) even on 80 days of storage were due to the inhibitory actions of the pickling solutions and these findings agree with those of Singh and Panda (1984) and Charoenpong and Chen (1980). Unlike in meat, there were significant (P<0.01) and gradual increases of counts in solutions upto 80 days of storage.

Chemical composition : Pickled samples, irrespective of formulation, had significantly (P<0.01) lower moisture % and higher (P<0.01) protein % and ether extractives than the fresh cooked sample. Storage of pickled meat from 20 days to 60 days had caused significant (P<0.01), but gradual decrease in moisture content and increases in proteins and

TABLE 2. EFFECT OF PICKLING FORMULATION AND STORAGE (32±3°C) ON THE CHEMICAL COMPOSITION AND ORGANOLEPTIC CHARACTERS OF CHICKEN MEAT

Character	Storage periods, days	Pickling formulations		
		Sol I	Sol II	Sol III
Moisture, %	0	64.46^{aA} ±0.28*	64.46^{aA} ±0.28	64.46^{aA} ±0.28
	20	62.51^{aB} ±0.20	61.95^{bB} ±0.21	61.00^{cB} ±0.03
	80	50.41^{bC} ±0.26	51.85^{cC} ±0.27	46.57^{aC} ±0.31
Ether	0	11.59^{aA} ±0.26	11.59^{aA} ±0.25	11.59^{aA} ±0.25
extractives, %	20	12.21^{aB} ±0.18	12.20^{aB} ±0.19	12.20^{aB} ±0.35
	80	14.09^{bC} ±0.29	13.86^{aA} ±0.36	14.79^{cC} ±0.22
Proteins, %	0	22.38^{aA} ±0.20	22.38^{aA} ±0.20	22.38^{aA} ±0.20
	20	23.52^{aB} ±0.27	23.52^{aB} ±0.32	23.69^{aB} ±0.17
	80	27.29^{bC} ±0.31	26.77^{aC} ±0.26	28.63^{cC} ±0.31
Colour	0	5.13^{aC} ±0.02	5.14^{aC} ±0.02	5.13^{aC} ±0.04
	20	7.02^{cA} ±0.02	7.32^{bA} ±0.03	7.39^{bA} ±0.02
	80	6.22^{bB} ±0.02	6.12^{aB} ±0.02	6.18^{abB} ±0.02
Flavour	0	5.16^{aC} ±0.02	5.17^{aC} ±0.02	5.17^{aB} ±0.02
	20	7.02^{aA} ±0.02	7.32^{bA} ±0.03	7.39^{bA} ±0.02
	80	6.52^{aB} ±0.03	6.79^{bB} ±0.02	7.30^{cA} ±0.02
Juiciness	0	6.55^{aB} ±0.06	6.56^{aA} ±0.07	6.54^{aB} ±0.05
	20	6.40^{aC} ±0.06	6.54^{bA} ±0.06	6.34^{aC} ±0.06
	80	5.76^{bA} ±0.06	6.13^{cB} ±0.05	5.69^{aA} ±0.04
Tenderness	0	5.14^{aC} ±0.06	5.14^{aB} ±0.05	5.14^{aC} ±0.06
	20	6.27^{bB} ±0.08	6.15^{aB} ±0.08	6.33^{cB} ±0.06
	80	6.55^{bA} ±0.07	6.34^{aA} ±0.07	7.15^{cA} ±0.08
Overall	0	5.42^{aC} ±0.08	5.42^{aC} ±0.07	5.42^{aC} ±0.08
acceptability	20	6.69^{bA} ±0.09	5.92^{aA} ±0.07	7.13^{cA} ±0.05
	80	6.48^{aB} ±0.08	6.65^{bB} ±0.04	6.95^{cB} ±0.06

Means bearing the same small letter row-wise and capital letter column-wise within each criterian do not differ significantly (P<0.01).

* Standard error

ether extractives. Reduction of moisture content in the chicken meat on pickling and during further storage has contributed to the higher levels of proteins and ether extractives of pickled meat (Singh and Panda 1984).

Organoleptic scores : Pickling significantly (P<0.01) increased the colour scores of meat in all formulations. Meat pickled in solution I gave higher (P<0.01) colour scores, followed by those in solutions III and II. The initial increased colour scores, however, got reduced on storage in all the formulations, but at a lesser phase in solution I (Table 2). This might be probably due to the low oxidative fading of pickled meat. Similar to the present findings, Singh et al (1982) and Singh and Panda (1984) also observed gradual decreases of colour scores in quail pickle, as the age of the product increased. Cut-up parts pickled in solution III had the highest (P<0.01) flavour scores, followed by those in solutions II and I. Pickled meat had higher (P<0.01) flavour scores at all storage periods than the fresh cooked meat, which might be due to the presence of additives specially salt and spices, which have flavour-enhancing quality (Sink 1979).

Pickled meat yielded significantly (P<0.01) lower juiciness scores than the fresh cooked meat, which might be due to the dehydration effect of acids, resulting in lowered water holding capacity. Significant decline in the juiciness score was noticed at 40 days of storage and thereafter, the reduction in scores was slow upto 80 days of storage. These results agree with the findings of Singh et al (1982).

Chicken meat pickled in solution III gave significantly (P<0.01) higher tenderness score followed by those in solutions II and I. Similar to the results of Charoenpong and Chen (1980), pickled meat significantly gave higher tenderness scores than the fresh cooked meat and further storage too had caused an increase in the scores. This might be due to the acidity of the pickling solutions and ageing effect on storage. The superior (P<0.01) overall acceptability rating in meat pickled in solution III may be the reflection of higher flavour and tenderness scores. Although samples stored for 80 days recorded lesser (P<0.01) scores than the 20 days stored samples, the scores were well above (P<0.01) the scores of fresh cooked meat.

Based on the present findings, it may be concluded that pickling of chicken meat can be done in both mustard oil and groundnut oil combinations and the product can be stored upto 80 days at ambient temperature.

References

AOAC (1980) Official Methods of Analysis 13th edn. Association of Official Analytical Chemists. Washington DC

Charoenpong C, Chen TC (1980) Qualities of pickled chicken gizzards as affected by salt and vinegar. Poult Sci 59: 537-542

Chestnut CM, Enswiler BS, Kotaula AW, Young EP (1977) Bacteriological quality of ingredients used in ground beef manufacture. J Animal Sci 4: 213-217

ISI (1962) Methods of Test of Dairy Industry. IS 15-1479 Bacteriological Analysis of Milk. Indian Standards Institution. Manak Bhavan, New Delhi

Jay MJ (1964) Release of aquous extracts by beef homogenates and factors affecting release volume. Food Technol 18: 129-131

Singh RP, Panda B, Kulshretha SB (1982) Preparation and keeping quality of pickled quail meat. J Food Sci Technol 19: 159-160

Singh RP, Panda B (1984) Preparation and storage stability of quail pickle. Poult Sci 19: 203-206

Snedecor GW, Cochran WG (1968) Statistical Methods, 6th edn. Oxford and IBH Publishing Co, Calcutta

Sink JD (1979) Meat flavour factors influencing the flavour of muscle foods. J Food Sci 44: 1-5

Witte VC, Krause GF, Bailey ME (1970) A new extraction method for determining 2-thiobarbituric acid values for pork and beef during storage. J Food Sci 35: 582-585

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Frozen Storage Performance of Edible Oyster *Crassostrea madrasensis* (Preston)

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Frozen storage performance of edible oyster *(Crassostrea madrasensis)* at - 18°C for 150 days was assessed by biochemical and microbiological parameters. During frozen storage, decrease in moisture, proteins and glycogen were noticed. Among the freshness parameters, total volatile bases, thiobarbituric acid number, free fatty acids increased, whereas alpha amino nitrogen decreased. Counts of viable organisms, staphylococci, motile aeromonads, total coliforms and faecal indicator organisms decreased with period of storage. Oyster meat kept well for 150 days at - 18°C without adversely affecting the keeping quality.

Keywords: Edible oyster, Frozen storage, Freshness parameters, Microbial quality, Keeping quality.

In recent years, processing of bivalve molluscs have undergone extensive development, because of the increasing demand in overseas countries for such delicacies. Oysters are one of the most valuable among molluscs found along Indian coast. Proper technology of oyster meat processing to satisfy consumer taste and meet quality standards of international market is a critical area requiring immediate attention. During frozen storage, oyster meat undergoes changes in chemical, microbial and organoleptic characteristics (Llobrera et al. 1986; Jeong et al. 1990; Abraham et al. 1994). Considering the importance of oyster meat as export commodity, it is necessary to establish the keeping quality during frozen storage. In an earlier study, the sensory quality and oxidative rancidity of farmed edible oyster during frozen storage were compared (Abraham et al. 1994). The present communication describes the biochemical and microbiological qualities of *Crassostrea madrasensis* during storage at - 18°C for 150 days.

Farmed edible oysters *(Crassostrea madrasensis)* were collected alive from the Tuticorin centre of Central Marine Fisheries Research Institute, Tuticorin, India. The oysters were depurated in running water for 24 h and shucked by placing in boiling water for 5 min. The picked meat was packed (300 g/pack) in low density polythene filmic bags and frozen at - 40°C for 90 min in contact plate freezer (Abraham et al. 1994; Balasundari et al. 1994). At monthly intervals, samples were randomly drawn in duplicate, packed in polythene pouches, thawed in running water (30±1°C) for 90 min and thaw drip was calculated (Mishra and Srikar 1989).

Moisture, proteins, fat and titrable acidity were determined according to AOAC (1975) methods. Glycogen content was estimated by using anthrone reagent (Carroll et al. 1956). The total volatile base-nitrogen (TVB-N) and trimethylamine-nitrogen (TMA-N) were determined by the method of Beatty and Gibbons (1937). The method of Pope and Stevens (1939) was followed for the determination of alpha amino nitrogen (AAN). Thiobarbituric acid (TBA) and free fatty acids (FFA) were determined by the methods of Woyewoda and Ke (1979) and Ke et al (1976), respectively. The pH of oyster meat was measured directly using a combined electrode pH meter, after blending 10 g meat in a mortar with 50 ml distilled water. APHA (1976) methods were followed for the enumeration of total plate counts (TPC), total coliforms (TC), faecal coliforms (FC), *Enterococcus faecalis* counts (EFC) and staphylococcal counts (SC). Enrichment methods were followed for the detection of *salmonellae* and *Vibrio cholerae*. Motile aeromonad counts (MAC) were enumerated by the method of Palumbo et al (1985). A simple correlation was used to correlate the storage days with various parameters and among parameters.

The physico-chemical and microbiological quality characteristics of oyster meat during frozen storage are presented in Table 1. The moisture content of *C. madrasensis* decreased significantly ($P<0.05$) during 150 days of storage, as noted earlier in other bivalves (Mishra and Srikar 1989). The decrease was, however, insignificant during the first 60 days of storage. The fat content of oyster meat fluctuated between 2.4 and 2.5% during storage ($r = -0.097$; $P>0.05$). The decrease in protein level was significant ($P<0.01$), indicating the breakdown of proteinaceous matter of oyster meat during storage. Oysters are known to contain high

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amounts of glycogen, which vary with season and the condition of the material. Glycogen content decreased significantly (P<0.05) from 6.5 to 5.8%, which accounted for a reduction of slightly above 10% during storage. These results corroborate with the observations of Mishra and Srikar (1989). The decrease may be attributed, to some extent, to the breakdown of glycogen by glycolytic enzymes in oyster muscle. Further loss through thaw drip could not be ruled out.

The pH values of live oyster meat were observed in the range of 6.19 – 6.28. Heat treatment during shucking increased the pH values to about 7.3. However, during frozen storage, the pHs decreased significantly (P<0.05) with a corresponding increase in titrable acidity (P<0.05). There existed a significant negative correlation (r=–0.997; P<0.01) between glycogen and titrable acidity. Thaw drip loss increased significantly (P<0.01) from 11 to 19% in frozen-stored oysters so also in clams (Mishra and Srikar 1989). Free drip to a certain extent reflects the degree of protein denaturation, resulting from surface dehydration, ice crystal formation and cell rupture. TMA–N was undetectable in fresh oyster as well as in frozen oyster meat during storage. A significant negative correlation (r = –0.863; P<0.05) was observed between proteins and TVB–N. TVB–N increased significantly (P<0.01) and never exceeded a value of 12.3 mg/100 g on 150 days of storage. The results are in agreement with those of Llobrera et al (1986).

