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REVIEW *J. Food Sci. Technol., 1999, Vol. 36, No. 6, 487-499*

Oxidative Problems in Meat and Meat Products and Use of Antioxidant Vitamins

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Meat consumption in India is considered as a status symbol, because of its appealing flavour and texture, high nutritional value and price. In recent years, meat industry has come under increasing scrutiny because of concerns such as those relating to saturated fat, cholesterol and heart disease, food safety, animal welfare and environmental protection. In general, there is now a greater demand than ever before by consumers for foods perceived as natural, fresh-tasting, healthy, more nutritious and without any residue of chemical preservations. One of the main factors limiting the quality and acceptability of meat and meat products is lipid oxidation, which has been of increasing interest now, especially in relation to improvement of the quality of ready-to-eat and convenient meat products. An attempt has been made in this review to appraise the oxidation problems in meat and meat products and use of antioxidant vitamins to prevent them.

Keywords : Meat and meat products, Lipid oxidation, Pigment oxidation, Antioxidant vitamins, Physico-chemical qualities, Microbiological qualities.

Introduction

Lipid oxidation in general and specifically oxidation of phospholipids has been found to be responsible for the characteristic rancid off flavour developing in pre-cooked meat (Ladikos and Longovois 1990; Salih et al. 1989; Wu and Sheldon 1988). Furthermore, it has been found that development of warmed over flavour (WOF) is related to the content of myoglobin in the muscle, chicken dark muscle being more unstable towards oxidation due to more myoglobin content than white muscles (Igene et al. 1979). Earlier, Tappel (1962) proposed that haematin compounds catalyze the decomposition of hydroperoxides, in effect propagating further lipid oxidation. Harel and Kanner (1985) and Johns et al (1989) found that heat denatured myoglobin appear to be more effective catalyst than inorganic iron for the cleavage of hydroperoxides in muscle tissues. The lipid oxidation processes lead to discolouration, drip losses, off-odour and off-flavour development and the production of potentially toxic compounds (Morrissey et al. 1994 a ; Gray et al. 1996). The oxidation effects on proteins, peptides and amino acids can impair texture, flavour and nutritional value (Spanier et al. 1992). Recently, Adegoke et al (1998) critically reviewed the aspects of antioxidants and lipid oxidation in foods.

The low oxidative stability of meat and pre-cooked and restructured meat products is a problem for all those involved in the meat production chain, including the primary producers, processors, distributors and retailers. Understanding and controlling the processes which lead to lipid oxidation is a major challenge for meat scientists. The above oxidative effects in meat can be minimised or prevented by chemical/synthetic antioxidants, but for the carcinogenic and other adverse effects of BHA (Ito et al. 1986), BHT (Takahasi 1992), TBHQ (Van Esch 1986) and other synthetic antioxidants, the health conscious meat consumers now disapprove the use of chemical preservatives in meat foods because of their residual effect. On the other hand, vitamins C and E are the two potent naturally occurring antioxidants.

Realising the importance of the problems related to the above research area and the public preferences, an attempt has been made to review the different aspects of lipid oxidation, pigment oxidation and use of ascorbic acid and tocopherol acetate to improve the quality, especially the oxidative stability of meat and meat products.

Lipid Oxidation

Food lipids oxidation is considered to be a risk factor for human health. Some lipid oxidation products and a few cholesterol oxides (COPs), in particular, are considered atherogenic agents and appear to have mutagenic, carcinogenic and cytotoxic properties. Furthermore, COPs appear to be able to replace cholesterol molecules in biomembranes perturbing permeability, stability and other membrane properties (Sevanian and Peterson 1986; Kubow 1990; Guardiola et al. 1996). Lipid oxidation is one of the primary causes of deterioration in the quality of meat and meat

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products during storage leading to the development of off flavour, loss of colour and texture and decrease in nutritive value (Buckley et al. 1995). It has been suggested that membranal phospholipids, which are high in polyunsaturated fatty acids are responsible for the initial development of oxidised flavour in raw and cooked products during storage (Gray and Pearson 1987; Pearson et al. 1977). The mechanisms of oxidation of polyunsaturated fatty acids, production of lipid oxidation, *in vivo* effects of peroxidation, factors affecting oxidation and control of lipid oxidation (Hsieh and Kinsella 1989); oxidation rancidity and discolouration of meat (Watts 1954); oxidative processes in meat and meat products: quality implications (Kanner 1994) had been overviewed. A review of the chemistry of free radicals in lipids was presented by Hamilton et al (1997).

The first step in lipid oxidation is the removal of a hydrogen from a methylene carbon in a fatty acid (RH). This becomes easier as the number of double bonds in the fatty acid increases, due to which polyunsaturated fatty acids are particularly susceptible to oxidation. The initiation step can be catalysed by HO• or by certain iron–oxygen complexes (e.g., ferryl or perferryl radicals).

$$RH + HO^{\bullet} \rightarrow R^{\bullet} + H_2O \qquad (1)$$

The fatty acyl radical (R•) reacts rapidly with O_2 to form a peroxyl radical (ROO•):

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \qquad (2)$$

The rate–constant (k_1) for this reaction is $3X\ 10^8 M^{-1}\ S^{-1}$ Because ROO•, is more highly oxidized than the fatty acyl radical or the fatty acid itself, it will preferentially oxidize other unsaturated fatty acids and propagate the chain reaction:

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet} \qquad (3)$$

The rate–constant (k_2) for this step is relatively low ($10^1\ M^{-1}S^{-1}$). Lipid hydroperoxides (ROOH) formed in the propagation reaction are both products of oxidation and substrates for further reaction with Fe^{2+} and Cu^{+} to yield ROO• and alkoxyl radicals (RO•) (Morrissey et al. 1994b). Fe^{2+} reductively cleaves ROOH (3) as follows:

$$Fe^{2+}\ ROOH \xrightarrow{fast} Fe^{3+} + RO^{\bullet} + OH \qquad (4)$$

and Fe^{2+} can be regenerated as follows:

$$Fe^{3+} + ROOH \xrightarrow{slow} ROO^{\bullet} + Fe^{2+} + H^{+} \qquad (5)$$

$O^{\bullet}_2$ also reduces ferric iron to ferrous and cupric copper to cuprous *in vivo*, allowing a redox cycle in which the transition metal ion is used several times:

$$O^{\bullet}_2 + Fe^{3+} \rightarrow Fe^{2+} + O_2$$

$$O^{\bullet}_2 + Cu^{2+} \rightarrow Cu^{+} + O_2$$

Other strong reductants such as ascorbic acid and paraquat also reduce Fe^{3+} to Fe^{2+}

Both ROO• and RO• can initiate further reactions [e.g. (3) and the following].

$$RO^{\bullet} + RH \rightarrow ROH + R^{\bullet} \qquad (6)$$

The RO• can also undergo β–scission and degrade to alkyl radicals ($R^1CH^{\bullet}_2$) and a range of aldehydes ($R^{11}CHO$) depending on the particular hydroperoxide present (Morrissey et al. 1994b).

$$RO^{\bullet} \rightarrow R^1CH^{\bullet}_2 + R''CHO \qquad (7)$$

($R'CH^{\bullet}_2$) can initiate further chain reactions resulting in the formation of ethane and pentane, while the aldehydes, including hexanal, malondialdehyde and 4–hydroxynonenal, can react readily with ε–amino groups of proteins to yield Maillard type complexes.

The products of lipid oxidation are responsible for unacceptable off flavours and off odour in cooked meats and limit the shelf life of such meats. In a raw meat, lipid oxidation occurs over days or weeks whereas in cooked meat, the reaction proceed rapidly (Tims and Watts 1958; Love and Pearson 1974; Motttram 1987). The susceptibility of specific cooked meat to lipid oxidation is influenced by intrinsic characteristics of the meat species including the unsaturated fatty acid contents and the presence of pro-oxidants and antioxidant components. Such characteristics may be influenced by production factor such as diet. The degree of unsaturation of muscle lipid and susceptibility of muscle to lipid oxidation can be influenced by diet in pigs (Monahan et al. 1992), cattle (Larick and Turner 1989). Dietary iron has been shown to affect iron levels in turkey muscle (Kanner et al. 1990).

Improving the neutral antioxidant status of muscle by dietary means has also attained considerable attention and it is established that vitamin E supplementation through diet increases the level of vitamin E and oxidation stability of muscle. Extrinsic factors such as addition of antioxidants during processing, cooking or packaging can be manipulated to enhance the oxidation stability of the cooked meat. Both synthetic and natural antioxidants have been used in meat products to control lipid oxidation (Chastain et al. 1982). In the preparation of cooked meat products, factors such as cooking duration and temperature can also affect the extent of lipid oxidation (Ang and Huang 1993; Mielche 1995).

Another approach to control lipid oxidation in the cooked meat is the elimination of oxygen from package. This may be achieved either by vacuum packaging or creating modified atmosphere, thus retarding the rate of development of oxidised flavour (Nolan et al. 1989; Cannon et al. 1995; Ho et al. 1995).

Pigment oxidation

Colour that determines meat quality depends on myoglobin, a pigment with several forms. In reduced form, myoglobin is purplish, in oxygenated form this ferrous pigment is bright cherry red and referred to as oxymyoglobin. Myoglobin oxidised to metmyoglobin is characterised by a change in pigment to a brown colour that is not acceptable to the consumer. This is the red to brown transition, which is responsible for the fading of meat. The three forms of myoglobin, deoxymyoglobin, oxymyoglobin and metmyoglobin are constantly being interconverted during storage, resulting in changes in meat colour (Giddings 1974; Van Laack et al. 1990). Their relative contents at the meat surface are governed by the relative rates of metmyoglobin reduction and myoglobin autooxidation (Ledward 1985; Renerre 1990). The accumulation of metmyoglobin at the surface of fresh beef depends on several contradictory mechanisms, such as the rates of oxygen diffusion (Brook 1929), oxygen consumption, autooxidation of the pigment in the presence of oxygen (George and Startman 1952; Brown and Mebine 1969) and the enzymatic reduction of metmyoglobin (Stewart et al. 1965a; Watts et al. 1966).

The rate of metmyoglobin accumulation is muscle dependent. Differences in colour stability between muscles are of considerable importance to the meat industry. Light, pH, temperature, bacterial contamination, lipid oxidation and PO_2 are known to influence the oxidation of myoglobin (Lawrie 1979). Metmyoglobin is constantly generated in muscle and is reduced to ferroderivative form by enzymic systems (Giddings 1974), which work in anaerobic conditions (Stewart et al. 1965b) or in aerobic ones (Ledward 1985). According to Giddings (1974), the loss of anaerobic metmyoglobin reducing activity (MRA) in post-rigor meat is due to factors such as decrease in tissue pH, depletion of substrates and co-factors such as co-enzymes (NADH), oxidative deteriorative changes, decreasing enzyme activities including disintegration of mitochondrial particles. DPN related enzyme systems of raw meat are able to utilise oxygen and in the absence of oxygen to reduce metmyoglobin (Watts et al. 1966).

MRA is known to be influenced by several additives and environmental factors (Stewart et al. 1965a). It increases with pH in the range pH 5.5 - 7.0 and with temperature from 3° to 35°C. NaÇl inhibits MRA. Ledward (1985) attributed the low colour stability of some beef muscles to low reducing capacity. On the other hand, Echevarne et al (1990) concluded that the most unstable muscles with respect to colour had the highest reducing activities and proposed a lack of effectiveness of the reducing system in colour stability regulations during meat storage. Studies by others demonstrated that the rate of meat discolouration was influenced more by factors such as autooxidation of myoglobin and oxygen consumption rate (Govindarajan 1973; O'Keefe and Hood 1982; Renerre and Labas 1987). Meat discolouration during storage is also affected by environmental factors such as temperature, light and oxygen partial pressure (Faustman and Cassens 1990; Ledward 1992; Renerre 1990). It has been known that other factors such as multivalent metal ions (Clydesdale and Francis 1976), pH (Adams 1976), tissue lipid oxidation (Green and Price 1975) and microflora (Ockerman and Cahill 1977) are also responsible for meat discolouration.

Zhu and Brewer (1998) observed that DFD pork had highest MRA and OCR, while the enzyme activity of PSE pork was lower than normal meat. MRA dropped slowly and OCR decreased sharply during storage (4°C). Both MRA and OCR declined more rapidly in the light condition of the display unit. Chan et al (1997) reported that the addition of catalase into the oxymyoglobin-liposome system significantly ($P<0.05$) decreased oxidation of MbO_2 and lipid suggesting a role for H_2O_2 in the interaction between oxymyoglobin and lipid. The addition of superoxide dismutase was without effect, suggesting that superoxide anion was not directly involved in mediating oxidation of MbO_2 and lipid.

The pH of ground chicken meat increased and Hunter 'a' and 'L' values decreased significantly ($P<0.05$), as the refrigerated (3°C) storage time increased. In case of cooked samples, the Hunter 'L' and 'b' values decreased and 'a' increased ($P<0.05$), as storage time progressed. Significant correlations between pH and Hunter colour values of cooked ground chicken meat were observed (Yang and Chen 1993). Mandigo (1982) reported that when the storage time of boneless meat cuts increased before processing, the discolouration of the finished, restructured cuts usually increased. Hunt and Kropf (1987) have indicated that oxidation

of meat pigments is favoured by low pH and seems to occur more rapidly in low-pH muscle.

There is an interaction between pigment and lipid oxidation. It has been found that development of WOF is related to the content of myoglobin in the muscle. Igene et al (1979) found that heat-treated chicken meat from dark muscles was more unstable towards oxidation than white muscles. Tappel (1962) proposed that haematin compounds catalyse the decomposition of hydroperoxides, in effect propagating further lipid oxidation. In contrast, Sato and Hegarty (1971) concluded from model experiments with cooked muscle tissue, that non-haem iron was responsible for the catalytic cleavage of hydroperoxides. This is in agreement with the results of Konopka et al (1995). Other studies, seem to indicate that metmyoglobin is responsible for the cleavage of hydroperoxides in muscle tissues. Harel and Kanner (1985) and Johns et al (1989) thus found that following heat denaturation, haemoproteins appear to be more effective catalysts than inorganic iron. More studies are needed to clarify the role of heat-denatured myoglobin in oxidation of cooked meat.

Vitamin C (Ascorbic Acid)

Ascorbic acid was first isolated by the American biochemists King and Waugh in 1932. It is a lactone of a sugar acid. It is required in the diets of man, monkey, guineapigs and insects. It is not present in microorganisms nor does it seem to be required. Ascorbic acid is a strong reducing agent, readily losing H^+ to become dehydroascorbic acid, which also has vitamin C activity. However, vitamin C activity is lost, when the lactone ring of dehydroascorbic acid is hydrolysed to yield diketogluconic acid. In food, it is largely destroyed by cooking.

Use in meat processing

It is used as a biological antioxidant to stabilize meat colour by way of pre-slaughter injection in beef (Hood 1975), exogenous addition in ground beef (Greene et al. 1971), dip treatment or surface application of beef steaks (Benedict et al. 1975; Harbers et al. 1981; Mitsumoto et al. 1991a; Okayama et al. 1987). In cured meat products such as cooked ham and smoked beef sausages, it reduces the requirement of nitrite, reduces nitrosamine formation (Counsell 1971) and also reduces the time of processing (Pearson and Tauber 1984). In dry fermented sausage, it greatly enhances colour stability (Alley et al. 1992).

Function in meat system

Ascorbic acid acts as an antioxidant by inhibiting radical formation at double bonds of mono or polyunsaturated fatty acids, quenching free radicals, scavenging oxygen and serving as a reductant (Cort 1982; Cabelli and Bielski 1983); Ascorbic acid is a good antioxidant for peroxidations initiated in the aqueous phase, but does not trap peroxy radicals in the lipid phase (Doba et al. 1985). In aqueous systems containing metals, ascorbic acid can act as a peroxidant by reducing the metals, which become more active catalysts of oxidation in their lower valence state (Uri 1961). In the absence of added metals, ascorbic acid is an effective antioxidant at high concentration (Cort 1982). In non-aqueous media, ascorbic acid and esters are not good antioxidants (Porter 1980), Ascorbyl palmitate is a good antioxidant in lipid phase. The multiple effects of ascorbic acid and ascorbyl palmitate include: (a) hydrogen donation to regenerate the stable antioxidant radical, (b) metal inactivation to reduce the rate of initiation by metals; (c) hydroperoxide reduction to produce stable alcohols by non-radical processes and (d) oxygen scavenging.

Pigment oxidation

Sodium ascorbate, when added to ground beef at 500 ppm level, showed lower surface and extract metmyoglobin content of 26.7 and 24.3%, respectively during 7 days illuminated display at 4°C, whereas control samples had corresponding values of 60 and 70%, respectively (Mitsumoto et al. 1991b). Surface spreading with 0.1 ml of 10% vitamin C solution to 20 g beef steaks had lower MMb (40%) as compared to the control sample (60%) after 4 days of refrigerated display (Mitsumoto et al. 1991a). In an aqueous carotene linoleate solution, vitamin C upto 176 ppm acted as a pro-oxidant, while at higher than 176 ppm level it acted as antioxidant (Kanner et al. 1977). The pro-oxidant activity of vitamin C increased with ferric (Fe^{+++}) iron concentration. Vitamin C incorporation maintained good colour in ground beef upto 5 days refrigerated display (Greene et al. 1971 ; Shivas et al. 1984), when added at concentrations of 500 ppm and 1000 ppm as determined by visual colour scores and spectrophotometric colour analysis. The colour stability of sodium ascorbate treated fresh beef muscles, particularly *M. psoas major and M. gluteus medius* held at 5°C was significantly better than the control samples (Hood 1975). The

magnesium salt of ascorbic acid showed a similar protective effect to sodium ascorbate against fading of cooked cured meat colour. This magnesium salt of ascorbic acid-3-phosphate was hydrolysed to A.A by acid phosphatase contained in the water soluble fraction of pork, beef and mutton (Takagi et al. 1971). A comparative study between sodium ascorbate and sodium erythorbate for the technological properties and processes of cooked cured pork loin was made. Residual ascorbate or erythorbate concentration, nitrate and nitrate concentration, colour properties and sensory quality were assessed at intervals during storage. No significant differences were observed between samples made with ascorbate and those made with erythorbate (Blunk 1993).

Sodium erythorbate, a sterioisomer of sodium ascorbate is cheaper to manufacture than sodium ascorbate. It crystalizes in the monohydrate form and its antioxidant activity develops, when it is dissolved in water. It is a highly effective antioxidant owing to its ene-diol structure in various meat products (Anon 1995). Ground buffalo meat containing 500 ppm SA had significantly higher colour score, lovibond tintometer red colour units and lower metmyoglobin contents as compared to other levels of sodium ascorbate. The metmyoglobin was positively correlated with TBARS number (Sahoo and Anjaneyulu 1996a, 1997a).

Addition of reducing agents, i.e., ascorbic acid, isoascorbic acid and their salts improve colour stability and extend storage shelf life of the meat products (Ladikos and Lougovois 1990).

Lipid oxidation

Ascorbic acid treatment showed inhibition of lipid oxidation in beef steaks stored in a cold room at 4°C for 13 days (Okayama et al. 1987). On the other hand, Benedict et al (1975) reported that 50 ppm vitamin C addition to ground beef caused greater TBA value than controls. Exogenous addition of ascorbate delayed lipid oxidation in ground beef (Shivas et al. 1984; Greene et al. 1971). Vitamin C treatment showed high lipid stability in ground beef, recording TBA value of 1.52 as compared to control samples (4.0) during illuminated display at 4°C for 7 days (Mitsumoto et al. 1991b).

Combination of ascorbate and phosphate minimised flavour deterioration in PVC packaged refrigerated cooked beef-carrageenan patties. Reduced fat patties containing ascorbate or phosphate had much less lipid oxidation than their counterparts without additives (Kulshrestha and Rhee 1996). SA showed a protective effect against photoxidation (measured by TBA test), but it did not have an influence on the pate samples in darkness (Perlo et al., 1995). Ground buffalo meat treated with 500 ppm SA had significantly higher odour score and lower TBARS number as compared to control and other levels of SA. The TBARS number was inversely correlated with odour scores (Sahoo and Anjaneyulu 1996a, 1997a). Sato and Hegarty (1971) used SA (0.5%) in ground beef and reported reduced TBA values. High levels of AA (1000, 10000 ppm) inhibited, whereas lower level (3-500 ppm) increased warmed over flavour of muscle tissue. On the other hand, Benedict et al (1975) reported that AA catalysed development of lipid oxidation at low levels upto 250 ppm but inhibited the reaction at high level (500 ppm). Angelo et al (1990) also observed that at high concentration, ascorbates reduced TBARS number in beef patties, whereas at low concentration (250 ppm) increased the TBARS number from 1.99 - 6.55 mg malonaldehyde/kg. At low concentration, 0.02 % ascorbyl palmitate along with 1.5 % sodium chloride increased TBA value in turkey steaks from 0.49 to 1.24 mg malonaldehyde/kg (Akamittath et al. 1990). Ahn et al (1993) also observed that combination of ascorbic acid and tripolyphosphate reduced TBA from 1.24 to 0.60 mg malonaldehyde/kg.

Physico-chemical quality

The improving action of L-ascorbic acid on gel formation of fish may be due to the oxidation of sulphydryl groups in fish protein by dehydro-L-ascorbic acid (Yoshinaka et al. 1972). In addition to colour stabilization and lowering nitrate levels, ascorbic acid can reduce cooking losses in both fat-free and fat containing meat products (Reichert 1994). Ground buffalo meat containing 500 ppm sodium ascorbate had significantly higher pH, lower cooking loss as compared to other levels of sodium ascorbate (Sahoo and Anjaneyulu 1997a). Addition of sodium ascorbate to brine was shown to suppress N-nitrosodimethylamine (NDMA) from added DMA in experimental curing of pork middles. NDMA is formed due to canning or freezing of the cured meats. However, lowest amounts of NDMA were found in the bacon cured in the presence of ascorbate (Mottram et al. 1975). Ascorbate addition proved beneficial in cured meat products (cooked ham, smoked beef sausages), as it helped to reduce nitrite contents to less than 50% and the residual nitrite could be maintained at a lower level. Use

of sodium ascorbate was proved to be superior to sodium erythorbate in the above products (Counsell 1971). The heat stability of ascorbic acid in food during cooking was studied. It was observed that ascorbic acid was oxidised faster in utensils made up of standard aluminium than in enamelled cast iron or standard aluminium coated with pure aluminium. Therefore, cooking utensils made up of standard aluminium and coated with aluminium of 99.99% purity was recommended (Evenshtein 1971).

Sensory quality

In the salami type sausages, made from 74% lean pork and 26% fat ripened for 45–90 days under different temperatures and ventilation conditions, the aroma and taste of sausages (i) containing 25 mg $NaNO_3$ and 100 mg ascorbic acid/kg were found superior to those of sausages, (ii) made with 250 mg sodium nitrate and 150 mg sodium nitrite per kg (Ubertalle and Faccini 1971). No significant difference was observed between the cooked cured pork loin samples made with ascorbate and those made with erythorbate in respect of residual ascorbate or erythorbate concentration, nitrate and nitrite concentration, colour properties and sensory quality during storage (Blunk 1993).

Microbiological quality

No significant effect was observed on the microbiological (aerobic mesophiles count, psychrotrophic plate counts) quality of ground buffalo meat treated with sodium ascorbate at different levels (Sahoo 1995). Shivás et al (1984) and Greene et al (1971) also reported that microbial numbers were not different for ascorbic acid treatments in ground beef. Ascorbic acid did not appear to inhibit bacterial spoilage of beef.

Vitamin E (α-Tocopherol acetate)

Vitamin E was isolated from wheat germ and was given the name tocopherol (a Greek word, tokos meaning child birth). Vitamin E is not synthesized by the animal/human and so a constant dietary supply is required. Its activity is derived from a series of compounds namely, the tocopherols and tocotrienols from plant origins. These have been called nature's antioxidants.. Tocopherols include α, β, γ and δ homologs and the corresponding tocotrienols. The most active and abundant is α-tocopherol. Tocopherols are present in appreciable quantities in all vegetable oils. On the other hand, animal fat contains almost no tocopherol.

Use in meat processing

Vitamin E as an antioxidant has now received much attention for its ability to maintain meat colour, extend shelf life, improve taste, reduce drip loss and offer health benefits (Armstrong 1993). Reduced heart attack risks in uses of vitamin E supplements (Stampfer et al. 1993; Rimm et al. 1993) and reducing susceptibility of food lipoprotein to oxidation (Jialal and Grundy 1992) have encouraged many food scientists to have concerted research efforts in the area of vitamin E supplementation either through diet of meat animals or by exogenous addition or by directly adding to the muscle foods for increasing lipid stability during storage and improving flavour and taste of meat products. The *in vitro* addition of vitamin E appeared to be over 400 times more efficient than *in vivo* supplementation to enhance the quality and stability of rendered fowl fat (Brekke et al. 1975). Vitamin E is very widely used in restructured meat products. In the processed meats such as cooked rabbit meat (Castellini et al. 1998), minced beef (Formanek et al. 1998), cured turkey products (Walsh et al. 1998), pork chops (Asghar et al. 1991), ground buffalo meat and buffalo meat nuggets (Sahoo 1995), vitamin E had been used successfully to enhance product quality and to extend shelf life during storage conditions.

Mode of action in meat system

Vitamin E acts as biological antioxidant in the process of lipid oxidation. Rate and extent of lipid oxidation are dependent on the level of polyunsaturated fatty acids (PUFA) present in the muscle system. It is now generally recognized that the phospholipid present in the sub-cellular membrane, i.e., microsomes, mitochondria, rather than triglycerides are responsible for initial development of oxidised flavour in raw and cooked meat products during storage (Younathan 1985; Gray and Pearson 1987) because phospholipids fraction is highly unsaturated and contains fatty acids with more than 3 double bonds (Pikul et al. 1984a,b). In addition, sub-cellular membrane contains an enzyme system capable of initiating the peroxidative reactions and are thought to be the origin of free radicals to initial lipid oxidation (Monahan et al. 1990).