The initial level of AAN observed in this study appears to be high, probably due to the heat process given at the time of shucking. The AAN level of oyster meat decreased significantly (P<0.01), while in thaw drip, its level increased (P<0.01) with period of storage at –18°C. There existed a negative correlation between proteins and thaw drip AAN (r = –0.854; P<0.05), and positive correlation between thaw drip loss and thaw drip AAN (r = 0.961; P<0.01). The decrease in AAN in oyster meat could be attributed to the deamination of amino acids and possible loss through thaw drip.

Lipid oxidation and hydrolysis are the major causes of deterioration in the quality of processed bivalve meat during frozen storage (Jeong et al.

TABLE 1. PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF FROZEN STORED EDIBLE OYSTER

Parameters	Storage, days						Correlation co-efficient# (r)
	0	30	60	90	120	150	
Physico-chemical characteristics							
Moisture, %	79.2	78.2	78.0	77.4	76.3	77.1	–0.897*
Proteins[a], %	9.7	9.3	9.2	9.2	9.1	9.0	–0.902**
Glycogen[b], %	6.5	6.1	6.0	5.9	6.0	5.8	–0.858*
pH[c]	7.3	7.1	6.9	6.8	6.8	6.9	–0.815*
Thaw drip loss[d], %	11.0	13.3	15.6	16.3	17.3	19.0	0.981**
Titrable acidity[e], as % acetic acid (1 x 10^{-2})	0	4.6	5.3	6.6	5.9	6.8	0.831*
Total volatile base-nitrogen[f], mg/100g	8.5	9.1	9.7	10.1	11.2	12.3	0.983**
Alpha amino nitrogen[g], mg/100g	261.1	251.7	230.9	203.3	171.6	149.8	–0.989**
Alpha amino nitrogen in thaw drip[h], mg/100g	26.8	40.7	72.6	103.9	131.5	171.1	0.993**
Thiobarbituric acid value[i], µ moles melanoldehyde/kg oyster	8.7	9.8	11.8	17.0	20.5	22.3	0.962**
Free fatty acids[j], as % oleic acid/kg fat	1.0	2.5	2.6	3.6	5.0	5.1	0.975**
Microbiological characteristics							
Total plate counts, cfu/g x 10^3	22.0	36.0	12.0	8.8	1.7	3.4	ND
Staphylococcal counts, cfu/g x 10^2	7.1	4.4	6.2	6.1	6.4	5.6	ND
Motile aeromonads counts, cfu/g	20.0	56.0	6.0	2.0	4.0	4.0	ND
Enterococcus faecalis counts, cfu/g	10.0	6.0	2.0	10.0	10.0	2.0	ND
Total coliforms, MPN/g	21.7	21.7	1.3	0.4	0.9	<0.2	ND
Faecal coliforms, MPN/g	21.7	5.6	0.2	0.3	0.3	<0.2	ND

Values are average of 4 observations except the thaw drip loss and microbiological characteristics (n = 2)

Fat content fluctuated between 2.4 and 2.5% during storage (r = –0.097; P>0.05)

Trimethylamine-nitrogen was undetectable in fresh oyster and in frozen stored oyster meat

: Correlation co-efficient (r) computed between storage days and the respective parameter

ND : Not done; 'r' values between a and f = –0.863*; a and g = 0.835*; a and h = –0.854*; a and i = –0.816*; a and j = –0.911**; b and c = 0.988**; b and e = –0.997**; d and g = –0.945**; d and h = –0.961**; *: P<0.05; **: P<0.01

Salmonellae and *Vibrio cholerae* were absent in frozen oyster meat samples

1990). A progressive increase in FFA ($P<0.01$) was observed during storage, probably due to the action of hydrolytic enzymes on lipids. The levels of TBA also increased significantly ($P<0.01$), due to the oxidation of lipids during storage. These results are in conformity with earlier reports (Ablett et al. 1986; Mishra and Srikar 1989; Jeong et al. 1990). Abraham et al (1994) noted a significant decrease in sensory quality with increases in FFA and TBA values during the frozen storage of oyster meat.

The process of freezing brought about 89.9% reduction in TPC immediately after freezing, from 2.17×10^5/g recorded on shucked meat. On further storage at -18°C, there were reductions in the TPC and also in the SC, EFC, MAC, TC and FC. APHA (1976) recommended that freshly frozen bivalve meat should have plate counts below 5.0×10^5/g meat. The results of this study conform to the above standard. The faecal coliform counts were high initially (MPN 21.7/g), compared to the recommended level (≤ 2.3/g for frozen oyster. This may be due to cross contamination at the time of shucking. However, its counts reduced below 1/g on the 60th day of storage. *E. faecalis* and staphylococci showed higher resistance to frozen storage than faecal coliform.

There have been very few studies on the stability and survival of motile aeromonads to freezing and frozen storage. In a study on frozen oyster, Llobrera et al (1986) recorded 5.3 - 33.3% *Aeromonas* group among the total bacterial population. The results of this study also have revealed that the motile aeromonads are relatively resistant to freezing and survive during frozen storage. *Salmonallae* were present in fresh oyster meat, but not in frozen meat. They were destroyed during the process of shucking by heat. *V. cholerae* was not encountered in both fresh and frozen oyster meat. These results have revealed that the frozen oyster meat samples are safe from organisms of public health significance.

Frozen bivalve meat could keep well for 9 months or longer, if prepared from freshly harvested and shucked shellstock (Ablett et al. 1986). In the present study, the oyster meat kept well for 150 days at -18°C without adversely affecting the keeping quality. This investigation, however, has revealed that edible oyster meat under frozen condition undergoes a progressive decline in freshness quality. Hence, addition of antioxidants and other chemical preservatives are indispensible to prevent quality loss during frozen storage.

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References

Ablett RF, Gould SP, Sherwood D (1986) Frozen storage performance of cooked cultivated mussels *(Mytilus edulis).* Influence of ascorbic acid and chelating agents. J Food Prot 51: 1118-1121

Abraham TJ, Balasundari S, Indra Jasmine G, Jeyachandran P (1994) Influence of antioxidants on the sensory quality and oxidative rancidity of frozen edible oyster. J Food Sci Technol 31: 168-170

AOAC (1975) Official Methods of Analysis, 12th edn. Association of Official Analytical Chemists, Washington, DC

APHA (1976) Compendium of Methods for the Microbiological Examination of Food. American Public Health Association, New York

Balasundari S, Abraham TJ, Shanmugam SA, Jeyachandran P (1994) Depuration of edible oyster, *Crassostrea madrasensis* (Preston). Poll Res 13: 153-160

Beatty SA, Gibbons NE (1937) The measurement of spoilage of fish. J Biol Board Can 3: 77-91

Carroll NVR, Longley W, Rao JH (1956) The determination of glycogen in liver and muscle by use of anthrone reagent. J Biol Chem 220: 583-593

Jeong BY, Ohshima T, Koizumi C, Kanou Y (1990) Lipid deterioration and its inhibition of Japanese oysters, *Crassostrea gigas,* during frozen storage. Bull Jap Soc Sci Fish 56: 2083-2091

Ke PJ, Woyewoda AD, Reyier CW, Ackman RG (1976) An improved titrimetric method for determination of free fatty acids in fish oil. Fisheries and Oceans, Halifax, Nova Scotia, Canada, New Series Circular No 61: 1-4

Llobrera AT, Bulalacao ML, Sunaz N (1986) Effects of storage on the microbial quality of slipper oysters, *Crassostrea iredalei.* In: Maclean JL, Dizon LB, Hosillos LV (eds), Proceedings of the First Asian Fisheries Forum, Asian Fisheries Society, Manila, pp 437-442

Mishra R, Srikar LN (1989) Shelf life of frozen stored clam *(Meretrix casta)* meat. J Food Sci Technol 26: 201-204

Palumbo SA, Mayino F, Williams AC, Buchanan RL, Thayer DW (1985) Starch-ampicillin agar for the quantitative detection of *Aeromonas hydrophila.* Appl Environ Microbiol 50: 1027-1030

Pope CG, Stevens MF (1939) The determination of amino-nitrogen using copper method. Biochem J 33:1070-1077

Woyewoda AD, Ke PJ (1979) Quality assessment of fatty fish by the 2-thiobarbituric acid distillation method. Fisheries and Oceans, Halifax, Nova Scotia, Canada, New Series Circular No 65: 1-5

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Denaturation of Seer Fish Actomyosin Subjected to Heating Regimes at 40°C and 85°C

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Seer fish (*Scomberomorus* spp) actomyosin solution (AM) was subjected to heating regimes at 40 and 85°C and the extent of denaturation was elucidated by determining protein solubility (PS), reduced and relative viscosities and Ca^{2+} ATPase activity. PS increased with duration of heating, whereas viscosity values and Ca^{2+} ATPase activity decreased considerably, indicating the conformational changes in the active sites of AM molecules.

Keywords: Seer fish, Actomyosin, Denaturation, Protein solubility, Viscosity, Ca^{2+} ATPase activity.

Surimi prepared from minced fish meat has unique functional properties due to which it is being used now-a-days as a valuable ingredient in a wide range of food products. The unique functional properties of *surimi* are due to the presence of myofibrillar proteins (MFP), particularly, the actomyosin (AM), as the major constituent of fish muscle proteins. One of the important functional properties of *surimi* is gelation, which is responsible for the formation of Kamaboko gel. AM, which is a salt soluble protein, is the main component that forms the Kamaboko gel (Suzuki 1981). In the formation of Kamaboko gel, the viscous sol obtained from mixing of *surimi* with salt is readily converted to an elastic gel through setting with or without heat. There are three different setting processes, viz., cold setting, partial-heat setting and full-heat setting (Lee 1984). Cold setting occurs at either 0-4°C or room temperature (22°C). Time required is very long with no thermal denaturation occurring. Here, the gel strength is time dependent and the type of bond involved is primarily hydrogen bonding (Suzuki 1981). Partial heat-setting is achieved at 40-50°C, which is accompanied by mild denaturation of proteins. Bonding involved are hydrogen bonds (Niwa et al. 1983), to a lesser extent, disulphide (S-S) bonds (Niwa 1986) and hydrogen bonds (Suzuki 1981). Full-heat setting can be brought about with or without partial heat setting and the sol receives full heat treatment at 80-95°C for a period of time sufficient for cooking (Lee 1994).

In the present investigation, AM, the MFP mostly responsible for gelation properties of *surimi* was isolated and subjected to two heating regimes, 40°C, a partial heat setting temperature and 85°C, a full-heat setting temperature. The influence of the two heating regimes on the extent of protein denaturation and its consequence on gelation behaviour of *surimi* was studied by determining the changes in PS, reduced and relative viscosity and Ca^{2+} ATPase activity of the actomyosin.

Actomyosin from freshly caught seer fish (*Scomberomerous* spp) was isolated at a temperature of 0-4°C, following the procedure of Niwa et al (1989). AM precipitate was stirred in 25 ml of tris maleate buffer, containing 0.5 M KCl. This AM solution was held in water baths maintained at 40°C and 85°C, respectively. Samples were drawn from both treatments at intervals of 10 min upto 60 min and further samples from 40°C treatment were drawn at an interval of 30 min from 60 to 180 min and analyzed for protein solubility (PS), relative and reduced viscosities and Ca^{2+} ATPase activity. PS was determined using biuret reagent by the method of Gornall et al (1949). Relative and reduced viscosities were determined according to Bradbury (1970), while Ca^{2+} ATPase activity was determined according to Perry (1955), by estimating inorganic phosphate (Kates 1972). The results are presented as mean of 3 estimates along with the standard deviation.

In AM held at 40°C, the initial PS was 2.40 mg/ml, which decreased to 1.70 mg/ml after heating for 20 min and then increased to 3.10 mg/ml at the end of 180 min. At 85°C, PS increased from 1.70 to 2.40 mg/ml after 50 min. although a low PS of 1.45 mg/ml was recorded at the end of 10 min (Table 1).

The initial relative and reduced viscosities in AM held at 40°C were 120.98 centipoise and 0.87 dl/g, respectively, which decreased to 108.48 centipoise and 0.27 dl/g after heating for 180 min. However, least viscosity value was in the sample, heated for 60 min. In case of AM held at 85°C, reduced viscosity decreased by about 50% from 0.61 to 0.35 dl/g after heating for 60 min (Table 1).

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TABLE 1. EFFECT OF HEATING REGIME ON PROTEIN SOLUBILITY AND VISCOSITY OF SEER FISH AM

Temperature	40°C			85°C		
		Viscosity			Viscosity	
Holding time, min	PS, mg/ml	Relative, centipoise	Reduced, dl/g	PS, mg/ml	Relative, centipoise	Reduced, dl/g
0	2.40 (0.00)	120.98 (1.03)	0.87 (0.05)	1.70 (0.00)	110.36 (0.20)	0.61 (0.01)
10	1.90 (0.00)	107.96 (0.13)	0.37 (0.05)	1.45 (0.05)	106.45 (0.28)	0.45 (0.02)
20	1.70 (0.00)	107.58 (0.13)	0.44 (0.01)	1.70 (0.00)	107.26 (0.22)	0.49 (0.02)
30	1.70 (0.00)	106.25 (0.09)	0.37 (0.01)	2.20 (0.00)	108.22 (0.05)	0.37 (0.00)
40	1.80 (0.10)	106.42 (0.74)	0.36 (0.04)	2.00 (0.00)	107.79 (0.18)	0.39 (0.01)
50	1.95 (0.05)	104.89 (0.74)	0.25 (0.04)	2.40 (0.00)	109.32 (0.13)	0.39 (0.01)
60	1.95 (0.05)	104.28 (0.65)	0.22 (0.03)	2.40 (0.00)	108.88 (0.55)	0.35 (0.02)
90	2.30 (0.10)	106.22 (0.31)	0.27 (0.01)	- -	- -	- -
120	2.60 (0.00)	108.07 (0.47)	0.30 (0.02)	- -	- -	- -
150	2.83 (0.13)	110.50 (1.13)	0.37 (0.04)	- -	- -	- -
180	3.10 (0.00)	108.48 (0.57)	0.27 (0.02)	- -	- -	- -

* Values are mean of 3 estimations ± SD in parentheses; - denotes not determined. PS - Protein solubility

The Ca^{2+} ATPase activity decreased from 4.45 to 2.49 μ mole P/mg AM/min after 180 min at 40°C with the first maximum activity at 50 min (5.04 μ mole P/mg AM/min) and second maximum activity at 60 min (5.37 μ mole P/mg AM/min). In AM solution heated to 85°C, the ATPase activity decreased from 5.98 to 2.64 μ mole P/mg AM/min at the end of the heating regime (Fig. 1).

Protein solubility showed an overall increase

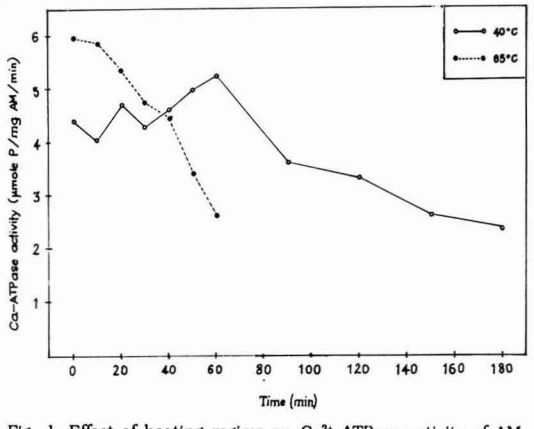

Fig. 1. Effect of heating regime on Ca^{2+} ATPase activity of AM solution

with the increase in holding time at both the temperatures. The increase in PS observed could be due to higher charge frequency (Bigelow 1967) and dissociation and solubilization of filaments (Liu et al. 1982). Moreover, increased salt concentration and pyrophosphate are known to increase the extractability of proteins from myofibrils (Samejima et al. 1985). A simple increase in protein solubility, however, is not always associated with improved gelation properties (Samejima et al. 1986). Reduced and relative viscosities decreased in both the treatments considerably. Similar results were obtained by Kawai et al (1993), who observed that the structure of AM, arrow-headed filaments and straight filamentous F- actin were transformed with an increase in temperature over a range of 30-45°C. Gill et al (1992) reported that myosins from different fish species aggregated to different extents as temperature increased. Thus, the decrease in viscosity was probably due to thermal induced aggregation of myosin sub-fragments. Heating AM solution at 40 and 85°C resulted in reduction of ATPase activity. Similar trend was observed by Sano et al (1994), who found that reactive SH increase from 20-50°C, suggesting that SH groups inside the AM molecule emerged to the surface as a result of unfolding, thereby causing a gradual decrease in ATPase activity with the increase in temperature, followed by a rapid loss in enzyme activity from 40-50°C, indicating conformational changes in active sites in AM.

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References

Bigelow CC (1967) On the average hydrophobicity of proteins and relation between it and protein structure. J Theor Biol 16:187-211

Bradbury JH (1970) Viscosity, In: Leach SJ (ed), Physical Principles and Techniques in Protein Chemistry, Part B. Academic Press, New York, pp 99-145

Gill TA, Chan TK, Phoncharcon KF, Paulson AT (1992) Efect of salt concentration and temperature of heat induced aggregation and gelation of fish myosin. Food Res Intl 25:333-342

Gornall AG, Bradawill CJ, David MM (1949) Determination of proteins by means of biuret reaction. J Biol Chem 177:751-766

Kates M (1972) Techniques in lipidology, In: Work TS, Work E (eds), Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 3. North-Holland Publishing Co., London

Kawai Y, Nakasato T, Hatano M (1993) Effect of heat treatment on the solubility and viscosity of salt extracted protein from mature Chum salmon (*Oncorhynchus keta*). Bull Fac Fish Hakkaido University, Japan 44:247-253

Lee CM (1994) Surimi processing from lean fish, In: Shahidi F, Botta JR (eds.) Seafoods- Chemistry, Processing Technology and Quality. Blackie Academic and Professional. Glasgow, UK, pp 263-287

Liu YM, Lin TS, Lanier TC (1982) Thermal denaturation and aggregation of actomyosin from Atlantic Croaker. J Food Sci 47:1916-1919

Niwa E (1986) Functional aspects of surimi, In: Martin RE, Collette RC (eds) Proceedings of the International Symposium on Engineered Seafoods including *Surimi*. National Fisheries Institute, Washington DC, pp 141-147

Niwa E, Nakayama T, Hamada I (1983) The third evidence for participation of hydrophobic interaction in fish flesh gel formation. Bull Jap Soc Sci Fish 49:1763-1771

Niwa E, Kanoh S, Osaka Y, Nakayama T, Watabe S, Hashimoto K (1989) Changes in surface hydrophobicity of fish actomyosin induced by urea. Bull Jap Soc Sci Fish 55:143-146

Perry SV (1955) Myosin Adenosinetriphosphatase, In: Colowick SP, Kaplan NO (eds) Methods in Enzymology, Vol II. Academic Press Inc., New York, USA, pp 582-587

Samejima K, Egelundsdal B, Fretheim K (1985) Heat gelation properties and protein extractability of beef myofibrils. J Food Sci 50:1540-1543, 1555

Samejima K, Oka Y, Yamamato K, Asghar A, Yasui T (1986) Effects of temperature, actin-myosin ratio, pH and salt and protein concentration on heat induced gelling of cardiac myosin and reconstituted actomyosin. Agric Biol Chem 50:2101-2110

Sano T, Ohno T, Fuchino HO, Matsumoto JJ, Tsuchiya T (1994) Carp natural actomyosin: Thermal denaturation mechanism. J Food Sci 59:1002-1008

Suzuki T (1981) Frozen minced meat (Surimi), In: Suzuki T (ed) Fish and Krill Protein Processing Technology. Applied Science Publisher Ltd., Essex, England, pp 15-147

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Effect of Defatted Soy Flour on the Quality of Buns

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High protein buns were prepared by replacing wheat flour with 5, 10 and 15% defatted soy flour (DSF). The farinograph water absorption of dough increased from 60.4 to 67.2% and dough development time from 2.0 to 5.5 min with increasing addition of soy flour. The mixograph area decreased from 75.9 to 71.0 cm^2, the extensograph resistance to extension, extensibility and area values decreased gradually from 990 to 780 BU, 138 to 115 mm, 182.8 to 136.0 cm^2, respectively, indicating adverse effect on the dough properties. Though the specific volume of buns decreased from 3.80 to 3.61 ml/g with 15% addition of soy flour, the firmness of crumb decreased from 5.50 to 3.64 N, indicating improvement in softness. The protein contents of buns increased from 11.01 to 18.89%. Addition of sodium stearoyl-2-lactylate (SSL) or soy lecithin (SL) improved the quality of buns, containing soy flour.

Keywords: High protein buns, Defatted soy flour, Bun crumb firmness, Storage.

India produces about 2.3 million tonnes of soybean, which is about 2% of the total world soybean production (Nawab Ali 1993). Utilization of soyflour to increase the protein content and improve the amino acid balance of the proteins in baked products has been well recognised for many years (Marnett et al. 1973). Tsen et al (1971) reported that a mixture of wheat flour fortified with 12% defatted soy flour (DSF) increased the lysine content by 2 times that of wheat flour alone and that the protein content of the bread made from such a blended flour increased by approximately 35%. Sinha et al (1993) reported that supplementation of wheat flour with DSF improved the shelf life of baked goods in addition to increase in protein content.

Baking industry is one of the largest organised food industries in the country. The growth of bakery industry is about 10% per annum and the products are increasingly becoming popular among all sections of people (Anon 1992). Protein malnutrition is a major health concern in India and most of the developing countries (Sinha et al. 1993). There exists a considerable scope for introducing protein-rich baked goods to combat protein mal-nutrition. High protein buns are ideal to meet such requirement. They can be popularized through school feeding programmes and making available to geriatric subjects.

With this background, investigations were carried out to study the effect of DSF on the rheological characteristics, bun making quality and shelf life of buns and the results are presented in this communication.

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A commercial wheat flour (*maida*) procured from the local market and DSF obtained from Sakthi Soy Limited, Coimbatore were used in the studies.

Chemical characteristics: Total ash, crude proteins (Nx5.7), dry gluten, sedimentation value, falling number, diastatic activity were determined according to AACC (1983) procedures.

Rheological characteristics: Dough characteristics as affected by the substitution of wheat flour with 5, 10 and 15% DSF were studied according to AACC (1983) procedures, using farinograph and extensograph.

Preparation and quality of buns : Bun making quality of wheat flour was studied, using the formulation- flour 100 g, compressed yeast 3.0 g, salt 1.5 g, fat 5.0 g, and glycerol monostearate 0.5 g. The processing conditions were fermentation time. 90 min, proof time, 45 min and baking, 15 min at 220°C. The effects of DSF (5, 10 and 15%), sodium stearoyl lactylate (SSL-0.5%) and soy lecithin (SL-0.5%), respectively on the quality of buns were studied. Duncan's new multiple range test as described by Harter (1960) was used for finding out the stastical significance of the results.