α-tocopherol is associated with biomembranes and protects them by neutralizing oxidative induced free radicals (Buttrish and Diplock 1988). It acts as an antioxidant by donating a hydrogen atom

to a free radical (Tappel 1962). A logical hypothesis is that α-tocopherol quenches free radicals originating from lipid oxidation and in turn, protects oxymyoglobin oxidation. α-tocopherol inhibits free radical oxidation by reacting with peroxyl radicals to stop chain propagation and with alkoxyl radicals to inhibit the decomposition of the hydroperoxides and decreases formation of aldehydes. α-tocopherol behaves as a chain breaking antioxidant by competing with the substrate for the chain carrying peroxy radicals, normally present in the highest concentration in the meat system (Frankel 1996). It was suggested that vitamin E would protect lipids from oxidation through formation of a semi-quinoid structure (Greene 1969).

Lipid oxidation

Vitamin E treatment resulted in moderate increase of TBA values from 0.45 to 2.16 as compared to control from 1.25 to 4.6 in ground beef during refrigerated storage (4°C) for 7 days (Mitsumoto et al. 1991b; Yin et al. 1993). Several investigators reported that lipid oxidation was delayed with increased concentration of α-tocopherol in meat (Faustman et al. 1989; Arnold et al. 1992). α-tocopherol provides a direct antioxidation effect in the lipid phase and indirectly protects water soluble components such as oxymyoglobin from pro-oxidant free radicals (Yin et al. 1993). The different levels of vitamin E used in the processed meat products by different researchers were 200 ppm in restructured pork (Miles et al. 1986), 100 and 200 ppm in ground pork (Whang et al. 1986), 50 ppm in ground beef (Benedict et al. 1975), 800 ppm solution for dipping beef steaks (Okayama et al. 1987), 10 ppm in ground buffalo meat and buffalo meat nuggets (Sahoo and Anjaneyulu 1997b). High concentration of vitamin E has been shown to exert a pro-oxidant effect (Mahoney and Graf 1986; Cillard et al. 1980). Hence, too much addition of vitamin E to ground meat may accelerate lipid oxidation as well as pigment oxidation. Added α-tocopherol showed oxidation both in raw and cooked ground pork during refrigerated or frozen storage (Whang et al. 1986; Aberle et al. 1985). Similar observation was reported in fresh ham slices and loin chops (Specht-Overholt 1995). Mitochondria and microsome lipids isolated from pork loins from swine receiving the long term treatment (200 mg tocopherol/kg diet for 6 weeks) were more stable to oxidation than from control ones. Retention rates of vitamin E in the meat at 30 and 60 days of storage (2°C) were 61.5% and 28.9%, respectively. The vitamin E contents in pork during storage at -2°C decreased sharply (Kin et al. 1994). Vitamin E supplementation improved immune response, increased serum and muscular α-tocopherol levels, causing delayed metmyoglobin formation in a dose-dependent manner. Supplemental vitamin E was more effective in meat from beef steers than from dairy cattle (Garber et al. 1992). In the muscle from vitamin E supplemented pigs, the development of TBARS remained low during storage at 4°C. The tocopherol levels of muscles tissue did not change during storage (Pfalzgraf et al. 1995). Vitamin E supplementation (250 mg/kg) or spraying directly onto turkey meat after slaughter resulted in lower TBARS values than controls during storage at 2-4°C (Sante and Lacourt 1994). Exogenously added α-tocopherol reduced lipid oxidation and decreased myosin denaturation during heating (Shiao-Jing and King 1996). Use of 10 ppm α-tocopherol for pre-blending of ground buffalo meat significantly reduced TBARS number and increased odour score during refrigerated storage (Sahoo and Anjaneyulu 1997b). Cured reformed cooked turkey ham and cured restructured cooked turkey patties manufactured from turkeys breast meat obtained from turkeys supplemented with dietary α-tocopherol acetate showed better oxidative and colour stability, when compared to non-supplemented controls during refrigerated storage at 4°C (Walsh et al. 1998). α-tocopherol levels in plasma and muscle were significantly higher in the supplemented group of rabbits, which also showed an increased oxidative stability in both raw and cooked meats (Castellini et al. 1998).

Pigment oxidation

Supplementary swine diet with α-tocopherol acetate and 15% ground flaxseed improved pigment stability in the fresh ham slices and loin chops throughout a 6 days retailed shelf life display (Specht-Overholt 1995). Increased colour stability and decreased drip loss were observed on keeping pork chops previously frozen for 3 months at 4°C under fluorescent light for 10 days (Asghar et al. 1991). Vitamin E supplementation to the beef cattle caused delay in metmyoglobin formation of beef in a dose-dependent manner during storage (Garber et al. 1992). Longissimus muscles for Holstein steers supplemented with vitamin E at 500 or 2000 mg per day showed less surface metmyoglobin (MMb) accumulation than control during 12 days storage at 4°C. Vitamin E also diminished the

adverse effect of temperature abuse of muscle at 25°C for 24 h (Chan et al. 1995). Vitamin E supplementation or spraying directly on turkey meat resulted in a lower myoglobin oxidation ($P<0.05$) and a higher Hunter 'a' value at day 2 of storage at 2–4°C (Sante and Lacourt 1994). Ground buffalo meat samples treated with 10 ppm α-tocopherol acetate significantly lengthened the desired visual colour, had higher lovibond tintometer red colour units, chroma and lower MMb contents, indicating pigment oxidative stability during refrigerated storage at 4±1°C (Sahoo and Anjaneyulu 1997b). There was a positive correlation between MMb content and TBARS number. Hutchins et al (1967) also observed a significant positive correlation between MMb and malonaldehyde in stored refrigerated meats.

Physico-chemical quality

Muscle tenderness or muscle pH was not affected due to α-tocopherol acetate in ham slices and loin chops during retail display conditions (Specht-Overholt 1995). TA at 10 ppm level in ground buffalo meat showed lower cooking loss during refrigerated storage (Sahoo and Anjaneyulu 1997b). Dietary α-tocopherol acetate supplementation (200 mg/kg diet) to the hybrid rabbits improved the physical traits of the meat, significantly reducing shear force and increasing WHC, n-3 fatty acids in raw as well as cooked meats, while decreasing thrombogenic index ($P<0.05$) (Castellini et al. 1998).

Addition of antioxidant tocopherols (natural) or BHT (synthetic) prior to irradiation of chicken meat showed synergistic effect in lowering FFA content and TBARS number during chilled storage (Kanatt et al. 1998). Adjusting pH values of ground raw poultry meat to neutral or alkaline showed increase in TBA values during refrigerated storage (Chen and Waimaleongora-Ek 1981).

Microbiological quality

No difference ($P<0.05$) in bacterial load was observed in longissimus muscle from Holstein steers supplemented with vitamin E at 500 or 2000 mg/day during storage (Chen et al. 1995). Similar observation was made in ground buffalo meat treated with 10 ppm α-tocopherol acetate during refrigerated storage (Sahoo and Anjaneyulu 1997b). In general, use of high vitamin E beef versus control beef in patty manufacture had no major effect on populations of aerobic bacteria, coliforms, sorbitol negative bacteria or *L. monocytogenes* in ground beef patties displayed at 4 or 12°C (Cabedo et al. 1998).

Sensory quality

In case of pigs and poultry, high n-3 fatty acids concentration in meat are associated with fishy flavours, whose development can be prevented with high dietary levels of the antioxidant vitamin E (Wood and Enser 1997). There is a relationship of TBA values to the development of off flavours.

Interactive effect of vitamins C and E

The mixture of TA and AA exhibiting a strong synergistic effect is well recognised (Uri 1961; Tappel et al. 1961). Evidence based on pulse radiolysis technique (Packer et al. 1979) and electron spin resonance studies (Bascetta et al. 1983; Niki et al. 1984; Loliger et al. 1986) supports a redox mechanism, involving reduction of the tocopheroxyl radical intermediate by ascorbic acid to regenerate α-tocopherol. By this synergistic mechanism, TA and AA can mutually reinforce one another by regenerating the oxidised form of the other. Another mechanism for synergism involves the metal inactivating effect of ascorbic acid. These are readily decomposed in the presence of metals and their activity is significantly improved by adding a metal inactivator (Frankel et al. 1959). The lipophillic derivative ascorbyl palmitate is also known for its synergistic activity with natural tocopherols in vegetable oils, where it is more active than BHT and BHA (Cort 1974). The mixture of TA, AA or ascorbyl palmitate and phospholipids are also known for their good synergistic activities (Bourgeois 1981).

Pigment as well as the lipid oxidation was inhibited by combined treatment of TA and ascorbate in oxymyoglobin liposome model (Yin et al. 1993) and in ground beef (Mitsumoto et al. 1991b). Ascorbate alone enhanced lipid oxygen radical propagation but its prooxidant effect was reversed, when TA was incorporated (Liebler et al. 1986). Vitamin E acted as primary antioxidant to scavange free radical in lipid phase and ascorbate effectively regenerated α-tocopherol acetate, reacting with vitamin E radical and thus prolonged the antioxidant life of α-tocopherol acetate (Niki et al. 1982; Yin et al. 1993).

Dipping the beef loin steaks into ethanol containing solution of α-tocopherol acetate and ascorbate delayed lipid oxidation significantly, compared to either α-tocopherol acetate or ascorbate alone during 13 days in cold room at 4°C (Okayama

et al. 1987). Ten ppm vitamin E + 500 ppm vitamin C showed the strongest synergism to inhibit pigment and lipid oxidations of beef (Mitsumoto et al. 1991b).

The use of vitamin E and/or C (ascorbic acid or ascorbyl palmitate) for oxidative stability of meat products under typical processing and storage conditions was reviewed. Experimental studies were used to obtain a more detailed understanding of the interaction mechanism of these vitamins and to further optimise the use of these antioxidants in processed meats. The benefits of combining natural tocopherols with vitamin C in turkey meat products are demonstrated (Brunn–Jensen 1997). In ground buffalo meat, the combined treatment of 500 ppm SA, 10 ppm TA and 0.5% sodium tripolyphosphate improved pigment and lipid stabilities and functional properties during refrigerated storage (Sahoo 1995). Similar observations were made in pre-blended vacuum-packaged ground buffalo meat (Sahoo and Anjaneyulu 1996b, 1997c). The above workers in another experiment with buffalo meat nuggets (BMN) treated with the above additives noticed a significantly ($P<0.05$) higher pH, W–B shear force, moisture, protein, better sensory attributes and lower free fatty acid contents in comparison with control samples. Use of natural antioxidants and vacuum-packaging extended the shelf life of BMN from 10–30 days under refrigerated storage (Sahoo and Anjaneyulu 1997d).

Conclusion

Lipid oxidation, particularly oxidation of phospholipids limits the keeping quality of the meat and meat products during storage, especially of pre-cooked meats due to the development of unacceptable warmed over flavour (WOF) by autooxidation processes. The meat containing more myoglobin is more prone to such oxidative changes, because the oxidised meat pigment is the more potent catalyst than inorganic iron for the initiation reaction of the autooxidation process. Moreover, different free radicals generated during such oxidative changes, are injurious to human health. There is an urgent need to prevent the pigment and lipid oxidations by use of natural antioxidants, because of a public disapproval of chemical/ synthetic antioxidants, e.g., BHA, BHT, TBHQ etc. in meat due to their residual effect. Vitamins C and E are two such naturally occurring potent biological antioxidants. Vitamin C is more effective in aqueous phase and E in lipid phase to inhibit oxidative changes in meat. Ascorbic acid reduces tocopheroxyl radical to form α–tocopherol, thereby increasing the antioxidant potency of vitamin E. Therefore, a synergistic antioxidant effect is observed, when vitamins C and E are used in combination in meat products. However, the optimal level of these vitamins to minimise the pigment and lipid oxidations is different in different meat systems, which needs further research.

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Prediction of Freezing Time for Shrimp Blocks by Numerical Modelling

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A three-dimensional numerical heat transfer model formulated through a combined fully implicit (FI) and Crank-Nicolson (C-N) schemes was validated with freezing of shrimp and Tylose blocks carried out in a laboratory freezing unit under various experimental conditions. The model predicted the time-temperature profiles fairly well with the help of a computer program based on a single algorithm. The C-N scheme provided an accuracy of prediction better than the FI scheme for all the freezing conditions and with Tylose blocks. Prediction by C-N model (5.6-13.2%) in case of shrimp blocks. The correlation coefficient between the experimental and predicted temperature during shrimp block freezing was better with C-N model (0.95-0.97) than that with FI model (0.93-0.95). Similar results were obtained with Tylose blocks. Refinement of spatial interval and time increment improved further the accuracy of prediction, but with a higher computational time and memory.

Keywords: Shrimp freezing, Three-dimensional modelling, Numerical heat transfer model, Finite difference technique, Freezing time prediction

Numerical modelling by finite difference (FD) techniques has been used by a number of workers to solve heat conduction equations for the freezing process. These investigations are mostly based on approximations made on the thermophysical properties of food products, constant average values of heat transfer coefficient and initial and boundary conditions correlated to specific food materials (Bonacina and Comini 1971; Bonacina et al. 1973; Heldman 1974; De Michelis and Calvelo 1982). Numerical models are applied to predict heat transfer at the surface of mostly one dimensional infinite slabs, infinite cylinder and spheres, which approximated the food geometries but not of block-shaped food products (Succar and Hayakawa 1984; Succar 1987). A three-dimensional numerical heat transfer model was formulated through FD fully implicit (FI) scheme and a computer program was used to predict the time-temperature profiles of a shrimp block freezing process by the present authors (Mishra and Bandyopadhyay 1996). The model was validated by comparing the predicted freezing time with the existing models of modified Plank's equation (Nagaoka et al. 1955) and of Ramaswamy and Tung (1984) and with experimental data taken during freezing of a shrimp block in a deep freezer, where cooling temperature was maintained at -10°C (Mishra and Bandyopadhyay 1996). The experimental determination of freezing time was, however, of limited operating conditions in this study. Therefore, the present work was undertaken with the objectives (i) to validate the three-dimensional model by predicting freezing time of shrimp blocks under various experimental conditions in a bench scale freezing unit, and (ii) to compare the result with that of Tylose block freezing.