Changes in quality of buns during storage: The buns after cooling for 3 h were packed separately in polypropylene bags (150 gauge) and stored at temperature and humidity of 27°C and 65% RH, respectively. Evaluation of buns after 24 h and 48 h of storage was carried out for crust and crumb characteristics. The bun crumb firmness was determined, using Instron Texturometer (Model 4301) as per AACC (1983) procedures with the following settings: plunger-36 mm dia, crosshead speed- 100 mm/min, chart speed- 500 mm/min,

TABLE 1. EFFECT OF DEFATTED SOYFLOUR ON THE RHEOLOGICAL CHARACTERISTICS OF WHEAT FLOUR

Parameters	Defatted soyflour, %			
	0	5	10	15
Farinograph				
Arrival time, min	1.5	1.5	1.5	1.5
Departure time, min	6.5	6.5	7.0	8.0
Stability, min	5.0	5.0	5.5	5.5
Mixing tolerance index at 20 min, BU	75	90	90	85
Valorimeter value	46	46	54	58
Extensograph				
Resistance to extension, R, BU	990	950	900	780
Extensibility, E, mm	138	135	130	115
Ratio figure, R/E	7.25	7.09	6.92	6.78

compression load cell - 100 kg max, sample thickness - 25 mm, position of upper crosshead limit - 1 mm above centre surface of the sample and position of lower crosshead limit at 25% compression. The force required for 25 % compression of a crumb size of 25 mm thickness was read off directly from the chart and expressed as firmness of bun. The crumb firmness was measured separately after 24 h and 48 h of storage.

Chemical characteristics: The flour used for the study had total ash (0.45%), proteins (11.8%), dry gluten (10.40%) sedimentation value (21), falling number (500) and diastatic activity (240 mg of maltose/10 g flour). The protein content of DSF was 45.4%.

Rheological characteristics: Table 1 gives the dough properties studied, using farinograph and extensograph. There was a gradual increase in farinograph water obsorption from 60.4 to 67.2%, as the soy content increased from 0 to 15% in the blend. The dough development time increased from 2.0 to 5.5 min, indicating increasing mixing requirements. The stability of the dough was unaffected. Sahni and Krishnamurthy (1975) reported similar findings, while studying the effect of DSF on the farinograph characteristics of Indian wheat varieties like 'Shera', 'Hira', 'Kalyan Sona' and 'Moti'. With increased addition of soy flour, extensograph resistance to extension, extensibility and area decreased by 21%, 17% and 26%, respectively, indicating the adverse effect on the dough properties. Similar observation on the adverse effect of soy flour on dough properties was reported (Kulp et al. 1980; Tsen and Hoover 1973).

Bun making quality: The baking studies (Table 2) showed that specific volume of buns decreased by about 7%, as soy content increased from 0 to 15%. Kulp et al (1980) also reported that the inclusion of soy flour in white bread formulation tended to reduce the bread making potential of wheat flour. The crust and crumb colour changed from golden brown to dark brown and creamish white to light brown, respectively. There was a marginal decrease in crumb score from 7.0 to 6.5. However, the crumb firmness (Table 2) decreased by 34%, as soy content increased, indicating improvement in crumb softness. Gaur and Jamuna (1993) reported similar observation that soy flour, when used at the optimum levels led to improved

TABLE 2. EFFECT OF SODIUM STEAROYL-2-LACTYLATE (SSL) AND SOY LECITHIN (SL) ON THE QUALITY OF BUNS CONTAINING DEFATTED SOY FLOUR

Defatted soy flour	Specific volume, ml/g			Crust colour			Crumb grain score*			Protein,** Nx5.7 %	Crumb firmness N					
	A***	B***	C***	A***	B***	C***	A***	B***	C***		A***		B***		C***	
											24h	48h	24h	48h	24h	48h
0	3.80^{e}	3.92^{i}	3.94^{j}	Golden brown	Golden brown	Golden brown	7.0	7.5	7.5	11.01	5.50	8.98	5.25	8.50	5.46	8.79
5	3.75^{d}	3.88^{h}	3.86^{g}	Dark brown	Dark brown	Dark brown	7.0	7.5	7.5	13.77	4.62	6.63	4.14	5.85	4.31	5.96
10	3.71^{c}	3.86^{g}	3.84^{f}	Dark brown (+)	Dark brown (+)	Dark brown (+)	6.5	7.0	7.0	15.82	4.11	5.96	4.05	5.57	4.03	5.40
15	3.61^{a}	3.71^{c}	3.69^{b}	Dark brown (++)	Dark brown (++)	Dark brown (++)	6.5	7.0	7.0	18.89	3.64	4.66	3.35	4.27	3.66	4.17

SEM ± 0.01
(df=36)

* Maximum score : 8

** Expressed on 14% moisture basis

*** A: Control, B: Control + 0.5% SSL and C: Control + 0.5% SL
Means of the first three columns followed by different letter differ significantly ($P<0.05$) according to Duncan's Multiple Range test

texture and tenderness of the crumb of the bread, doughnuts and cookies.

Effect of SSL and SL on the quality of buns: It can be seen from Table 2 that the addition of SSL and SL separately improved the quality of buns significantly. The specific volume (ml/g) increased by 3.2%, 3.5%, 4.0% and 2.8% for SSL and 3.7%, 2.9%, 3.5% and 2.2% for SL of the buns with 0, 5, 10 and 15% DSF separately. The crust colour, shape and crumb colour were unaffected. However, there was a marginal increase in crumb grain score by 0.5. The improvement in crumb softness was observed, when 0.5% SSL was added, as indicated by the decrease in crumb firmness value by 0.35, 0.48, 0.06 and 0.29 N for buns, containing 0, 5, 10 and 15% respectively. Similarly, with 0.5% SL, the decrease in crumb firmness value was by 0.04, 0.31, 0.08 and 0.02 N. (Table 2). Tsen et al (1971), Kulp et al (1980) and Sahni and Krishnamurthy (1975) reported that addition of SSL and other dough conditioners separately compensated the volume-depressing effect of the soy flour and improved crumb softness.

Changes in the quality of buns during storage: The effect of storage on the crumb firmness is presented in Table 2. The crumb firmness values increased by 63%, 44%, 45% and 28% during 48 h of storage for buns, containing 0, 5, 10 and 15% DSF from their initial 24 h stored values, respectively. However, the values increased by only 62%, 41.3%, 37.5%, 27.4% and 61%, 38.3%, 36%, 13.9%, when SSL and SL were added, respectively, which indicated slower rate of crumb firming. Gaur et al (1993) reported that soy flour increased water retention capacity of the dough, thereby increasing the yield and shelf life of bakery products.

Protein contents of buns: The protein contents of the buns containing 0, 5, 10 and 15% soy flour are presented in Table 2. The results indicated that protein content increased by 25.07%, 43.7% and 71.5% for the buns with 5, 10 and 15% DSF, respectively. Marnett et al (1973) also reported increases in protein contents of breads by 35%, when wheat flour was substituted with 12% DSF.

It can be concluded from the results that high protein acceptable quality buns with increased softness can be prepared by replacing wheat flour with 10% defatted soy flour and using SSL or SL as additive.

References

AACC (1983) Approved Methods of Analysis. 8th edn. American Association of Cereal Chemists, St. Paul, Minnesota

Anon (1992) Future Perspectives for Wheat and Wheat-Based Industries. A Study by Wheat Products Promotion Society

Gaur MK, Jamuna KV (1993) Soy fortified bakery products.The Indian Baker 24:15-19

Harter HL (1960) Critical values for Duncan's new multiple range test. Biometrics 16:671-685

Kulp K, Volpe T, Barrett FF, Johnson K (1980) Low protein wheat flour utilised in soy fortified bread. Cereal Foods World 25:609-612

Marnett LF, Tenney RJ, Barry VD (1973) Methods of producing soy-fortified breads. Cereal Sci Today 18:38-43, 51

Nawab Ali (1993) Soy Food Technology: What it can do for India, Guide on Food Products Year Book, pp 72-73

Sahni SK, Krishnamurthy K (1975) Development of high protein bread. Part II- Soy flour utilization. J Food Sci Technol 12:178-183

Sinha LK, Singh G, Nelson AI (1993) Utilization of defatted soy flour to fortify bread and *chapati* flour: Prospect in India. The Indian Baker 24:21-30

Tsen CC, Hoover WJ, Phillips D (1971) High Protein breads. Bakers Dig 45:20-23

Tsen CC, Hoover WJ (1973) High protein bread wheat flour fortified with full fat soy flour. Cereal Chem 50:7-16

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Characterization of Virulence Factors Among Enterotoxigenic Strains of *Aeromonas hydrophila* Isolated from Fish

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Of the 11 isolates of *Aeromonas hydrophila* from 120 freshwater fish samples, one produced cytotoxic and 3 produced both cytotoxic and cytotonic heat labile enterotoxins, indicated by fluid accumulation in rabbit ligated ileal loop, oedema in mouse foot pads, increased vasopermeability response in rabbit skin and cytopathic changes in vero cells. None of the isolate was invasive, mouse lethal and haemolytic, while all enterotoxigenic strains had strong cell surface hydrophobicity. Mannose resistant haemagglutination was caused by all, but only one enterotoxigenic strain had mannose sensitive fimbriae. Multiple drug resistance was common among enterotoxigenic strains.

Keywords: *Aeromonas hydrophila*, Enterotoxins, Invasiveness, Haemagglutination, Multiple drug resistance

Aeromonas hydrophila, an emerging foodborne pathogen has frequently been isolated from fish, crabs, prawns, shelfish, beef, quails, poultry products, pork, pigs, polluted water etc. (Kirov 1993). *Aeromonas* causes diarrhoeal outbreaks and isolated cases especially in summer season, mainly affecting children and aged (Wilcox et al. 1992), probably by releasing cytotoxin and enterotoxin in human intestine (Kirov 1993). However, other virulence factors have not been studied in detail and the information is scantily available on environmental and food isolates. This study was, therefore, conducted to analyze the virulence factors of *Aeromonas hydrophila* isolated from fish.

Isolation : A total of 120 fresh water fish samples from various retail shops in and around Bareilly city were collected and processed to resuscitate the injured organisms (ICMSF 1978). After selective enrichment at 37°C for 24 h for *A. hydrophila* (Kirov 1993) it was plated on ampicillin dextrin agar (ADA, Havelaar and Vonk 1988) and xylose lysine desoxycholate agar (XLDA, Merck) incubated at 37°C for 24 h. The oxidase positive, isolated yellow colonies surrounded by yellow zone were examined for detailed morphological, cultural and biochemical characteristics and were confirmed as *A. hydrophila*, on the basis of criteria described by Popoff (1984). All the control strains used in the study were obtained from the National *Salmonella* Centre (IVRI), Izatnagar. The control and *A. hydrophila* isolates were maintained in laboratory by sub-culturing at regular interval of 90 days on buffered nutrient agar slants.

Aeromonas hydrophila isolates were tested for enterotoxin production by growing in brain heart infusion (BHI) broth. Enterotoxicity of cell-free culture filtrate (CFCF) was assessed by rabbit ligated ileal loop (RLIL) test (De and Chatterjee 1953), suckling mouse assay-SMA (Dean et al. 1972), vasopermeability factor test (VPFT) on abdominal skin of albino rabbits (Sandefur and Peterson 1976), mouse foot pad (MFP) test (Singh and Kulshreshtha 1992) and on vero cells mono layers in 96 well tissue culture plates (Spiers et al. 1977). In the above tests, CFCFs of known enterotoxigenic (heat stable -P16 and heat labile-T 96) and non-enterotoxigenic (J53) *E. coli* strains were used as positive and negative controls, respectively. Aliquots of CFCFs were heated at 72°C for 15 sec and at 63°C for 30 min to detect the heat stability of enterotoxic factors.

Antibiogram of *Aeromonas* isolates was analyzed according to disc diffusion method of WHO (1961) by using Hi-Media (Mumbai) antibiotic discs. Haemolysin production was determined by inoculating the test strains on nutrient blood agar (10% defibrinated sheep blood) plates (Cruickshank et al. 1973). Mouse lethality of all the isolates was tested in adult swiss albino mice by intraperitoneal

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injection of 0.1, 0.2 and 0.5 ml 18 h BHI broth culture (Cruickshank et al. 1973), and the autoclaved broth culture of the *Aeromonas* was used as negative control. Invasive ability was assessed in guinea pig eye model (Sereny 1955) and with congo red dye adsorption test-CRDT (Ishiguro et al. 1985), *Shigella dysenteriae* (E-218) was used as invasion positive control. Cell surface hydrophobicity was determined by salt aggregation method of Lindahl et al (1981). Fimbrial haemagglutination was performed according to the modified method of Simmons et al (1988).

Of the 11 isolates of *A. hydrophila*, none was invasive in the Sereny's test and only one showed congo red eye adsorption (Table 1). The latter has been reported to be the more suitable test to detect invasion in *Aeromonas* isolates (Ishiguro et al. 1985). However, CRDT results had no correlation with lethality in mice. Absence of invasion and mouse lethality traits in *A. hydrophila* strains indicated rarity of these traits in environmental and food isolates (Singh and Sanyal 1992). While, cell surface hydrophobicity (CSH) of *A. hydrophila* strains (Table 1) indicated the positive correlation with enterotoxigenicity. Similarly, it has been reported earlier that *A. veronii*, isolates with high CSH colonize better in the intestinal tract and cause acute as well as persistent diarrhoea (Kirov 1993).

All *A. hydrophila* strains under study agglutinated fresh guinea pig and rabbit erythrocytes, but tanned erythrocytes were poorly agglutinated and finally haemolyzed within 30 min of incubation. The HA pattern of *Aeromonas* strains (Table 1) revealed the presence of type one mannose sensitive fimbriae in only one enterotoxigenic strain, which was lacking in mannose resistant types of fimbriae, present in all the others. Fimbriated *Aeromonas* strains colonize better in intestine (Quinn et al. 1993). However, none of the *Aeromonas* isolates could be proved to have colonization factor antigen.

Interestingly, 3 enterotoxigenic strains of *A. hydrophila* haemolyzed tanned guinea pig erythrocytes, but none of the isolate could haemolyse untanned erythrocytes either of guinea pig or rabbit or sheep (in blood agar). This phenomenon of hemolysis of tanned erythrocytes by enterotoxigenic isolates needs further investigation. Most of the

TABLE 1. PATHOGENIC ATTRIBUTES OF *A. HYDROPHILA* STRAINS ISOLATED FROM FISH

Attributes	Strains				
	I-1	I-12	SF-2	SF-10	7NT
Haemagglutination of fresh guinea pig erythrocytes	MR	MR	MS	MR	MR
Tanned guinea pig erythrocytes	MR*	MR*	-	MR*	MR
Fresh rabbit ethyrocytes	MR	MR	MS	MR	MR
Molar salt concentration of $(NH_4)_2SO_4$ for agglutination	0.9	0.1	0.75	0.75	≤1.2
Invasiveness assays					
Sereny's test	-	-	-	-	-
Congo-red dye binding test	-	-	-	+	-
Mouse lethality (%)	0.0	0.0	0.0	0.0	0.0
Cell-free culture filtrate reaction					
Rabbit ligated ileal loop test index	1.1	1.8	1.2	1.0	≤0.5
Mouse foot pad test index	1.5	2.2	1.8	1.0	≤1.2
Suckling mouse assay index	1.2	1.2	1.2	1.2	≤7
Vasopermeability factor test (Zone of blueing in mm)					
Rapid	12	12	12	8	≤7
Delayed	18	25	15	20	≤7
Cytopathic effect (CPE) in vero cell lines					
Cytotoxic	+	+	+	-	-
Cytotonic	+	+	+	+	-
Resistance to	A,Ce,CF Co,F,O	A,Ce,CF No,O	A,Ce,O	A,Ce,CF F,O	A,Ce
Sensitive to	C,Na,No	Co,F,C Na	CF,Co,F C,Na,No	Co,C,Na No	CF,Co F,O,C Na,No

MR: mannose resistant; MS: mannose sensitive; * Haemagglutination response was weak and vanished within 30 min of incubation at 25°C due to haemolysis of erythrocytes; - ; NT: non-enterotoxigenic; A: ampicillin; Ce: Cephalexin; C: Chloramphenicol; CF: Cephataxime; Co: Cotrimoxazole; F: Furazolidone; Na: Nalidixic acid; No: Norfloxacin O: Oxytetracycline

earlier studies reported hemolysin production in enterotoxigenic *A. veronii* strains, and rarely in *A. hydrophila* strains, particularly of environmental origin (Kirov 1993; Singh and Sanyal 1992).

All the four enterotoxigenic *A. hydrophila* strains elicited strong heat-labile enterotoxic response in RLIL, MFPT and VPFT (Table 1), but none produced heat-stable enterotoxic effect in any of the bioassays including sucking mouse assay (SMA) and cytopathic effect (CPE) in vero cell-line assay (VCA). Rapid and the delayed types of heat-labile VPF responses could be observed with enterotoxic CFCFs. Detachment and death of vero cells, was associated with rapid VPFT response. However, CFCF only with delayed VPFT response (SF10) caused rounding (cytotonic response) of vero cells. Our preliminary observations regarding correlation between rapid and delayed VPFT with cytotoxic and cytotonic response in vero cell lines appear to be important, but need to be investigated further. These studies confirm the multiplicity of enterotoxic factors produced by *Aeromonas* strains frequently reported earlier (Kirov 1993).

Irrespective of enterotoxigenicity, all *Aeromonas* isolates were sensitive to nalidixic acid and chloramphenicol, but resistant to cephalexin and ampicillin (Table 1). Variable resistance to cephataxime, furazolidone, oxytetracycline, norfloxacin and contrimoxazole was observed in the enterotoxigenic strains only. Similar multiple drug resistance has been reported earlier in clinical and environmental *Aeromonas* isolates (Kirov 1993). This indicates the circulation of antibiotic drug resistant enterotoxigenic strains of *Aeromonas* in environment, food and diarrhoeal patients. Isolation of multiple drug resistant, enterotoxigenic and adhesive strains from fishes for human consumption is of public health significance and requires appropriate attention, while using antibiotics in fish ponds.

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References

Cruickshank R, Dugauid JP, Marmion BP (1973) Medical Microbiology 12th edn. English Language Book Society, Edinburgh, UK

Dean AG, Ching Y, Williams RG, Harden LB (1972) Test for *E. coli* enterotoxin using infant mice; Application in a study of diarrhoea in children in Honolulu. J Infect Dis 125:407-411

De SN, Chatterjee DN (1953) An experimental study of mechanism of action of *Vibrio cholerae* on intestinal mucous membrane. J Pathol Biol 66:559-562

Havelaar AH, Vonk M (1988) The preparation of amplicillin dextrin agar for the enumeration of *Aeromonas* in water. Letters Appl Microbiol 7:169-171

ICMSF (1978) Microorganisms in foods. 2nd edn. International Commission on Microbiological Specifications for Foods. University of Toronto Press, Toronto, Canada

Ishiguro EE, Ainsworth T, Trust TJ, Kay WW (1985) Congo red agar, a differential medium for *Aeromonas salmonicida* detects the presence of cell surface protein, assay involved in virulence. J Bacteriol 164:1233-1237

Kirov SM (1993) The public health significance of *Aeromonas* spp. in foods. Int J Food Microbiol 20:179-198

Lindhal M, Faris A, Wadstrom T, Hjerten S (1981) A new test based on salting out to measure relative surface hydrophobicity of bacterial cells. Biochem Biophys Acta 677:471-476

Popoff M (1984) Genus III *Aeromonas*. In: Krieg NR, Holt JG (eds). Bergey's Manual of Systematic Bacteriology, Vol 1, Williams and Wilkins, Baltimore, pp 545-548

Quinn DM, Wong CYF, Atkinson HM, Flower RLP (1993) Isolation of carbohydrate reactive outer membrane proteins of *Aeromonas hydrophila*. Infect Immun 61:371-377

Sandefur PD, Peterson JW (1976) Isolation of skin permeability factors from culture filtrates of *Salmonella typhimurium*. Infect Immun 14:671-679

Sereny B (1955) Experimental *Shigella keratoconjunctivitis*, a preliminary report. Acta Microbiol Acad Sci Hung 2:293-296

Simmons KW, Wooley RE, Brown J (1988) Comparison of virulence factors and R plasmids of *Salmonella* spp isolated from healthy and ill swine. Appl Environ Microbiol 54:760-767

Singh BR, Kulshreshtha SB (1992). Preliminary examination on the enterotoxigenicity of isolates of *Klebsiella pneumoniae* from seafoods. Int J Food Microbiol 16:349-352

Singh DV, Sanyal SC (1992) Haemolysin and enterotoxin production by *Aeromonas caviae* isolated from diarrhoeal patients, fish and environment. J Diarrhoeal Dis Res 10:16-20

Spiers JI, Stavric S, Knowalchuk J (1977) Assay of *Escherichia coli* heat-labile enterotoxin with vero cells. Infect Immun 16:617-622

WHO (1961) Standardization of methods for conducting microbic sensitivity tests. Technical Report Series 210, World Health Organization, Geneva

Wilcox MH, Cook AM, Elcy A, Spencer RC (1992) *Aeromonas* spp as a potential cause of diarrhoea in children. J Clin Pathol 45:959-963

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Influence of Binders and Refrigerated Storage on Certain Quality Characteristics of Chicken and Duck Meat Patties

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Patties were prepared with 3 different types of binder combinations by utilizing spent chicken and duck meat and evaluated for quality on refrigerated storage (5 ± 1 °C). Chicken meat patties had (P<0.05) higher pH, with high percentage of cooking losses, crude proteins, ether extractives and higher colour scores, whereas the duck meat patties had higher (P<0.05) protein contents and superior sensory scores. Storage of patties for 6 days resulted in (P<0.05) higher cooking losses, pH, TBA values and lesser gain in thickness, proteins, ether extractive and sensory scores.

Keywords: Chicken and duck meat patties, Refrigerated storage, Binders, Sensory scores.

Consumer preference for broiler meat is hampering the marketing of spent chicken and ducks, leaving a wide scope for the development of different varieties of poultry products. Comminuted poultry products such as patties are one of the choices in utilizing the spent layers and ducks. Addition of binders to the comminuted product will improve acceptability of the product by increasing emulsifying, water binding and slicing characteristics. The protein content was found to be higher, when patties were extended with egg albumen (Hussain et al. 1988) and with soy protein substitution (Lyon et al. 1978). The present work was undertaken to study the effect of binders and storage on the quality of chicken and duck meat patties.

Spent 'White Legborn' layers and spent 'desi' ducks aged about 1 1/2 years, of 25 each were slaughtered conventionally in 5 batches. The processed ready-to-cook carcasses were partially cooked in a pressure cooker at 1 kg/cm^2 of pressure for 5 min. to facilitate easy deboning. The manually deboned meat was minced in a mincer, using a 4 mm dia sieve. The minced meat was divided into three equal parts and mixed separately with different binders, namely 3% gram flour (G), 1.5% gram flour + 1.5% egg albumen (G+E) 1.5% *maida* + 1.5% egg albumen (M+E). Three types of patties were prepared with meat 89.5%, vegetable oil 5.0% binders 3.0%, salt 1.0% and spice mixture 1.5%, according to Hussain et al (1988). The prepared patties of chicken and duck meats were wrapped in polythene bags separately and stored in a refrigerator (5±1 °C) for 6 days. The samples were drawn initially on the 0 day and subsequently after 3 and 6 days of refrigerated storage and evaluated for quality. The patties were cooked to an external temperature of 80°C for 25 min. Cooking loss was calculated by finding the difference between pre- and post-cooking. Gain in thickness and shrinkage in dia were measured with the help of a Vernier caliper. pH of the patties was estimated by using a digital pH meter. Thiobarbituric acid (TBA) values of the samples were determined as per the method of Witte et al (1970) methods. The proximate composition was estimated as per AOAC (1970) methods. Organoleptic evaluation was done on a 9-point Hedonic scale with 5 taste panelists for colour, flavour, juiceness, tenderness and overall acceptability. The data obtained on various parameters were subjected to statistical analysis (Sendecor and Cochran 1968).