Heat transfer model and numerical solution

The numerical model (FD equations) was developed by discretisation of the heat conducting body in a complete three-dimensional grid network of the finite volume (Fig. 1). The grid spacings δx, δy, δz of the volume elements were in the X,Y,Z axes representing axial directions in length, breadth and thickness of the block, respectively (Mishra and Bandyopadhyay 1996). A temperature T^t (I,J,K) was assigned to each node (I,J,K), which represented the average nodal temperature of the volume element. The superscripts t and $t+\delta t$ in model equation (1) represented the temperature at the current (known) time level and the future (unknown) time level after a time increment δt, respectively.

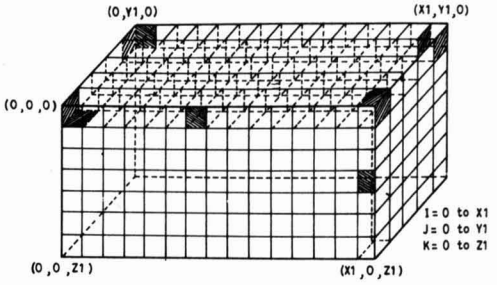

Fig. 1. Three-dimensional discretisation of block

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The discretisation of the shrimp block resulted in different nodal equations that governed the time-temperature profiles at successive time increments, depending upon the mode and direction of heat transfer.

The fully implicit (FI) and the Crank-Nicolson (C-N) FD schemes are the most commonly used numerical tools for solution of partial differential equations (Cleland and Earle 1984). The C-N scheme provides a better accuracy over the FI scheme for the same spatial and time increments, but involves a somewhat more complicated calculation procedure. In the present investigation, the two schemes were combined into a single scheme by using an weighed parameter so that advantages of both the schemes can be taken by a single algorithm. The value of the weighed parameter was 1 for the FI and 0.5 for the C-I scheme, respectively. The whole set of equations describing the time-temperature profiles of the discretised block were solved simultaneously to yield the future nodal temperatures. The average as well as local heat transfer coefficients were evaluated at each time increments on the basis of the average nodal temperatures for the respective surfaces. The correlations of natural and forced convection heat transfer for the flow conditions have been used for this purpose (Burmeister 1983). In the matrix notation, the whole set of equations is written as:

$$A^{t+\delta t}T^{t+\delta t} = A1^{t}T^{t}+C \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where $A^{t+\delta t}$ and $A1^{t}$ are the square matrices of the variable coefficients for the future and current level nodal temperatures, respectively, T's are the column vectors of the nodal temperatures, and C is the constant vector containing the surface heat transfer coefficient terms. Solutions for the $T^{t+\delta t}$ were obtained by applying the matrix inversion technique.

A computer program was written for generation and solution of equations derived from the FD approximations. The numerical algorithm includes estimation of temperature dependent physical properties of shrimp (k,ρ,c), evaluation of time-variant as well as asymmetric surface heat transfer coefficients (h), and generation of variable coefficient matrices, nodal temperatures and constant vectors. Sub-routine for the prediction of the properties k,ρ,c was developed by using the nodal temperature at that instant and the corresponding equations. Similarly, the surface heat transfer coefficients were evaluated by utilising the average surface temperature of the block, cooling medium temperature and its physical properties. Sub-routines for matrix multiplication and inversion, based on Gauss-Jordan elimination method, were used in the main program to generate time-temperature profiles for the entire nodal system of the test block for successive time steps. The freezing process was assumed to complete, when the node at the geometric centre of the block attained the desired final freezing temperature, thus predicting the freezing time for the shrimp block. The final freezing temperatures were -16° and -10°C for shrimp and Tylose block, respectively (Table 1). In the present study, no attempt was made to define the thermal centre of the test block. It has been observed that during non-symmetric freezing (as in the present case), position of the thermal centre changes continuously throughout the entire freezing process (Succar and Hayakawa 1984; Pham 1987).

TABLE 1. VARIOUS EXPERIMENTAL CONDITIONS OF BLOCK FREEZING

Run No	Block dimension, cm	Cooling medium: Temperature, °C	Cooling medium: Velocity, m/s	Mode of wrapping	Porosity
Material:Shrimp, Initial temperature:14°C, Final temperature:16°C					
S_1	8 x 4 x 2	-25	4		0.21
S_2	8 x 4 x 1	-25	4	With	0.25
S_3	8 x 4 x 2	-21	4	polyethylene	0.23
S_4	8 x 4 x 2	-25	5	film	0.21
Material : Tylose, Initial temperature : 5°C, Final temperature:10°C					
T_1	8 x 4 x 4	-23	4	Without	0
T_2	8 x 4 x 2	-23	4	wrapping	0
T_3	8 x 4 x 4	-17	4		0
T_4	8 x 4 x 4	-17	4	With wrapping	0

Materials and Methods

Materials : To check the accuracy of the numerical models, experiments were conducted with shrimp and Tylose blocks. Shrimps (*Penaeus* spp.) selected for the study were peeled, deveined and beheaded. The tail meat was blanched in boiling 7% NaCl solution for 5-10 sec, cooled and kept packed under refrigeration for further use. Blocks of different dimensions were prepared by arranging the tail meat inside a 0.17 mm polyethylene film wrapper, taking care to leave minimum void space between the meat and the wrapper. The firm shape of the blocks was ensured by forming each block inside a metallic mould, which was removed before putting it inside the freezer.

Tylose, the model food material and also known as "Karlsruhe test substance", has been

extensively used for testing freezing time prediction models (Cleland and Earle 1984). Tylose was prepared as a gel by adding water to carboxymethyl cellulose powder (SD Fines LR, India) in the weight ratio of 77:23 and moulding as a homogeneous block without any void space. Tylose blocks were prepared with and without wrapping.

The block length and breadth, 8 x 4 cm, were chosen for convenience of the experimental unit. The block having the largest dimension and exposed to the lowest cooling temperature (S_1 and T_1) were considered as the general condition (Table 1). The other experimental conditions were chosen for studies on variation in block thickness, cooling medium temperature, velocity and packaging. The initial and final temperatures were kept same for each test material, but while the former was varied within the range followed in the industrial practice, the latter was merely the experimental convenience.

Specific heat, thermal conductivity and density of shrimp meat samples were determined at different temperatures from 30° to -30°C. The specific heat was determined by the differential scanning calorimetry (DSC) and thermal conductivity by transient probe methods and also predicted from suitable models (Karunakar et al. 1998). True densities of shrimp samples were measured at different temperatures by liquid displacement method using toluene and also matched with predicted values (Mishra et al. 1997). Porosity of shrimp block was calculated from the formula:

$$\text{Porosity (P)} = 1 - \frac{\text{density of the block}}{\text{true density of shrimp}}$$

The density of block was calculated from its mass/net volume. The values of the porosity attained in shrimp blocks by manual packing ranged between 0.21 and 0.25. The thermophysical properties (TP) of the shrimp block were determined from the relationship:

$$TP=P*TP_a+(1-P)*TP_s$$

where subscript a and s represent air and shrimp, respectively (Mishra 1996).

Experimental procedure : The schematic diagram of the bench scale freezing unit is shown in Fig. 2. The test block was kept inside the freezing chamber on a raised platform. The bottom surface of the block was kept insulated from the platform surface so that heat transfer took place through convection only along the five surfaces. Cold nitrogen vapour obtained by pressurising liquid nitrogen with the help of nitrogen gas was used as a convenient cooling medium for freezing at a temperature within the range of -17 to -25°C. Temperature of the cooling medium was controlled within the freezing chamber by flow control of cold nitrogen vapour through a solenoid valve and relay system of a 10-channel temperature scanner-cum-data logger. The cooling medium was circulated

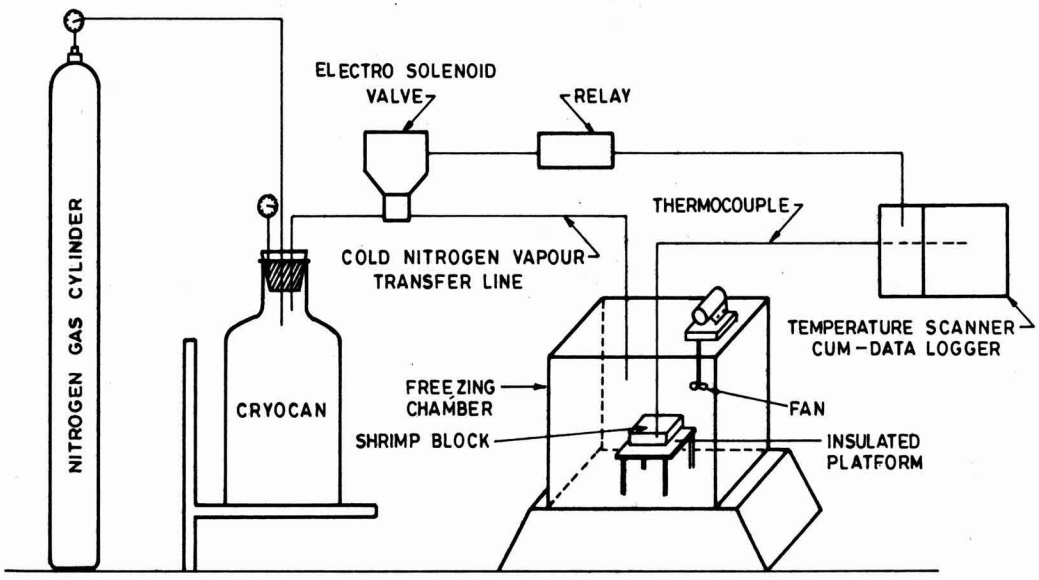

Fig. 2. Schematic diagram of experimental freezing unit

over the block surfaces with the help of a fan fitted inside the chamber. The cooling medium velocity was determined from the fan speed. The fan speed measured with the help of an rpm counter was calibrated with an air flow meter. The cooling medium velocities necessary for forced circulation over the blocks have been assumed to be uniform over the exposed surfaces. Temeperature at the centre of blocks was measured through copper-constantan thermocouple by the temperature scanner with 0.1°C resolution and accuracy of ±0.5%.

Results and Discussion

Time-temperature profiles of one shrimp block (S_3) and one Tylose block (T_3) at the geometric centres-both experimental and those predicted by the two FD models are represented as typical results in Fig.3. The time-temperature profiles were fitted with the help of a computer graphics software at the geometric centre of the blocks with the data obtained from the freezing experiments and those predicted from the computer program. The experimental results of these examples and other conditions (Table 1) showed that the initial freezing temperature varied within -1.5° to -1.9°C, and -1.8° to -2.1°C for shrimp and Tylose blocks, respectively, with supercooling occurring near the initial freezing temperature and most of the phase change terminating around -5°C. However, the supercooling effect could not be accommodated in the numerical model and hence the effect was not observed in the predicted profiles.

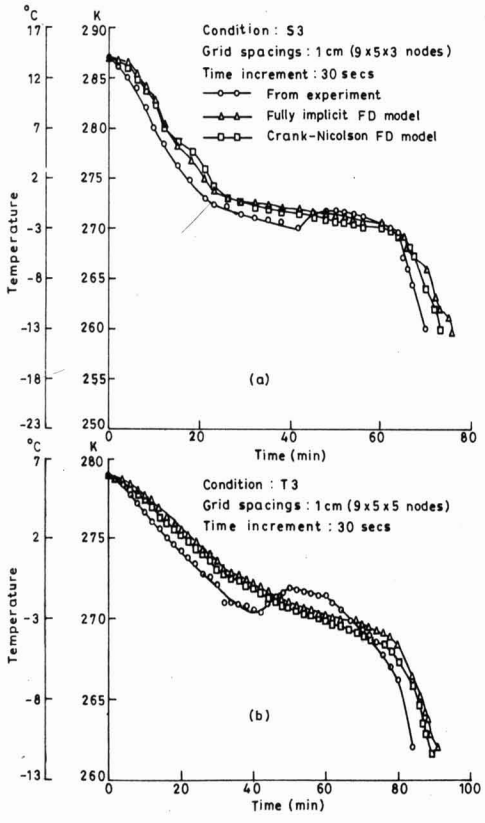

Fig. 3. Comparison of time-temperature profiles at the geometric centre of (a) Shrimp and (b) Tylose blocks

For most of the freezing conditions of the test blocks, the predicted time-temperature profiles matched fairly well with the experimental profiles. The deviations occurred were more pronounced in the phase change and tempering zones than in the pre-cooling zone. The reason may be due to use of effective specific heat in the numerical models to represent the latent heat of fusion of the test blocks. The supercooling effect of the test materials in the phase change zone and the deviation between the experimental and predicted thermophysical properties in the phase change and tempering zones might have also contributed to the prediction error. Table 2 shows the comparison between experimental and predicted freezing times. It was found that for the same value of model parameters of grid spacing and time increment, C-N model resulted in deviation from the experimental value lower (3.2-7.8%) than the FI model (5.6-13.2%). The results also showed that mean temperature difference between the experimental and predicted profiles was lower in the C-N model (1.09-1.26 K) than in the FI model