Physical characteristics: The mean % cooking losses were significantly (P<0.05) higher and gain thickness was lower in chicken meat patties than in duck meat patties, irrespective of binders and storage (Table 1). The lesser gain in thickness of chicken patties may be due to their higher cooking loss. Similar levels of shrinkage in dia were observed in chicken patties by Thind et al (1988). Patties recorded significantly (P<0.05) higher gain in thickness, when they were extended with egg albumen. This might be due to the foaming activity of egg albumen. These results are in accordance with those of Hussain et al (1988) in rabbit meat patties.

Irrespective of species and binders, patties under refrigeration for 6 days recorded significantly (P<0.01) higher cooking losses and lower gain in thickness than those stored for 3 days and fresh (0 days). This may be attributed to the lowering of water

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holding capacity of meat and loss of moisture on storage. Similar shrinkage was observed by Judge et al (1974) in beef patties on frozen storage.

The pH of chicken meat patties was significantly (P<0.05) higher than the pH of duck meat patties in majority of combinations (Table 1). Higher pH in chicken meat patties might be due to the lower glycogen content and glycolytic activity, resulting in lower lactic acid production. Similarly, higher pH was noticed in chicken meat patties by Anjaneyulu et al (1990). The mean TBA values of chicken meat patties were significantly (P<0.01) lower than those of duck meat patties in all the three combinations and storage periods (Table 1). Higher moisture content in duck patties might have accelerated fat oxidation, resulting in higher TBA values.

pH and TBA values of patties increased significantly (P<0.05) with increase of storage periods. The increase of pH in patties during storage might be due to liberation of metabolites, resulting from bacterial activity. These results are in accordance with the findings of Thomson et al (1983) in chicken patties.

Proximate composition: The moisture content of chicken meat patties was significantly (P<0.05) lower than the duck meat patties (Table 2), which may be due to the spent chicken and the ducks used in this experiment (Molonon et al. 1976). Crude proteins, ether extracts and total ash contents were higher (P<0.05) in chicken meat patties than in duck meat patties (Table 2).

Protein contents increased (P<0.05) due to the addition of egg albumen and lipid content due to the addition of gram flour in chicken as well as duck meat patties (Table 2), falling in line with the results of Lyon et al (1978) and Padda et al (1987) in chicken meat patties.

Refrigerated storage had significantly (P<0.05) reduced both crude protein and ether extract contents in patties (Table 2), as also reported by Cunningham and Howers (1977) in chicken meat patties.

Organoleptic evaluation: Chicken meat patties had significantly (P<0.05) higher colour and flavour scores, whereas patties made with duck meat recorded higher (P<0.05) juiciness, tenderness and overall acceptance scores (Table 2). Dark brown colour of duck meat patties was less attractive due to over browning. Higher fat content in the chicken meat patties improved their flavour scores and the higher moisture contents produced more juicy and tender duck meat patties (Table 2).

TABLE 1. PHYSICAL CHARACTERISTICS, PH AND TBA VALUES OF PATTIES AS INFLUENCED BY BINDERS, SPECIES AND STORAGE PERIOD AT 5±1°C

Storage period, days	Binders					
	3% gram flour		1.5% gram flour 1.5% egg albumen		1.5% *maida* + 1.5% egg albumen	
	Chicken	Duck	Chicken	Duck	Chicken	Duck
	Cooking loss					
0	34.45^{bA} ±1.54	32.07^{bA}±1.72	37.88^{cA} ±2.10	29.14^{aA} ±1.88	37.21^{cA}±0.94	28.75^{aA} ±1.76
3	39.52^{bB} ±1.72	36.15^{aB}±0.71	38.48^{bA} ±0.92	35.81^{aB} ±0.88	40.32^{bB}±1.92	36.45^{aC} ±1.39
6	44.92^{cC} ±2.10	37.40^{bC}±1.18	44.97^{cB} ±2.22	37.39^{bC} ±0.87	47.61^{cC}±1.72	34.19^{aB} ±1.57
	Gain in thickness					
0	18.90^{abA}±2.00	29.00^{dA}±2.80	21.20^{bA} ±2.10	29.30^{dA} ±2.02	27.70^{cA}±1.92	30.20^{dA} ±2.22
3	24.60^{aB} ±1.70	23.00^{aB}±2.20	27.20^{bB} ±2.18	27.50^{bB} ±2.04	22.20^{bA}±2.21	24.91^{bB} ±1.98
6	17.61^{aC} ±1.19	17.86^{aC}±2.12	21.90^{bC} ±2.08	29.90^{dA} ±1.26	22.20^{bB}±2.21	24.91^{cC} ±1.98
	Shrinkage in diameter					
0	15.28^{bA} ±1.72	12.09^{aA}±0.57	15.50^{bA} ±0.84	12.96^{aA} ±0.51	16.16^{bA}±1.56	12.57^{aA} ±1.27
3	15.93^{bA} ±0.64	14.85^{aB}±0.81	15.86^{bAB}±0.40	16.02^{bC} ±0.99	15.80^{bA}±0.32	15.66^{abB}±0.50
6	15.86^{bA} ±0.83	13.59^{aB}±1.00	16.26^{bB} ±0.96	14.66^{abB}±0.67	15.82^{bA}±0.80	14.28^{abB}±0.78
	pH					
0	5.85^{aA} ±0.12	5.81^{aA}±0.08	5.83^{aA} ±0.09	5.98^{bA} ±0.08	5.91^{bA}±0.10	6.07^{bA} ±0.07
3	6.04^{bB} ±0.14	5.90^{aB}±0.05	6.10^{bB} ±0.13	6.00^{bB} ±0.10	6.11^{cB}±0.06	6.03^{bA} ±0.06
6	6.27^{cC} ±0.13	6.13^{bC}±0.09	6.28^{cC} ±0.13	6.10^{bC} ±0.08	6.27^{cC}±0.22	6.07^{aA} ±0.12
	TBA values					
0	1.51^{bA} ±0.13	1.56^{bA}±0.23	1.47^{aA} ±0.08	1.87^{aA} +0.20	1.69^{cA}±0.22	1.92^{dA} ±0.30
3	1.66^{bB} ±0.21	1.90^{cB}±0.14	1.63^{aB} ±0.20	2.09^{dB} ±0.23	2.03^{dC}±0.31	2.19^{eB} ±0.32
6	1.65^{aB} ±0.28	2.14^{cC}±0.10	1.78^{bC} ±0.35	2.29^{eC} ±0.23	1.77^{bB}±0.25	2.58^{dC} ±0.28

Means bearing same small letters in a row and capital letter in a column within each criterion are not significantly different (P<0.05)

TABLE 2. PROXIMATE COMPOSITION AND ORGANOLEPTIC SCORES OF PATTIES AS INFLUENCED BY BINDERS, SPECIES AND STORAGE PERIOD AT 5±1°C

Storage period, days	Binders: 3% gram flour		1.5% gram flour + 1.5% egg albumen		1.5% *maida* + 1.5% egg albumen	
	Chicken	Duck	Chicken	Duck	Chicken	Duck
	Moisture					
0	60.33^{aC} ±1.47	60.55^{cA} ±1.28	58.03^{aB} ±1.26	66.28^{bA} ±0.73	64.09^{cA} ±0.80	66.82^{cA} ±1.48
3	54.41^{aA} ±1.35	66.25^{cA} ±1.10	55.74^{aA} ±1.54	67.99^{cB} ±1.26	60.14^{bB} ±1.33	66.05^{cA} ±1.57
6	57.06 ±2.05	66.75 ±0.75	57.10 ±1.73	66.90 ±0.63	58.35 ±1.80	66.80 ±1.30
	Crude proteins					
0	24.97^{bC} ±1.28	22.39^{aB} ±1.27	25.92^{bB} ±1.24	22.02^{aB} ±1.70	27.21^{CB} ±1.59	22.24^{aB} ±1.38
3	22.48^{aB} ±1.39	21.91^{aB} ±1.93	23.38^{bA} ±1.24	21.86^{aB} ±1.36	24.81^{cA} ±1.11	22.20^{aB} ±1.14
6	19.46^{aA} ±1.59	19.28^{aA} ±1.28	22.07^{bA} ±1.01	19.71^{aA} ±0.95	24.12^{cA} ±1.21	19.64^{aA} ±0.68
	Ether extractives					
0	17.88^{cC} ±1.16	10.92^{aA} ±0.41	15.50^{bB} ±1.20	15.50^{aB} ±1.20	15.89^{bB} ±0.86	10.48^{aB} ±0.52
3	15.92^{cB} ±1.29	10.97^{aA} ±0.71	13.48^{bA} ±0.61	10.34^{aB} ±0.30	13.10^{bA} ±1.08	9.57^{aA} ±0.62
6	14.29^{cA} ±1.48	10.39^{aA} ±0.49	13.92^{cA} ±0.53	9.64^{aA} ±0.09	12.32^{bA} ±0.82	9.51^{aA} ±0.56
	Total ash					
0	1.65^{bA} ±0.15	1.39^{aA} ±0.03	1.85^{bA} ±0.16	1.45^{aA} ±0.10	1.78^{bA} ±0.10	1.29^{aB} ±0.11
3	1.72^{bA} ±0.18	1.79^{bB} ±0.09	1.72^{bA} ±0.12	1.85^{bB} ±0.16	1.66^{aA} ±0.14	1.48^{aA} ±0.14
6	1.73 ±0.17	1.73 ±0.18	1.63 ±0.14	1.75 ±0.12	1.68 ±0.16	1.39 ±0.05
	Colour					
0	6.93^{bB} ±0.07	6.41^{aA} ±0.14	6.90^{bB} ±0.07	6.39^{aA} ±0.05	6.93^{bB} ±0.07	6.47^{aB} ±0.10
3	6.33^{aA} ±0.13	6.35^{aAB} ±0.11	6.53^{bA} ±0.08	6.35^{aA} ±0.05	6.49^{bA} ±0.15	6.28^{aA} ±0.09
6	6.29^{bA} ±0.10	6.15^{aA} ±0.12	6.42^{cA} ±0.12	6.30^{bA} ±0.18	6.35^{bcA} ±0.14	6.28^{bA} ±0.11
	Flavour					
0	6.67^{cC} ±0.19	6.28^{aA} ±0.12	6.73^{cB} ±0.12	6.45^{bA} ±0.08	6.83^{dB} ±0.07	6.62^{cB} ±0.05
3	5.90^{aA} ±0.15	6.18^{bA} ±0.14	6.36^{cA} ±0.19	6.31^{cA} ±0.10	6.66^{dA} ±0.15	6.08^{bA} ±0.22
6	6.24^{aB} ±0.08	6.25^{aA} ±0.14	6.52^{cA} ±0.10	6.39^{bA} ±0.12	6.58^{cA} ±0.18	6.23^{aA} ±0.15
	Juiciness					
0	6.27^{cB} ±0.20	6.41^{bB} ±0.16	6.37^{abA} ±0.20	6.65^{cB} ±0.17	$6..50^{bB}$ ±0.12	6.89^{dB} ±0.15
3	5.97^{aA} ±0.21	6.34^{bAD} ±0.11	6.28^{bA} ±0.19	6.56^{cA} ±0.09	6.70^{dc} ±0.10	6.56^{cA} ±0.17
6	6.03^{aA} ±0.18	6.23^{bA} ±0.20	6.28^{bA} ±0.13	6.39^{bcA} ±0.16	6.29^{bA} ±0.12	6.48^{cA} ±0.21
	Tenderness					
0	6.27^{aA} ±0.12	$6..67^{cB}$ ±0.20	6.33^{abB} ±0.13	6.77^{cA} ±0.18	6.40^{bA} ±0.20	7.00^{dB} ±0.12
3	6.19^{aA} ±0.20	6.43^{cA} ±0.15	6.31^{bB} ±0.21	6.60^{dA} ±0.21	6.44^{cA} ±0.18	6.64^{dA} ±0.17
6	6.25 ±0.22	6.60 ±0.19	6.07 ±0.10	6.65 ±0.20	6.35 ±0.23	6.70 ±0.20
	Overall acceptability					
0	6.67^{bB} ±0.09	6.55^{ab} ±0.13	6.60^{abB} ±0.07	6.61^{abB} ±0.12	6.60^{abB} ±0.18	6.67^{bA} ±0.06
3	6.05^{aA} ±0.08	6.27^{bA} ±0.12	6.40^{cA} ±0.12	6.62^{adB} ±0.04	6.44^{cA} ±0.14	6.52^{cd} ±0.05
6	6.19^{aA} ±0.16	6.22^{abA} ±0.20	6.30^{bA} ±0.10	6.35^{bA} ±0.14	6.36^{bA} ±0.10	6.64^{cA} ±0.09

Means bearing same small letters in a row and capital letter in a column within each criterion do not differ siginificantly ($P<0.05$)

Addition of egg albumen improved the sensory quality of patties (Table 2), as also observed by Hussain et al (1988) in rabbit meat patties. Fat oxidation, as indicated by increased TBA values (Table 1) in stored patties was the reason for getting lower colour and flavour scores. Loss of moisture during storage (Table 2) caused the patties to obtain lesser juiciness and tenderness scores (Table 1). Thind et al (1988) also observed decreased organoleptic scores in stored chicken meat patties.