TABLE 2. COMPARISON BETWEEN EXPERIMENTAL AND PREDICTED FREEZING TIMES

Run No	Freezing time, min			Mean CV, %		Mean temperature difference between the profiles, K		r^2	
	Experimental	FI	C-N	FI	C-N	FI	C-N	FI	C-N
Material : Shrimp									
S_1	62	66	64	6.5	3.2	1.8	1.1	0.94	0.97
S_2	38	43	41	13.2	7.8	1.8	1.2	0.93	0.95
S_3	73	79	76	8.2	4.1	1.9	1.3	0.93	0.95
S_4	58	62	60	6.9	3.4	1.5	1.2	0.95	0.97
Material : Tylose									
T_1	61	67	64	9.8	4.9	1.4	1.2	0.94	0.96
T_2	39	42	41	7.7	5.1	1.3	1.2	0.96	0.97
T_3	84	1	88	8.3	4.8	1.4	1.3	0.97	0.97
T_4	107	113	111	5.6	3.8	1.2	1.1	0.97	0.97

TABLE 3. COMPARISON OF PREDICTED FREEZING TIMES OF TYLOSE BLOCKS WITH MODEL REFINEMENT

Model parameter		FI model			C-N model			Experimental
X, cm	t, s	Freezing time, min	Computational time, h	Time increase, %	Freezing time, min	Computational time, h	Time increase, %	freezing time, min
1.0 (9 x 5 x 5)	30	67	0.06	-	64	0.12	-	61
1.0 (9 x 5 x 5)	10	66	0.11	83.3	64	0.20	66.0	61
0.5 (17 x 9 x 9)	10	65	0.21	250	63	0.35	191	61

Computational time is based on users time

(1.21-1.94K). The correlation coefficient between the experimental and predicted temperature was, therefore, better in case of C-N model for all the freezing conditions, thus establishing its better accuracy over the FI model. However, in the finite difference predictions, the spread of errors reflects mainly the size of the experimental error in the data set and the likely causes suggested are the errors in estimation of surface heat transfer coefficient and thermophysical properties below the freezing zone and control of cooling medium temperature (Cleland and Earle 1984). In the present study, air blast freezing might have affected the ability to control the uniformity of surface heat transfer coefficient across a surface in comparison with plate and immersion freezing.

Table 2 shows also the effect of variations in product and process parameters on freezing time. The decrease in block thickness by 50% (from S_1 to S_2, and T_1 to T_2) did not lower the freezing time by the same proportion probably due to lowering of temperature differential and subsequent reduction in heat transfer rate. For Tylose block with wrapping increase in cooling temperature (from T_1 to T_4) caused great variation in the freezing time obviously due to increased resistance to heat transfer rate. For Tylose block with wrapping increase in cooling temperature (from T_1 to T_4) caused great variation in the freezing time obviously due to increased resistance to heat transfer. Increase in cooling medium velocity by 25% (from S_1 to S_4) lowered the freezing time by 6.4 only, which was, however, very marginal. Between the test materials, the predicted values of freezing time showed a higher degree of correlation for Tylose than that for shrimp blocks. Among all the freezing conditions, the maximum deviation of freezing time between the experimental and the model was 9.8% for Tylose and was 13.2% for shrimp block. The greater accuracy of prediction of final freezing time in Tylose block might have been due to its more uniform packing into a mould than meat or fish (Cleland and Earle 1984).

A comparison was drawn between the predicted and experimental freezing times of Tylose under the condition T_1 with refinement of model parameters (Table 3). Refinement of spatial interval and time increment increased the prediction accuracy, but there was a proportional increase in the computational time and storage. In fact, both time and space steps should be chosen sufficiently small to make truncation errors in FD schemes negligible (Cleland and Earle 1984). Although C-N model predicted the freezing times better than the FI model, the requirement of higher computational time and memory would be the deciding factor for using the C-N model for freezing time prediction.

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Comparative Study on Fatty Acids and Ether Lipids of Fresh and Sun-dried Bombay Duck *(Harpadon nehereus)*

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The lipids, fatty acids and 1-*O*-alkylglycerols (glycerol ethers) composition of fresh and sun-dried Bombay duck (*Harpadon nehereus*) were estimated. In both types of fishes, palmitic (16:0), palmitoleic (16:1) and oleic acids (18:1) were the predominant fatty acids and the fishes contained significant quantities of the ω3 polyunsaturated fatty acids (PUFAs), viz., eicosapentaenoic (20:5ω3) and docosahexaenoic acids (22:6ω3). They also contained higher levels of 1-*O*-alkylglycerols, in which chimyl alcohol (16:0), batyl alcohol (18:0) and 20:0 alcohol were predominant. After sun-drying no significant alterations in the lipid profile in these fishes were observed. However, in sun-dried fish, proportions of 22:6 fatty acid decreased. Inspite of this decrease, sun-dried samples retained 68.9% of the initial amount of ω3 PUFA.

Keywords: Bombay duck, Ether lipids, Lipids, Polyunsaturated fatty acids, Sun-dried.

Harpadon nehereus (Hamilton), commonly known as "Bombay duck", belonging to the family Harpadentidae is found in a discontinuous distribution along the Gujarat and Maharashtra region of West Coast and the West Bengal region of the East Coast (Jhingran 1983). The majority of the catch in these areas is sun-dried (Sarvaiya 1988) for preservation and export. New development in the role of fish oil in cardiovascular-oriented diets, including dried Bombay duck (Vinh et al. 1994), makes it important in human health and nutrition (Lands 1986). The polyunsaturated fatty acids of marine lipids received considerable attention because of their various biological activities in health and diseases (Wykes 1993). The non-saponifiable matter of the fish oil contains glycerol ethers. Glycerol ethers, including those of chimyl or batyl alcohol, have enormous economic potential because they exhibit bacteriostatic and fungistatic properties (Hallgren 1983), anti-inflammatory activities (Burford and Gowdey 1968) and hemopoietic effects (Brohult and Brohult 1989). With the recent interest in platelet-activating factor (PAF) (Benveniste et al. 1977) and the anti-hypertensive polar renomedullary lipid (APRL) (Benveniste and Vargaftig 1983), both of which contain a glycerol ether, which can also be considered as biochemical mediators (Demopoulos et al. 1979).

In the present investigation, fatty acids and 1-*O*-alkylglycerols of fresh and sun-dried Bombay duck were estimated from nutritional point of view and the results are reported in this paper.

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Materials and Methods

Bombay Duck *(Harpadon nehereus)* samples were collected from a local fisherman of Namkhana, West Bengal, India. The fishes were immediately frozen and transported to the laboratory. A portion of fishes was sun-dried according to the method described by Sarvaiya (1988).

Prior to extraction, the fins, heads, bones and viscera were discarded and only body flesh was weighed. Matured fishes of approximately the same lengths and weights were collected during the month of November 1997. Males and females were pooled together for this study.

The total lipids were extracted from the body flesh of fresh and dried Bombay duck, following the method of Bligh and Dyer (1959). BHT (butylated hydroxy toluene) was added at a level of 100 mg/L to the solvent as antioxidant. The isolated lipids were dissolved in hexane and kept at -20°C for future use.

The total lipids from fresh and dried fish were saponified according to Christie (1982). Fatty acids and non-saponifiables were separated after saponification of the lipids (Mishra et al. 1984). Fatty acids were methylated using diazomethane (Schlenk and Gallerman 1960).

Thin layer chromatography (TLC) was performed on 20 x 20 cm^2 chromatoplates coated with silica gel G (0.55 mm thickness). The non-saponifiables were fractionated by preparative TLC using light petroleum ether (40°-60°C): diethyl ether:acetic acid (50:50:1.5, v/v/v) (Ghosh and Beal 1979). Each

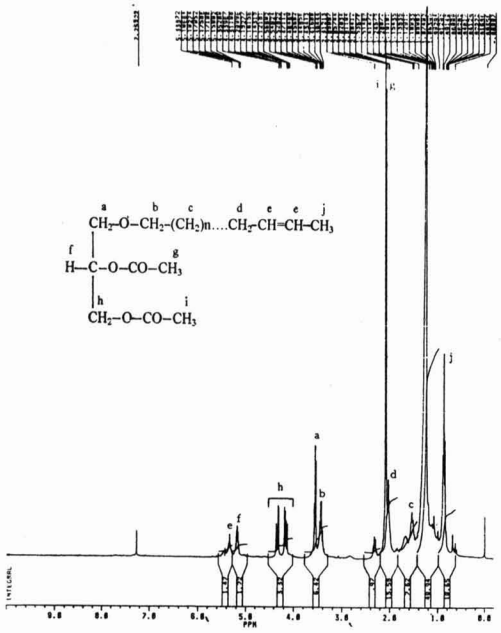

Fig. 1. ^{1}H NMR (300 MHz) spectrum of 1-*O*-alkyl-2,3-diacetylglycerol obtained from Bombay duck in $CDCl_3$ as solvent, with TMS as internal standard

class of lipids was estimated after recovery, by weighing in a micro-balance. Sterols obtained by preparative TLC was also estimated colorimetrically using ferric chloride reagent, as described by Kates (1986). The sterols and 1-*O*-alkylglycerols were acetylated according to Privett and Nutter (1967).

Gas liquid chromatography (GLC) was done on a Hewlett Packard instrument, model 5890 series II, equipped with a glass column (1.8 m x 2 mm i.d.) and a flame ionization detector (FID). Quantitation was done by an integrator (HP model 3394A) attached to GLC machine. Packed columns used were 3% OV-17 and 10% DEGS, both the liquid phases were supported on chromosorb-W(HP) 80-100 mesh and packed into glass columns. The column and standards were purchased from Hewlett Packard Co, Avondale, PA, USA and Sigma Chemical Co, St. Louis, MO, USA, respectively. Fatty acid methylesters, steryl acetates and 1-*O*-alkyl-2, 3-diacetylglycerols were analyzed and identified by GLC according to the method described by Banerjee et al (1997). Authentic standards were used for identification of component peaks in chromatograms.

The NMR spectra (δ, ppm) of 1-*O*-alkyl-2,3-diacetylglycerols were recorded on a 300 MHz Bruker (Switzerland) supercon NMR spectrometer (Model AM 300L) using $CDCl_3$ as solvent with tetramethylsilane (TMS) as internal standard.

IR spectra of 1-*O*-alkyl diacetylglycerols were recorded in an instrument model Impact 410, FT-IR by Nicolet Instrument Corporation, Madison, USA, with DTGS detector, with a resolution of 50 scans in the range 4000-6000 cm^{-1}.

Identification of IR and NMR spectra of 1-O-alkyl-2,3 diacetylglycerol : The IR spectra showed absorption bands (Kates 1986) at 1121 cm^{-1}, for -O-alkyl ether, -C-O-C-strechting and strong ester band at 1748 cm^{-1}.

^{1}H-NMR (300 MHz) spectrum (Fig. 1.) of 1-*O*-alkyl-2,3-diacetylglycerol showed signals at δ 5.18 (1H, m,-C<u>H</u>-O-), 4.15 (1H, d, *Jgem*=12.0 Hz, *Jvic*=6.5 Hz, H_A-3), 4.33 (1H, d, *Jgem*=12.0, *Jvic*=3.6 Hz, H_B-3), 3.54 (2H,d, *Jvic*= 5.2 Hz,H_2-1), 5.3 (m,-C<u>H</u>=C<u>H</u>-), 3.43 (2H, m,-OC<u>H_2</u>-CH_2-CH_2-), 1.54 (2H,m,-OCH_2-C<u>H_2</u>-CH_2-), 1.25 (broad s, x.-C<u>H_2</u>-), 2.04 (m, -C<u>H_2</u>-CH=CH-), 0.87 (3H, t, *Jvic* = 6.6 Hz, C<u>H_3</u>-CH_2-), 2.06 and 2.07 (3H each, s,-O-CO-C<u>H_3</u>), where s, d, t and m indicate singlet, doublet, triplet and multiplet, respectively.

Results and Discussion

The Bombay duck is a lean fish, with 2.1% lipid content and 25.4% non-saponifiables (NS), comparable to other fish of Sunderbans ecosystem (Banerjee 1994; Dutta et al. 1985). Appreciably high (47.2%) fatty acids (FA) are found in fresh Bombay duck. After sun-drying, there is an increase in the lipid content (29.1%) as evident from the data expressed on dry weight basis. NS (20.8%) and FA (40.9%) contents of sun-dried Bombay duck are more or less same as in the fresh fish, indicating that essential lipids and fatty acids are retained even after sun-drying. The products of NS of Bombay duck lipids are hydrocarbons (HC), 1-*O*-alkylglycerol, alcohols (AL) and sterols (ST). 1-*O*-alkylglycrol is the hydrolysed product of 1-*O*-alkyl-2,3- diacylglycerol, 1-*O*-alkyl-phospholipids whereas AL and ST are from wax esters and free and esterified sterols, respectively. The levels of HC, 1-*O*-alkylglycerols, AL and ST from the non-saponifiables in the fresh Bombay duck are 30.90%, 15.74%, 8.47% and 45.16%, respectively, whereas, those in the dried Bombay duck are 25.78%, 19.70%, 11.72% and 42.8%. Among ST, cholesterol is present to an extent of about 97% in both fresh and dried fish and this is in accordance with the findings of Patra (1995).

Fatty acids : The percentage distribution of the different fatty acids is represented in Table 1. In

TABLE 1. FATTY ACID COMPOSITION OF FRESH AND DRIED BOMBAY DUCK, ANALYSED AS METHYL ESTERS BY GLC

Components[a]	Fresh[b]	Dried[b]
16:0	34.65	30.63
16:1ω9	12.44	16.26
16:2ω9	0.41	0.25
18:0	7.05	9.21
18:1ω9	14.16	18.22
18:2ω6	0.73	0.93
18:3ω3	1.03	1.18
18:4ω6	0.20	0.21
18:4ω3	0.13	0.17
20:0	0.44	0.63
20:4ω6	3.19	2.66
20:5ω3	6.14	5.56
22:0	0.81	1.03
22:2ω6	0.40	0.89
22:4ω6	1.14	1.46
22:5ω6	1.54	1.44
22:5ω3	1.33	1.63
22:6ω3	13.69	6.84
24:1ω9	0.52	0.80
Σ SFA	42.95	41.50
Σ MUFA	27.12	35.28
Σ PUFA	29.93	23.22
Σ ω3 PUFA	22.32	15.38
ω3 PUFA/ω6 PUFA	2.93	1.96

[a]First and second figures represent, carbon chain length: number of double bonds. ω6 and ω3 represent the position of the first double from methyl end of the molecule
[b]Expressed as % w/w of toal fatty acids present in each sample

both fresh and dried fishes, 16:0, 16:1 and 18:1 are the predominant fatty acids and the fishes contained significant quantities of ω3 polyunsaturated fatty acids (PUFA), 20:5ω3 and 22:6ω3. The percentages of unsaturated fatty acids were higher than the saturated fatty acids (SFA). The ratio of PUFA (-ω3)/PUFA (-ω6) is 3 to 1, which emphasises the excellent quality of Bombay duck fat for the prevention of cardiovascular diseases (Kinsella 1987; Sanchez Muniz 1987; Rambjor 1996). Sun-drying of Bombay duck caused increase in the proportion of 16:1ω9, 18:0 and 18:1ω9. Decreases in 16:0 and 22:6ω3 are also observed. Consequently, the relative concentration of other fatty acids diminished or increased but in an uneven manner for each of them. In fact, the total SFA decreased by 3.4%, the monounsaturated fatty acid (MUFA) content increased by 30.1%, the PUFA content decreased by 22.4% and the dried fish retained 68.9% of the initial amount of -ω3 PUFA. Proportion of -ω3 PUFA was reduced in sun-dried fish due to degradation of 20:5ω3 and 22.6ω3, which are very much susceptible to autooxidation and relative percentage of other FA remains more or less same because of their stability due to the presence of common salt, which has a protective action against oxidation of fatty acids with lesser unsaturations. Considering the results reported by Keys et al (1965), Grundy (1987) and Grundy and Denke (1990), the composition of lipids seems to be more important than the quantity for protection from degenerative diseases. Given the negative effect of SFA on the development of cardiovascular diseases and the apparent capacity of MUFA to reduce plasma cholesterol (Grundy and Denke 1990; Oya 1993), the sun-dried Bombay duck lipids can be considered to have beneficial effect on cardiovascular diseases.

1-O-alkylglycerols : A perusal of Table 2 reveals that chain lengths of 1-*O*-alkyl-2,3-diacetylglycerols

TABLE 2. COMPOSITION OF 1-*O*-ALKYL-GLYCEROLS OF FRESH AND DRIED BOMBAY DUCK, ANALYSED AS DIACETYL DERIVATIVES BY GLC

Components[a]	Fresh[b]	Dried[b]
10:0	3.79	1.71
11:i	1.75	1.58
11:0	3.32	4.06
12:a	0.71	0.15
12:i	2.03	1.91
12:0	9.08	5.06
13:i	1.86	1.93
13:0	3.54	3.57
14:a	0.98	-
14:i	2.80	2.70
14:0	4.00	2.10
15:a	1.32	-
15:i	1.40	3.39
15:0	3.83	2.69
16:a	1.97	-
16:0	12.52	14.1
17:a	2.81	3.43
17:i	3.30	4.21
17:0	2.32	4.78
18:a	1.21	-
18:i	2.12	2.56
18:0	9.74	12.20
19:i	3.10	5.00
19:0	2.07	1.67
20:i	0.97	0.64
20:0	9.17	15.86
22:i	2.79	1.30
22:0	2.23	1.55
23:0	0.71	0.55
24:a	2.56	2.30

[a]The figures represent carbon chain length and i and a represent iso-and anteiso-components, respectively
[b]Expressed as % w/w of toal alkyl chain

are from C_{10} to C_{24} with odd and even carbon chain and containing saturated, iso and anteiso-moieties which are very much similar with the alkyl chains reported in the literature (Ratnayake et al. 1986; Banerjee et al. 1997). The major components found are 16:0 (chimyl alcohol), 18:0 (batyl alcohol) and 20:0 in both the samples. The long alkyl chains are derived from long chain fatty acids by reduction of their CoA derivatives to alcohols, probably via aldehydes. The ether bond is formed by the reduction of these alcohols with acyldihydroxyacetonephosphates (Mangold and Weber 1987). 1-O-alkylglycerols are more stable against chemical and enzymatic attack than ester lipids (Paltauf 1983), but they are more susceptible to autooxidation (Yanishlieva-Maslarova 1983). From the results in Table 2, it is obvious that there are no significant changes in overall 1-*O*-alkylglycerol compositions of fresh and dried fish. Although some individual 1-*O*-alkylglycerols show differences (Table 2), these could be partly due to errors in GLC calculations for small peaks and partly to diet and other natural factors affecting one or more of the fish in the lot analyzed. Ether lipids can be of value in the therapy of cancer patients (Berdel et al. 1985). The antineoplastic activity is probably caused by the generation of tumoricidal macrophages and a direct progressive destruction of tumour cells, whereas non-transformed cells are less affected (Munder 1983).

This study has shown that the fresh and dried Bombay duck are the rich sources of both PUFA and 1-*O*-alkylglycerols, which are considered to be beneficial to health.

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Response Surface Analysis of Enzyme Assisted Oil Extraction Factors for Sesame, Groundnut and Sunflower Seeds

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The effect of enzyme concentration (23.18-56.82 mg/100 g moisture-free seed), moisture content (7.95-28.05%, wet basis) and incubation period (6.95-17.05 h) on enhanching oil yield from sesame, groundnut and sunflower was evaluated by Response Surface Methodology (RSM). Mixed activity enzymes prepared from *Aspergillus fumigatus* were used. Statistical analysis of the responses was done using multiple regression technique. Depending upon the conditions of treatment, enhanced oil yield varied from 0.29 to 5.50 % in sesame, 1.06 to 3.32% in groundnut and 0.43 to 2.23% in sunflower seed. Optimization of the treatment parameters was performed and the seeds hydrolysed under optimal conditions were found to have enhanced oil yield up to 6.06 %, 3.08% and 2.29% in sesame, groundnut and sunflower, respectively. Moisture content, enzyme concentration and incubation period showed highly significant effects on oil yield from sesame.

Keywords: Enzymatic hydrolysis, Response surface modelling, Oilseed pre-treatment, Solvent extraction, Oil extraction, Optimization of extraction factors.

Oilseeds are generally given pre-treatment before oil extraction. Depending on the nature of oilseeds, the pre-treatment includes unit operations like dehulling, cracking/flaking, cooking or steam conditioning. The pre-treatment helps to rupture the cell structure of the oil-bearing materials and thereby, enabling easy removal of oil. An enzymatic hydrolysis is another option for pre-treatment of oilseeds. This method helps to break the structure of cotyledon cell walls making the structure more permeable (Rosenthal et al. 1996). Further, it breaks up the complex lipoprotein and lipopolysaccharide molecules into simple molecules, releasing extra oil for extraction (Tzen and Huang 1992). So far, a few studies on the effect of enzymatic hydrolysis as a pre-treatment have been carried out on two major oilseeds namely, rapeseed and canola (Fullbrook 1983; Sosulski et al. 1988; Sarker et al. 1998) and soybean (Fullbrook 1983; Smith et al. 1993; Kashyap et al. 1997). They reported enhanced oil recovery due to enzymatic hydrolysis pre-treatment. Increases in the oil yields due to enzymatic treatment from sesame, groundnut and sunflower were reported by Lanzani et al (1975) and Bhatnagar and Johri (1987). But, these studies have only considered the effect of pre-treatment on the oil yields due to different enzymes. No systematic study has been carried out so far on the influence of pre-treatment parameters like moisture content, enzyme concentration and treatment time on the oil yield. Therefore, the present study was conducted to investigate the effect of treatment parameters on the oil yield and optimize the hydrolysis parameters for maximum oil yield.

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Materials and Methods

Materials : Commercial varieties of sesame, groundnut and sunflower were obtained from the local market. The moisture content of the seeds was adjusted to 7% in order to commensurate with commercial practice by adding the calculated amount of distilled water. (Singh 1996). *Aspergillus fumigatus* nCIM 902, obtained from National Chemical Laboratory, Pune, was used for preparing the mixed activity enzyme on wheat bran medium (Bhatnagar and Johri 1987). Crude enzymes so prepared mainly contained cellulase, hemicellulase, chitinase, xylanase, pectinase and protease. The mixed activity enzyme was reported to be more effective than pure enzymes in improving oil yield (Bhatnagar and Johhri 1987). Cellulase and protease are the key enzymes that rupture the cell walls and lipoproteinous membrane leading to higher amount of oil recovery (Christensen 1989) and were, therefore, assayed. In addition, lipase activity of the enzyme solution was also measured, as it has a direct bearing on oil quality.

Cellulase activity was assayed by the method of Mandels et al (1976). Protease activity was measured following the procedure of Myers and Ahearn (1977). Lipase activity was assayed using the procedure of Breuil and Kushner (1975). The cellulase, protease and lipase activities of the enzyme solution were 0.3410 IU/ml, 0.15 IU/ml and 0.008 IU/ml, respectively.

TABLE 1. CODED AND UNCODED PARAMETER LEVELS

Parameters	Code				
	+1.68 (Augmented point)	+1 (Factorial point)	0 (Centre point)	-1 (Factorial point)	-1.68 (Augmented point)
Incubation period, h, (X_1)	17.05	15	12	9	6.95
Enzyme concentration, mg/100g moisture-free sample (X_2)	56.82	50	40	30	23.18
Moisture content during hydrolysis, % wb, (X_3)	28.05	26	23	20	17.95

Experimental design : Response Surface Methodology (RSM) (Khuri and Cornell 1987) was used to determine the effect of seed moisture content, enzyme concentration and treatment time on the oil yield. A second order central composite rotatable design was used in 3 variables at 5 levels. The coded and uncoded parameter values of the design are presented in Table 1 under which 6 replications at the centre point and 2 at the other points were made. The range of parameter values was based on earlier studies (Sosulski et al. 1988; Smith et al. 1993; Sarker et al. 1998).

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

Oil content : The extractable oil contents both in raw and hydrolyzed seeds were determined by extracting the oil from two samples ground to 0.5 mm particle size using a Soxhlet extractor. Petroleum ether was used at a flow rate of 150 drops per minute (AOAC 1984). The sample size was 5 g each. The ground samples were also analyzed for moisture contents to express the oil contents on moisture-free basis.

For each experiment, 20 g seeds were taken and moisture content was adjusted to by adding appropriate amounts of distilled water and enzyme solution, commensurate with the desired enzyme concentration (Singh 1996). The seeds were equilibrated in a refrigerator for 8 h. The hydrolyzed samples were dried in an oven at 70°C to inactivate the enzyme as also to readjust the moisture content to the desired level for extraction. Three gram samples in duplicate were drawn to check the moisture content and 5 g samples in duplicate to decide the Soxhlet extractable oil content. The Soxhlet extractable oil contents were also determined

TABLE 2. EXTRACTABLE OILS (EO) OF SESAME, GROUNDNUT AND SUNFLOWER HYDROLYSED ENZYMATICALLY UNDER DIFFERENT EXPERIMENTAL CONDITIONS

Coded parameters			Extractable oil, %, moisture-free sample			Increase in extractable oil due to hydrolysis, moisture-free basis		
X_1	X_2	X_3	Sesame	Groundnut	Sunflower	Sesame	Groundnut	Sunflower
Untreated seeds			51.15 ± 0.08	49.91 ± 0.12	54.89 ± 0.15	-	-	-
-1	-1	-1	52.07 ± 0.08	51.11 ± 0.08	56.15 ± 0.01	0.92	1.20	1.26
+1	-1	-1	52.19 ± 0.06	52.23 ± 0.06	56.64 ± 0.04	1.04	2.32	1.75
-1	+1	-1	53.08 ± 0.16	51.83 ± 0.04	56.57 ± 0.06	1.93	1.92	1.68
+1	+1	-1	53.89 ± 0.06	52.47 ± 0.03	56.84 ± 0.08	2.74	2.56	1.95
-1	-1	+1	52.28 ± 0.09	52.01 ± 0.09	56.18 ± 0.10	1.13	2.10	1.29
+1	-1	+1	54.84 ± 0.04	52.09 ± 0.08	56.28 ± 0.07	3.69	2.18	1.39
-1	+1	+1	53.96 ± 0.06	52.10 ± 0.07	56.34 ± 0.01	2.81	2.19	1.45
+1	+1	+1	56.66 ± 0.04	52.80 ± 0.15	56.56 ± 0.03	5.51	2.89	1.67
-1.68	0	0	51.44 ± 0.02	51.01 ± 0.02	55.60 ± 0.02	0.29	1.10	0.71
+1.68	0	0	54.75 ± 0.03	51.58 ± 0.04	56.72 ± 0.08	3.60	1.67	1.83
0	-1.68	0	51.95 ± 0.06	50.97 ± 0.09	55.32 ± 0.06	0.80	1.06	0.43
0	+1.68	0	53.18 ± 0.05	51.56 ± 0.02	56.23 ± 0.02	2.03	1.65	1.34
0	0	-1.68	52.96 ± 0.08	51.63 ± 0.01	55.51 ± 0.03	1.81	1.72	1.62
0	0	+1.68	56.60 ± 0.07	51.47 ± 0.06	55.79 ± 0.07	5.45	1.56	1.90
0	0	0	55.78	52.83	57.08	4.63	2.94	2.17
0	0	0	55.31	52.97	56.98	5.16	3.06	2.09
0	0	0	55.35	52.61	56.89	4.20	2.70	2.00
0	0	0	55.89	53.13	56.97	4.70	3.22	2.08
0	0	0	55.52	53.23	57.12	4.37	3.32	2.23
0	0	0	56.08	52.77	56.81	4.99	2.86	1.92

in unhydrolyzed samples. The data on the extractable oils of unhydrolyzed and hydrolyzed samples under different conditions (Table 2) were analyzed, using multiple regression techniques. Response surface models were developed to find the optimal conditions of parameter values in view of enhanced release of extractable oil content.