Based on the above observations it can be concluded that inclusion of egg albumen as binder in both chicken and duck meat patties is beneficial by improving the nutritional and organoleptic characters. Further, the prepared patties can be preserved under refrigeration for 6 days.

References

Anjaneyulu ASR, Kondap N, Salhudding MP, Pande B (1990) Quality of patties from chicken, mutton and their combination. Food Sci Technol 27:184-189

AOAC (1970) Official Methods of Analysis, 11th edn. Association of Official Analytical Chemists, Washington, DC

Cunningham FE, Bowers JA (1977) Composition, microbial content and stability of chicken patties held at refrigeration temperature. Poultry Sci 56:93-97

Hussain A, Keshri RC, Sharma N (1988) Effect of binders on organoleptic and physico-chemical properties of rabbit meat patties. Indian J Meat Sci Technol 1:91-93

Judge MD, Hangh CG, Zachariah GL, Parmelee CE, Pyles RL (1974) Soy additives in beef patties. Food Sci 39:137-139

Lyon BG, Lyon CE, Jownsond WE (1978) Characteristics of six patti formulas containing different amounts of mechanically deformed broiler meat and deboned fowl meat. Food Sci 43:1656-1661

Molonon B, Bowers JA, Cunningham F (1978) Eating quality of groundnut chicken soy patties. Poultry Sci 55:1553-1556

Padda GS, Sharma BD, Keshri RC, Sharma N (1987) Development of chicken patties with indigenous flavour profiles. Charion 16:182-184

Snedecor GW, Cochran WH (1968) Statistical Methods. 6th edn Oxford and IBH Publishing Co, Calcutta

Thind SS, Behia AS, Sekhon KS (1988) Partial replacement of chicken meat with giblets in the preparation of patties. Poultry Guide 25(4):46-48

Thomson LD, Arafa AS, Jony DM (1983) Stability of emulsions incorporating mechanically deboned poultry meat and soy flours. Poultry Sci 62:1368-1377

Witte VC, Kauze GF, Sailey HE (1970) A new extraction method for determining 2-thiobarbituric acid values for pork and beef during storage. Food Sci 35:582-583

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Virulence Factors of *Edwardsiella tarda* Isolated from Fish

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Of the 7 isolates of *Edwardsiella tarda* obtained from fish samples, 5 were lethal to mice, causing diarrhoea and bacteremia followed by death. Cell-free culture filtrates of these 5 isolates exhibited heat-labile enterotoxic effect in rabbit ileal loops, caused oedema in mouse foot pads, induced delayed vasopermeability in rabbit skin and cytotoxic effect in vero cells. All the 7 isolates exhibited type 3 fimbriae and were resistant to colistin and gentamicin, but sensitive to ciprofloxacin, chloramphenicol, nalidixic acid, nitrofurantoin, ofloxacin and streptomycin. All the 5 pathogenic strains had either type 1 fimbriae or colonization factor.

Keywords: Enterotoxicity, Verotoxicity, *Edwardsiella tarda*, Fish, Fimbriae types, Colonization, Pathogenicity.

Strains of *Edwardsiella tarda* are commonly associated with animals (Farmer and McWhorter 1984; Singh et al. 1994). The role of *E. tarda* as foodborne pathogen is uncertain. However, their high occurrence in fishes and common symptoms of diarrhoea among fish consumers indicated that fishes could be the reservoirs of this enteropathogen (VanDamme and Vadepitte 1980). In addition, *E. tarda* has also been isolated from human patients with septicemia, meningitis and internal abscesses (Clarridge et al. 1980). However, causal relationship of *E. tarda* with the above ailments has not yet been conclusively established and is not regarded as an inherent pathogen, but rather a rare opportunistic pathogen for humans (Farmer and McWhortar 1984). Considering the importance of culturing inland fisheries in India, this study was initiated to assess the presence of *E. tarda* in fresh water fishes. Besides, isolates *of E.* tarda were further characterized for the relevant virulence factors through the use of bioassay models.

Thirty whole fish samples of river origin were procured from various retail markets in and around Bareilly city of North India and processed according to standard method (ICMSF 1978). For swabbing cloacal regions of river fishes (67) and pond fishes (30), moistened sterilized cotton swabs were used. These were kept individually in sterilized glass vials containing 8-10 ml of strontium chloride B medium-SCBM (Farmer and McWhorter 1984). All samples were transported to the laboratory in an ice box within 30-45 min of collection. Tubes containing swabs were incubated at 35°C for 24 h. Fish samples in aliquots of 25 g were homogenized separately in 225 ml of 0.1% peptone-water (pH 7.2±0.2) and incubated for 8 h at 35°C, from which 1 ml of broth culture was transferred to 25 ml of SCBM and incubated at 35°C for 24 h.

The growth from SCBM was streaked on Hi-media xylose lysine desoxycholate agar (XLDA) and *Edwardsiella* agar (Singh et al. 1994) and incubated at 35°C for 24 h. Typical black centered pink colonies formed on *Edwardsiella* agar and dark or black centered pink colonies formed on XLDA were picked up and further identified on the basis of cultural and biochemical characteristics, according to Farmer and McWhorter (1984). Isolates of *E. tarda* strains were maintained in stabs of semi-solid nutrient agar, containing 1.0% agar in screw-capped vials for further testing.

Standard strains of *Escherichia coli* E 384 and E 358 and *Shigella dysenteriae* E-218 were obtained from National *Salmonella* centre, Indian Veterinary Research Institute, Izatnagar and maintained at 4°C by sub-culturing on nutrient agar slants at an interval of 21 days.

Isolates of *E. tarda* were characterized for a few of the important virulent factors. The invasive ability was determined by production of keratoconjunctivitis in guinea pig eyes model (Sereny 1957), using *S. dysenteriae* E-218 as positive control. Congo red dye binding assay was performed, as described by Ishiguro et al (1985) with *S. dysenteriae* E-218 and *E. coli* E-358 as positive and negative controls, respectively. Cell surface hydrophobicity responsible for bacterial adhesion was assessed among isolates of *E. tarda*. Strains of *E. coli* E-384 and E-358 were used as positive and negative controls, respectively (Lindahl et al. 1981). Types of fimbriae were characterized by modified haemagglutination (HA) test of Simmons et al

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(1988). Mouse lethality assay was performed on healthy swiss mice (Cruickshank et al. 1968). Ten mice, were injected through intraperitoneal route with 0.1 ml growth of *E. tarda* isolates, while in the negative set of mice, heat-killed culture broth was used. Antibiogram pattern of *E. tarda* isolates was tested by standard disc diffusion method (WHO 1961), using Hi-Media antibiotic discs. Haemolysis of the isolates was determined on blood agar plates, using guinea pig, rabbit and sheep defibrinated blood samples (Cruickshank et al. 1968).

Enterotoxigenicity of *E. tarda* isolates was confirmed by rabbit ligated ileal loop (RLIL) test of De and Chatterjee (1953), suckling mouse assay (SMA) of Dean et al (1972), vasopermeability factor test (VPFT) on abdominal skin of albino rabbits (Sandefur and Peterson 1976), mouse foot pad (MFP) test in hind foot pads of adult albino swiss mice (Singh and Kulshrestha 1992) and on vero cells monolayers in 96 well tissue culture plates (Spiers et al. 1977). For the above tests, cell-free culture supernatants (CFCS) were obtained by growing the isolates for 24 h at 35°C in brain heart infusion (BHI) broth. Samples of CFCS were divided into 3 aliquots, of which, 2 were individually heated at 63°C for 30 min and 72°C for 15sec, while the remaining aliquots were kept unheated (positive control).

In all, 7 isolates of *E. tarda* were obtained from 7 samples of fishes with the use of *Edwardsiella* agar, while only five samples were found positive for the organism with the use of XLDA, which proved the superiority of the former. The cultural and biochemical characteristics were in conformity with those documented earlier (Farmer and McWhorter 1984). It has been established that fishes are the main reservoirs of *E. tarda* (Van Damme and Vandepitte 1980). In the present study, not much difference was observed in relation to the occurrence of *E. tarda* between pond and river fishes. There appears to be an increase in the prevalence of *E. tarda* in fishes of Bareilly, probably due to its intensified fishery.

Of the 7 *E. tarda* isolates, none caused visible change in the eyes of guinea pigs. Only 2 strains, 4E2 and 6E6 yielded positive congo red adsorption test. Salt coaggregation test was negative with all the 7 antigens prepared according to Lindahl et al (1981), while bacterial antigens of strains 4E1, 4E2 and 6E4 prepared by growing in BHI broth yielded positive results. All the 7 strains had type 3 fimbriae. Besides, four (4E1, 6E3, 6E4, 6E6) and one (4E2) strains were also possessing colonization factor antigen and fimbriae type 1 antigen, respectively and were lethal to mice (Table 1). All the mice, which died following inoculation, had soiling of their tails with loose faecal matter. The CFCS of all the 5 mice lethal *E. tarda* strains caused rounding and conglomeration of cells, followed by detachment of monolayers of vero cells. The toxic CFCS preperations also induced fluid accumulation in RLIL, oedema in mouse foot pad and delayed vasopermeability response in rabbit skin (Table 2). None of the 7 CFCS, heat-treated either at 63°C for 30 min or at 72°C for 15 sec yielded positive response in suckling mouse assay or in any of the above bioassay models (Table 2). All the *E. tarda* strains were negative for haemolysis of rabbit, sheep or guinea pig erythrocytes.

Antibiotic sensitivity results revealed that all the 7 *E. tarda* isolates were resistant to colistin and gentamicin, two (6E3, 6E6) to cephalexin and one (6E6) to ampicillin, penicillin, cotrimoxazole, norfloxacin, streptomycin and tetracycline, while, all strains were sensitive to ciprofloxacin, chloramphenicol, nalidixic acid, nitrofurantoin and ofloxacin (results not shown). Re-isolation of *E. tarda* strains from blood and intestinal contents of mice

TABLE 1. RESULTS SHOWING VIRULENCE MARKERS OF *E. TARDA* ISOLATES FROM FISH

Strain No.	Source	Types of fimbriae present	Coloni -zation factor	With MA	CRDT	% mouse lethality on IP injection within 24h	48h	2 weeks
3E1	Cloacal swab of river fish	3	Absent	>2M	-	0	0	0
3E5	Cloacal swab of pond fish	3	Absent	>2M	-	0	0	0
4E1	Cloacal swab of pond fish	3	Present	0.1M	-	40	80	100
4E2	Cloacal swab of river fish	3, 1	Absent	0.1M	+	0	20	40
6E3	Whole fish (homogenized)	3, 4	Present	>2M	-	30	70	100
6E4	-do-	3, 4	Present	0.75M	-	40	90	100
6E6	-do-	3, 4	Present	>2M	+	50	100	100

MSC: molar concentration of ammonium sulphate required for salt coaggregation of bacterial suspension, LA: bacterial antigen prepared by the method of Lindahl et al (1981), MA: bacterial antigen prepared by the modified method, CRDT: congo red dye adsorption test, IP: interaperitoneal injection of about 10^4 *E. tarda* organism in 0.1 ml broth : **MSC was >2M in all cases**

TABLE 2. ENTEROTOXIGENICITY PATTERN OF *E. TARDA* ISOLATES FROM FISH

Strain No	Effect of CFCFs' in				
	RLILTI	MFPTI	SMAI	LVPFT Zone of blueing in mm	VCA
3E1	0.30	1.01	0.056	0	Nil
3E5	0.25	1.07	0.055	0	Nil
4E1	0.95	1.50	0.055	22	++
4E2	0.85	1.40	0.054	15	++
6E3	0.90	1.40	0.053	24	+++
6E4	0.95	1.50	0.054	22	+++
6E6	1.25	1.80	0.055	25	++++
Minimum limit of positivity	0.8	1.20	0.070	10	in CPE>40% cells

CFCF : cell-free culture filtrate, RLILI: rabbit ligated ileal loop test index, MFPTI : mouse foot pad test index, SMAI : suckling mouse assay index, RVPFT: rapid vasopermeability factor response (after 2-4 h of CFCS injection), LVPFT : delayed vasopermeability factor response (after 18 h of CFCS injection), VCA : Vero cell toxicity assay

Note : None of the above test gave positive response with heated CFCS either at 63°C for 30 min or at 72°C for 15 sec

RVPFT zone of blueing was zero in all cases

following IP inoculation indicated the possibility of invasive nature of organism. However, invasiveness of the organisms could not be substantiated by Sereny's or CRD tests. Similarly, no correlation between mouse lethality and hydrophobicity could be established.

Fimbriae type 3, important for survival of *E. tarda* in nature by anchoring the organism on aquatic plants and body surface of aquatic animals (Holmes and Gross 1990) did not appear to be related with pathogenicity. It appeared convincing to assume the role of fimbriae type 1 and colonization factor in pathogenicity of *E. tarda*, because all the 5 pathogenic strains possessed either of these two attributes.

Detection of *E. tarda* toxin causing marked changes in vero cells and other biomodels is significant, as it might be responsible for diarrhoea and other pathological lesions of mice. Further, detection of multiple drug resistance in strains 6E6 and 6E3 of *E. tarda* is a significant finding, as these types of strains may lead to severe public health problems. These strains may survive better in antibiotic-treated fish culture ponds. For elucidation of physico-chemical nature of *E. tarda* enterotoxin and cytotoxin, their genetic control and relative role in pathogenesis, further investigations are required.

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References

Clarridge JE, Musher DM, Fainstein V, Wallace Jr. RJ (1980) Extra-intestinal human infections caused by *Edwardsiella tarda*. J Clin Microbiol 11: 511-514

Cruickshank R, Duguid JP, Swain RHA (1968) Medical Microbiology. 11th edn. ELBS and E&S Livingstone Ltd, Edinburgh. pp 1016-1017

De SN, Chatterjee DN (1953) An experimental study of mechanism of action of *Vibrio cholerae* on intestinal mucous membrane. J Pathol Biol 66: 559-562

Dean AG, Ching Y, Williams RG, Harden LB (1972) Test for *Escherichia coli* enterotoxin using infant mice : Application in a study of diarrhoea in children in Honolulu. J Infect Dis 125: 407-411

Farmer JJ, Mcwhorter AC (1984) *Edwardsiella*. In: Krieg NR, Holt JG Williams W (eds), Bergey's Manual of Systematic Bacteriology, Vol 1, pp 486-491

Holmes B, Gross RJ (1990) Coliform bacteria. In: Parker, MT Duerden BI, Edwards A (eds), Topley and Wilson's Principles of Bacteriology, Virology and Immunity. 8th edn, London. pp 415-442

ICMSF (1978) Microorganisms in foods. 2nd edn., International Commission on Microbiological Specifications for Foods. University of Toranto Press, Toronto, Canada

Ishiguro EE, Ainsworth T, Trust TJ, Kay WW (1985) Congo red agar, a differential medium for *Aeromonas salmonicida* detects the presence of cell surface protein, assay involved in virulence. J Bacteriol 164: 1233-1237

Lindahl M, Faris A, Wadstrom T, Hjerten S (1981) A new test based on salting out to measure relative surface hydrophobicity of bacterial cells. Biochem Biophys Acta 677: 471-476

Sandefur PD, Peterson JW (1976) Isolation of skin permeability factors from culture filtrates of *Salmonella typhimurium*. Infect Immun 14: 671-679

Sereny B (1957) Experimental keratoconjunctivitis shigellosa. Acta Microbiol Acad Sci Hungary 4: 367-376

Simmons KW, Wooley RE, Brown J (1988) Comparison of virulence factors and R-plasmids of *Salmonella* spp isolated from healthy and ill swine. Appl Environ Microbiol; 54: 760-767

Singh BR, Kapoor KN, Bhilegaonkar KN, Kumar A (1994) *Edwardsiella tarda*, an emerging foodborne infection, Project report, 1993-94, Indian Veterinary Research Institute, Izatnagar. India. p 17

Singh BR, Kulshreshtha SB (1992) Preliminary examination on the enterotoxigenicity of isolates of *Klebsiells pneumoniae* from seafoods. Int J Food Microbiol 16: 349-352

Spiers JI, Stavric S, Knowalchuk J (1977) Assay of *Escherichia coli* heat-labile enterotoxin with vero cells. Infect Immun 16: 617–622

VanDamme LR, Vandepitte J (1980) Frequent isolation of *Edwardsiella tarda* and *Plesiomonas shigelloides* from healthy Zairese fresh water fish: A possible source of sporadic diarrhoea in the tropics. Appl Environ Microbiol 39: 475–479

WHO (1961) Standardization of methods for conducting microbial sensitivity test. Technical Report Series No. 210. World Health Organization, Geneva

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FOOD HYGIENE AUDITING - Edited by N. Chesworth, Published by Blackie Academic and Professional, First Edition 1997, Chapman and Hall, 2-6, Boundary Row London, SEI BHN, UK, ISBN 07514 02338, 1997, pp 198, Price £ 59/-

Food hygiene and its practice and maintenance are fundamental to the observance of good manufacturing practices and consequently to food safety. There is none of the latter without the former two. In an era, when every attempt at obtaining quality in food production is seen through the colours of ISO 9000 and/or HACCP principles, there is much in the way of organization, identification, documentation and performance of hygiene related acts, which factory owners, supervisors and floor workers need to know before their efforts will translate into a successful and safe end product each time and every time. The crucial and operative words are 'identification' and 'performance', which depend on organization and documentation. One must know what comprises food hygiene and the origin of factors or defects in the system, which can compromise the acceptable state of hygiene, one must know troubleshooting and how to take remedial action. Codes of hygienic practice of food manufacture (various situations) are described under the Bureau of Indian Standards, but are never easy to follow for the simple reason that they are no clear cut and specific directions to practise. The act of taking stock of and note of where and how to food hygiene or rather a food hygiene 'auditing' (which I am sure is a fall out of the term 'Quality auditing ' which is done to continue to bask under ISO 9000 certification) is now laid out in the book entitled 'Food Hygiene Auditing' edited by Mrs. N. Chesworth, who is a consultant food technologist, Tyne and Weark, Great Britain. This book is a collection of 11 articles, each one authored by a person connected to an academic institution of training or Technical Services related to food and its Science and Technology. Two major articles, very important from the point of country of origin discuss the regulatory aspects : Chapter 1 written by P. Wright, Head of Environmental Services in a Borough/Council describes the spirit and substance contained in Food Safety Act of 1990, the Food Safety (general food hygiene) Regulation 1995 and Food Safety (temperature control) Regulation 1955 as written by HMSO. Their Food Safety Act of 1990 sets out what is meant by food, food business, food premises, food for sale, who is to enforce the law and the power structures in the enforcement heirarchy and provides for quote 'due diligence system' unquote for defence in an ensuing legal matter. The Food Safety (general hygiene) Regulations 1955 places a major obligation on the proprietor of the food business, on each food handler, who knows or suspects that he is suffering from certain diseases transmissible through food. The Rules of Hygiene in these regulations give precise guidelines on the general requirements for food premises and includes such small, but essential details like the position and working of wash basins and flush toilets. The temperature control is an inescapable factor in every successful and safe food handling is emphasized by creating the Food Safety Regulations 1995. The food manufacturers in Great Britain are, therefore, well equipped to maintain and monitor all aspects of Food Hygiene by the detailed description of all important provisions in the Act.

Food Law in the United States is discussed in Chapter 2 by Dr. O.P. Snydes Jr., who is the President of Hospitality Institute of Technology and Management in Saint Paul, Minnesota USA. The Food and Drug Act of 1906, the Meat Inspection Act of 1906 and major amendments to the Federal Food Drug and Cosmetic Act 1938 are very clearly spelt out. Regulations for fair packaging, nutrition labelling, colour additives and pesticides are listed in the individual acts or amendments. This is indeed a ready reference source for such valuable information presented in a concise manner. These food manufacturers, who are targeting their products for the export market, will benefit by being conversant with this type of information. These two chapters complement our own Prevention of Food Adulteration Act (PFA) 1954.

The point of purpose of the book is exemplified and introduced by the subject of chapter 3, which is HACCP and factory auditing presented by WJ Crossland, Technical Manager in F.T. Sutton and Son Ltd of UK. There have been many articles, which described the 7 principles of HACCP exercise, but to include basic definitions and application-oriented questions and CCP decision free under one source is in my opinion a very useful and practical approach to understanding and practising the HACCP principles. The factory auditing takes off with an explanation of the purpose of audit, which broadly is quote 'to assess that the facility is physically suitable and capable to produce a safe product that legal constraints on the product are

being met that effective control measures are in place to ensure consistent performance, the general ability to produce a consistant specified quality, the management commitment to quality and safety, whether measures are in place to support a Due Diligence Defence, or a focus on a particular problem or quality issue' unquote.

The specifies of Pre-audit meeting, preparations for audit auditing, results and documentation, post-audit review and summary, audit report and the very important auditee response (after affirming any corrective actions and timescales) are given in a lucid narrative manner. The actual instruments of food hygiene auditing are presented in the 8 subsequent chapters. These pertain to premises (design and fabrication, raw materials, process equipment and machinery, personnel hygiene standards, preventive pest control, cleaning and disinfecting systems and management controls.

In a compact book of 192 pages plus 3 pages for subject index, the chapters are neatly laid out with appropriate sub-headings and easy to locate specific topic description. It is a hard cover book with a loose jacket made out in bright green with touches of blue and white. There is the list of 11 contributors, but I am disappointed that there is no preface by anyone or the editor herself. Prefaces give an indication of things to come and the purpose and need for choosing a subject for a book. Though the subject of the book itself covers a very current topic of interest, I am at a loss to know what provoked (motivated and inspired?). Mrs. Chesworth to put together such an informative bunch of articles, which in my considered opinion, can be used as a reference manual both in a factory and in a training class, workshops or by a prospective auditor for self education. That in UK, there are perhaps no such manuals available right now, may be a good supposition from the style of writing the Food Hygiene Auditing. Be that as it may, it certainly will be very useful to teachers and students of food science and technology and to factory managers and supervisors in India. It is a very good book indeed to reach for, when in doubt about what comprises auditing. HACCP, food laws or remedial action. The price of the book is affordable to academic libraries, big and small manufacturers and others, who take a keen interest in the subject.

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AFST (I) News

Notice for the Annual General Body Meeting (AGBM)

This is in continuation of the earlier circular dated 1/7/1997 sent out to our members regarding AGBM and election for the year 1997-98. It may be noted that the Annual General Body Meeting of the Association will be held at Multipurpose Hall of BARC Training School Hostel in Mumbai on **Friday, the 26th September 1997 at 5.30 pm.**

M.S. Krishnaprakash
Hony. Exec. Secretary

INDIAN FOOD INDUSTRY

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