Results and Discussion

All the oil contents reported here are expressed on moisture-free basis. The extractable oil (EO) refers to Soxhlet extractable oil content of a sample as determined by the standard method discussed earlier. The EO contents in unhydrolyzed samples were 51.15, 49.91 and 54.89% in sesame, groundnut and sunflower seeds, whereas, these ranged from 51.44 to 56.66%, 50.97 to 53.23% and 55.32 to 57.12%, respectively in enzymatically hydrolyzed samples depending on the conditions of treatments. This showed that enzymatic hydrolysis enhanced EO by 0.29 to 5.5% in sesame, 1.06 to 3.32% in groundnut and 0.43 to 2.23% in sunflower seed.

Increases in EO for treated samples had been attributed to the mixed activity enzyme action mainly due to the action of carbohydrase and protease. The carbohydrases degrade the cell walls and lipopolysaccharide, rendering easy extraction and improvement in oil recovery. Protease acts on lipoprotein molecule making more oil available for extraction (Bair and Snyder 1980; Fullbrook 1983; Sosulski et al. 1988; Tzen and Huang 1992).

To optimize the enzymatic hydrolysis process parameters, response surface models for the increases in EO of treated samples were developed, employing multiple regression technique. A linear model and second order models with and without parameter interaction terms were tested for their adequacies to describe the response surface, using the lack of fit Fisher's F-test at 95% confidence level (Singh 1996). For all the oilseeds, second order model with interaction was found adequate, possessing no highly significant lack of fit (Table 3). The response functions, so developed were:

For the increase in EO for :

Sesame seed

$$Y_s = 4.672 + 0.8612X_1 + 0.6066X_2 + 0.9253X_3 + 0.1037X_1X_2 - 0.5412X_1X_3 + 0.0987X_2X_3 - 0.9084X_1^2 - 1.0962X_2^2 - 0.3114X_3^2 \quad (1)$$

Groundnut seed

$$Y_g = 2.9897 + 0.2563X_1 + 0.2016X_2 + 0.08X_3 + 0.0175X_1X_2 - 0.1225X_1X_3 - 0.02X_2X_3 - 0.407X_1^2 - 0.4177X_2^2 - 0.8167X_3^2 \quad (2)$$

TABLE 3. ANOVA OF RESPONSE FUNCTIONS 1,2 AND 3

Sources of variation	Sum of squares	Degree of freedom	Mean sum of squares	F-value (calculated)
For Response Function 1				
Regression	56.1426	9	6.2381	47.3300
Lack of fit	1.2494	5	0.2499	1.8939*
Pure error	0.6591	5	0.1318	
R^2 value = 0.9617				
Table F=Value with Df (5,5), 0.01 = 10.97				
*Non-significant at 1% level				
For Response Function 2				
Regression	6.9752	9	0.7750	14.5676
Lack of fit	2.4662	5	0.4932	9.2749*
Pure error	0.2658	5	0.0532	
R^2 value = 0.7072				
*Non-significant at 1% level				
For Response Function 3				
Regression	3.5771	9	0.3974	29.8796
Lack of fit	0.6102	5	0.1220	9.1629*
Pure error	0.0665	5	0.0133	
R^2 value = 0.8409				
*Non-significant at 1% level				

Sunflower seed

$$Y_{sf} = 2.075 + 0.217X_1 + 0.1897X_2 - 0.271X_3 - 0.0125X_1X_2 - 0.555X_1X_3 - 0.0225X_2X_3 - 0.2254X_1^2 - 0.3618X_2^2 - 0.0518X_3^2 \quad (3)$$

where

Y_s, Y_g and Y_{sf} denote increases in EO in sesame, groundnut and sunflower due to enzymatic hydrolysis pre-treatment, % of moisture-free sample.

X_1 = incubation period, h

X_2 = enzyme concentration, mg/100 g moisture free sample

X_3 = moisture content during hydrolysis, % wb.

Optimum enzymatic conditions for maximum increase in the extractable oil as calculated by partially differentiating eqs 1, 2 and 3 with respect to each parameter and setting equal to zero. The results are given in Table 4.

The maximal increase in EO predicted by response functions 1, 2 and 3 for sesame, groundnut and sunflower at the optimum values of the

TABLE 4. OPTIMUM CONDITIONS FOR MAXIMUM INCREASE IN OIL YIELD

Parameters	Optimum conditions		
	Sesame	Groundnut	Sunflower
Incubation period, X_1, h	14.41	12.93	13.64
Enzyme concentration, X_2, mg/100g moisture-free sample	43.35	42.46	42.71
Moisture content, X_3, % wb	27.48	23.17	21.16

TABLE 5. ANALYSIS OF VARIANCE FOR THE OVERALL EFFECT OF THE PROCESS VARIABLES

Process variables	Degrees of freedom	Mean sum of squares			F-ratio		
		Sesame	Groundnut	Sunflower	Sesame	Groundnut	Sunflower
Incubation period, X_1	4	5.37	0.68	0.29	28.16***	2.49	4.38
Enzyme concentration, X_2	4	5.41	0.68	0.58	28.41***	2.49	8.67*
Moisture content, X_3	4	3.87	0.33	0.02	20.29***	1.20	0.28

* Significant at 10 % level
** Significant at 5 % level
*** Significant at 1 % level

parameters were 6.27%, 3.03% and 2.17%, respectively. The experimental verification of these optimum condition resulted in extractable oil increase of 6.06, 3.08 and 2.29% for the above oilseeds.

Further statistical analysis (Table 5) was then done. The analysis was a joint test on all the parameters, involving one particular factor. For example, test for incubation period, X_1, test the hypothesis that the parameters of X_1X_2, X_1X_3 and X_1^2 are all zero (Floros and Chinnan 1987). Results showed that all the process variables affected the oil yield from sesame seed significantly and had lesser effect on other oilseeds. Incubation period and enzyme concentration had the most significant effect, while moisture content was the least in sesame seed. Smith et al (1993) also reported similar effect on soybean seed. It was also observed that the enzymatic pre-treatment affected the oil yield more in sesame seed as compared with other two oilseeds. Similar results have been reported with seeds containing higher or equal amounts of oils compared with proteins. Satisfactory results have not been obtained with seeds containing higher protein than oil.

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Suitability of Some Date Cultivars for Jelly Making

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The optimum processing conditions as well as the suitability of nine Saudi date cultivars for jelly making have been standardized. The storability of the prepared date jelly was also studied. The results showed that the 50/50 date juice/sugar ratio gave the best jelly. It was also found that 'Sefri' and 'Barni' dates were more suitable than the other seven cutltivars for jelly making. Storage at room temperature (25±5°C) upto 32 weeks had no effect on pH, moisture, °Brix, acidity and sensory properties of the prepared date jelly. While a slight increase was observed in the colour intensity of the stored date jelly, a marked change was noticed in its sugar composition. However, the 32 weeks stored date jelly was still acceptable and was rated as equal to a fresh imported apple jelly.

Keywords: Date cultivars, Jelly making, Storage, Physico-chemical properties, Sensory evaluation.

Jellies made from a variety of fruits are popular among the local population. There are different types of fruit jellies other than dates available in the market, most of which are imported.

Methods of preparation for jellies of different toughness characteristics are well documented (USDA 1974; Salonkeh et al. 1991; AFRC 1989; Woodroof and Luh 1986; Anon 1981). Factors affecting setting temperature and setting time of pectin jellies were reviewed by Ahmad (1981). Furthermore, Freedman and Francis (1984) studied the effect of ascorbic acid on the colour of jellies. They found that the addition of ascorbic acid to strawberry, apple and orange jelly did provide a lighter coloured product. Yousif et al (1987) investigated the possibility of processing jelly from Saudi dates. The pH and °Brix values for the prepared date jellies were in conformity with Saudi standards for jam and jellies (SASO 1980). The results showed that the prepared date jellies possessed high quality attributes and were well accepted by the panel members.

The optimum processing conditions i.e., ingredients, pH and °Brix for processing of date jelly were also carried out by Yousif et al (1990). The prepared date jelly samples were packed in glass jars and stored up to 24 weeks at 25°C. The effect of storage on the chemical and sensory properties of the date jellies was conducted.

Since dates are rich in sugars and other important nutrients and there is a surplus of second quality dates, this study was undertaken to prepare date jelly and recommend the most promising date cultivars for this industry.

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Materials and Methods

Fruit jelly was prepared by mixing fruit juice with sugar, citric acid and pectin. Samples of nine date cultivars, at the tamar stage, i.e 'Rezeiz', 'Khnazi', 'Bkerah', 'Khudri', 'Shagra', 'Sullag','Kusbah' and 'Barni' were used in this study. The date fruits were cleaned, pitted, packed in 1 kg plastic bags and kept refrigerated prior to further treatment and analysis.

Date paste and date juice preparation : The paste was prepared as mentioned by Yousif et al (1996). The date juice was prepared by adding water to date paste at a ratio of 3:1 (w/v) as recommended by Yousif et al (1987, 1990). The mix was boiled gently with continuous stirring for 5 min, filtered through cheese cloth to remove the fibre and other impurities. The °Brix and pH were then determined for the date juice before being used in jelly making.

Date jelly preparation : Date jellies were prepared using the formula as described by Yousif et al (1987, 1990). In the formula, date juice/sugar ratio was: 50/50 (v/w), citric acid and pectin were: 0.66 and 1.25 %, respectively (of the total solids used in the recipe). In the preparation of date jelly, sugar, was dissolved in the hot date juice followed by boiling and continuous stirring and scumming, until a Brix of 65° reached. Pectin and citric acid after being dissolved in small quantities of the hot syrup were then added. Boiling was continued for a few minutes. °Brix was determined at two minute intervals and boiling was stopped, when the °Brix reached between 65 and 70°. The prepared jelly was filled hot into glass jars and cooled. The quality of date jelly was studied by judging date

juice/sugar ratio, effect of date cultivar and storage on jelly.

Effect of date juice/sugar ratio on the quality of the produced jelly : In studying this variable, 'Rezeiz' dates were used in preparing the date juice. Three levels of date juice/sugar ratios were selected i.e., 50/50; 75/25; and 100/0.0 After processing, the three date jelly samples were coded and given to a panel of 10 judges, who evaluated the quality of the jelly by ranking preference test.

Effect of date cultivar on the quality of the produced date jelly : The same nine date cultivars as mentioned before were used to study this variable. According to the date juice/sugar ratio variable results, the 50/50 date juice/sugar ratio was used in preparing the jelly samples. Date jelly samples were prepared from the juice of the date cultivars, then coded and evaluated in terms of °Brix and pH and sensory qualities. The nine date jelly samples were devided into two categories. Each group with a reference sample, which was an imported apple jelly available in the local market was given to a team of 12 panelists. The multiple comparison difference test as recommended by Larmond (1982) was followed. Jellies of the two date cultivars attaining the best scores were selected and used for the storage studies.

Effect of storage on the quality attributes of date jelly : Ten kg each of 'Barni' and 'Sefri' date jellies were prepared, mixed well before being filled hot in glass jars and cooled. The jars were stored at room temperature (25±5°C) upto 32 weeks. The physico-chemical and sensory properties of the freshly as well as the stored jellies (0.0, 4, 8, 16 and 32 weeks) were determined.

Moisture, ash, total soluble solids (°Brix), pH, total acidity as tartaric acid, and protein were determined using the standard AOAC (1990) methods. Minerals were analyzed using a Perkin Elmer atomic absorption spectrophotometer (model 3030). Colour was measured using an extraction procedure as described by Maier and Schiller (1960). The sugar monomers were determined by high pressure liquid chromatography (HPLC) as described by Yousif (1989).

Data were analyzed by analysis of variance using the SAS system of the University of Jordan Computer Center. Duncan multiple range test was used to determine the significant differences between the means (SAS, 1987).

Results and Discussion

The results regarding the processing of date jelly on laboratory scale are presented in Tables 1-3.

Effect of date juice/sugar ratio on the quality of the prepared date jelly : A preliminary evaluation was carried out for the jellies prepared using three date juice/sugar ratios (50/50, 75/25 and 100/0.0). It is obvious from this experiment that sucrose is an essential ingredient in jelly making. In the 100/0.0 date juice/sugar treatment, a product of an unacceptable consistency and colour was obtained. The situation was better with the 75/25 treatment and a product with an acceptable colour and consistency was produced. Using the ratio 50/50 date juice/sugar, an excellent product having an attractive colour and good consistency was obtained Accordingly, the ratio 50/50 date juice/sugar was used in the following experiments of the date jelly study.

Effect of date cultivar on the quality of date jelly: The second studied variable was the suitability of a selected date cultivar for jelly making. The °Brix

TABLE 1. TOTAL SOLUBLE SOLIDS (°BRIX), pH AND SENSORY EVALUATION SCORES FOR DATE JELLIES PREPARED FROM SOME DATE CULTIVARS

Date cv	Code	Values means* °Brix		pH		Sensory evaluation scores		
		Date juice	Date jelly	Date juice	Date jelly	Total	Mean	Rate
'Shagra'	560	20.50^{a}	66.50^{c}	6.26^{c}	3.11^{a}	75^{a}	6.25	9
'Kusbah'	914	17.50^{d}	68.50^{a}	6.38^{b}	3.09^{a}	61^{d}	5.08	6
'Sefri'	134	19.40^{b}	68.50^{a}	6.24^{f}	3.02^{b}	52^{f}	4.33	1
'Reziez'	310	18.60^{c}	68.30^{b}	5.98^{g}	2.93^{c}	56^{e}	4.67	3
'Khudri'	440	17.50^{d}	64.70^{g}	6.31^{d}	2.92^{c}	57^{e}	4.75	5
'Bkerah'	601	17.40^{d}	65.50^{f}	5.54^{i}	2.89^{cd}	68^{c}	5.67	7
'Khnazi'	74	17.60^{d}	67.90^{d}	5.61^{h}	2.86^{d}	73^{b}	6.08	8
'Sullag'	26	19.50^{b}	68.10^{c}	6.43^{a}	3.02^{b}	56^{e}	4.67	3
'Barni'	123	20.60^{a}	67.80^{d}	6.35^{c}	3.07^{a}	55^{e}	4.58	2

* means having the same letters in the same column are not significantly different

TABLE 2. CHEMICAL, PHYSICAL AND SENSORY PROPERTIES OF FRESH DATE JELLY

Composition	Values means*		
	'Barni' date jelly	'Serfi' date jelly	Imported apple jelly
Moisture,%	31.5[a]	31.7[a]	-
pH	3.11[b]	3.1[b]	3.16[a]
°Brix	66.9[b]	64.8[c]	68.6[a]
Titratable acidity (ml 0.1 N NaOH/100g)	75.9[a]	75.8[a]	71.8[b]
Ash,%	0.29[a]	0.30[a]	-
Proteins,%	0.32[a]	0.26[b]	-
Colour (mg pigment/g dry matter)	1.19[b]	1.35[a]	0.49[c]
Total sugars, %	58.2[a]	53.1[c]	54.0[b]
Sucrose,%	25.6[a]	24.4[b]	13.6[c]
Fructose,%	16.0[b]	14.1[c]	19.0[a]
Glucose,%	16.8[b]	14.6[c]	21.6[a]
Sensory evaluation (mean scores)	4.8[a]	4.5[b]	-
K, mg/100g	132[b]	168[a]	71[c]
Ca, mg/100g	16[c]	24[b]	30[a]
Mg, mg/100g	32[a]	22[b]	13[c]
Na, mg/100g	36[a]	36[a]	24[b]
Zn, mg/100g	20[a]	0.0[b]	0.0[b]

* Means having the same letters in the same row are not significantly different

for the prepared date juices ranged from 17.4 for 'Bkerah' date juice to 20.6 for 'Barni' date juice. The processed date jellies had °Brix values ranging 64.7 to 68.5, whereas their pH values ranged between 2.86 and 3.11 (Table 1). These °Brix and pH values, however, were in agreement with the Saudi standards for jam and jelly making (SASO 1980). It is evident from the sensory evaluation scores (Table 1) that the two date cultivars, 'Sefri' and 'Barni', gained the best scores. As a result, the date juices of these two date cultivars were used in preparing large quantities of jelly for the storage studies.

Storability of the date jelly : Tables 2 and 3 show the analytical data regarding the third studied variable (storability of date jelly) i.e., the chemical, physical, and sensory evaluation results for both 'Barni' and 'Serfi' date jellies either as fresh or after 4,8,16 and 32 weeks of storage. Table 2 also has the analytical data for an imported apple jelly, which was used as a reference in studying the sensory properties of the date jelly throughout this study.

'Barni' date jelly had almost the same characteristics as those of the 'Sefri' date jelly. This means that the date cultivars did not significantly P>0.05) affect the chemical, physical and sensory properties of the prepared date jelly.

It is also interesting to observe the close similarity in many of the chemical properties (Table 2) of the date jelly and those of the imported apple jelly. The similarities can be detected in the pH, total sugar content, magnesium, sodium and calcium contents. On the other hand, some differences could be observed between the date jelly and the imported apple jelly with regard to their colour, sugar composition and sensory properties. Less darkness (0.49) was the characteristic of the apple jelly compared with date jelly (1.19-1.35 mg pigment/ g dry matter). The date jelly also contained more sucrose and potassium and less fructose and glucose than the apple jelly.

Data on the sensory evaluation (Table 3) reveal that the panelist gave a mean scores of 3.3 to 3.9, which were rated as good. This means that the panelists preferred date jelly as moderately better than the imported apple jelly.

Results in Table 3 show that storage at room temperature (25±5°C) upto 32 weeks had no effect on the moisture, pH, °Brix, titrable acidity and the sensory properties of the prepared date jelly. Slight

TABLE 3. EFFECT OF STORAGE ON SOME CHEMICAL, PHYSICAL AND SENSORY PROPERTIES OF DATE JELLY

Composition	Date jelly	Storage period, weeks				
		0.0	4	8	16	32
Moisture %	'Barni' date jelly	31.5[b]	31.3[b]	33.5[a]	31.2[b]	33.4[a]
	'Sefri' date jelly	31.7[b]	31.7[b]	31.8[b]	31.4[b]	34.5[a]
pH	'Barni' date jelly	3.1[b]	3.5[a]	3.1[b]	3.2[b]	3.4[a]
	'Sefri' date jelly	3.1[d]	3.5[a]	3.2[c]	3.2[c]	3.4[b]
°Brix	'Barni' date jelly	66.9[b]	65.8[c]	69.1[a]	69.5[a]	67.1[b]
	'Sefri' date jelly	64.8[d]	64.9[d]	69.6[b]	69.3[c]	76.2[a]
Titratable acidity (ml 0.1N NaOH/100g)	'Barni' date jelly	75.9[b]	75.5[bc]	82.4[a]	75.3[bc]	75.0[c]
	'Sefri' date jelly	75.8[a]	75.9[a]	82.6[a]	77.5[b]	75.4[a]
Colour,mg pigment/g dry matter	'Barni' date jelly	1.46[a]	1.22[a]	1.20[a]	1.54[a]	1.63[a]
	'Sefri' date jelly	1.4[c]	1.4[c]	1.4[c]	1.9[b]	2.6[a]
Total sugars,%	'Barni' date jelly	58.2[b]	58.5[b]	57.2[c]	61.2[a]	50.4[d]
	'Sefri' date jelly	53.1[c]	57.0[b]	58.3[a]	56.6[b]	50.0[d]
Sucrose,%	'Barni' date jelly	25.6[a]	24.1[b]	21.3[c]	19.6[d]	9.2[e]
	'Sefri' date jelly	24.4[a]	24.4[a]	22.8[b]	17.0[c]	10.3[d]
Fructose,%	'Barni' date jelly	16.0[c]	16.8[d]	17.6[c]	20.1[a]	19.1[b]
	'Sefri' date jelly	14.1[c]	15.9[d]	17.4[c]	19.8[a]	18.4[b]
Gluose,%	'Barni' date jelly	16.8[d]	17.7[c]	20.0[b]	21.6[a]	22.2[a]
	'Sefri' date jelly	14.6[e]	16.8[d]	18.2[c]	19.9[b]	21.3[a]
Sensory evaluation (mean scores)	"Barni" date jelly	2.8[b]	3.7[a]	3.7[a]	3.9[a]	4.3[a]
	"Sefri" date jelly	2.50[b]	3.33[a]	3.83[a]	3.93[a]	4.00[a]

* Means having the same letters in the same row are not significantly different

increase could be observed in the colour intensity, whereas marked changes could be seen in the sugar composition. However, these changes were expected and might be ascribed to the sucrose inversion process. Fortunately, the 32 weeks stored jelly was still acceptable and was rated as equal to a fresh imported apple jelly.

It may be concluded from the study that there is a good possibility for utilization of surplus dates in jelly making. The date jellies prepared were rated better than the imported jellies and had characteristics of high nutritive value as compared with the Saudi standards. The results of the storage study revealed the possibility of storing date jelly at 25°C upto 32 weeks without affecting their quality.

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Comparative Oil Uptake by Potato Chips During Frying Under Different Conditions

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Effect of different frying media viz., vanaspati ghee, refined oils (groundnut, cottonseed, sunflower seed, soybean, and rapeseed), pre-treatment of potato slices with carboxymethyl cellulose and initial frying temperature on oil uptake during deep-fat-frying of potato chips was determined. The oil uptake expressed as oil uptake ratio in chips fried in different frying media ranged from 0.34 to 0.41. Higher initial temperature of frying media, in general, resulted in increased oil pick up by the chips. Carboxymethyl cellulose (0.1%) was found to be effective in reducing oil uptake. Chips fried in cottonseed oil picked up less oil, were more crisp and retained better quality during storage.

Keywords: Deep-fat-frying, Potato chips, Frying media, Temperature, Carboxymethyl cellulose, Oil uptake, Sensory quality, Storage.

During deep-fat-frying, heat transfer is accompanied by mass transfer which is characterized by the penetration of oil into the product and exit of water as vapour from the product (Blumenthal 1991). Some deep-fat-fried food products absorb large amounts of fat, often reaching 49% of the total product (Makinson et al. 1987). Although, there are no accepted standards for the levels of fat in deep-fat-fried products, the consumer is conscious of the fat contents of the foods *vis-a-vis* health. Besides, the shelf life is shortened and the cost of the fried products is increased due to higher oil pickup (Sandhu and Bawa 1993). The nature of food product, the frying medium, temperature, frying duration, product composition, shape, porosity, moisture content, pre-treatment of the food product and other factors are reported to affect the oil uptake (Smith et al. 1985; Selman and Hopkins 1989).

The quality of potato chips is affected by tuber variety, storage condition prior to processing, slice thickness, blanching time and temperature, nature of oil used, temperature and period of frying (Mottur 1989). Typically, potato chips pick up oil in the range of 30-40% during frying. The extent of oil pick up by potato chips may differ with the type of oil/fat used for frying (Blumenthal 1991). Water contents of potato slices also affect oil pick up. A linear relationship between oil uptake and water removed has been reported (Gamble et al. 1987). Percentage of oil pick up may be reduced by using potatoes of high specific gravity, manipulating slice thickness, partial drying of slices prior to frying, high oil temperature (170-180°C) and less frying time (Mottur 1989). Different cellulosic materials have been used in or on food products to reduce oil uptake during deep-fat-frying (Pinthus et al. 1993).

The present study was undertaken to determine the effect of different frying media, frying temperature and pre-treatment of potato slices with carboxymethyl cellulose on the oil uptake by potato chips during deep-fat-frying.

Materials and Methods

Raw materials : Potatoes (unknown variety), refined oils (groundnut, sunflower seed, soybean, cottonseed and rapeseed), and vanaspati (hydrogenated vegetable fat) were purchased from the local market. Carboxymethyl cellulose (CMC) was of laboratory grade (Loba Chemie Pvt. Ltd., Mumbai).

Preparation of potato chips : Potatoes of uniform sizes were thoroughly washed, peeled, washed again and sliced into ~ 1.3 mm thick slices using a manual mechanical slicer. The slices were washed three times using fresh water each time to remove excess adhering starch. The washed potato slices were dabbed with muslin cloth (starch-free) to remove excess surface water and kept in muslin cloth, until fried. The frying conditions are shown in Table 1. These parameters were observed throughout the frying operation for all the frying media.

Treatment with carboxymethyl cellulose (CMC) : Potato slices after thorough washing in water, were steeped in plain water (control), aqueous solution (0.1, 0.25%) of the sodium salt of CMC for 20 min before frying in refined rapeseed oil (180°C) using the conditions listed in Table 1.

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TABLE 1. FRYING CONDITIONS FOR POTATO CHIPS

Parameter	Condition (1)	Condition (2)
Oil, kg	1	1
Potato slices, kg	0.15	0.15
Oil temperature during frying, °C		
Starting	180	190
Ending	145-150	145-150
Frying time, min	5	4.5
Draining time, min	1	1

LPG was used to heat the frying medium

Analysis of samples : Moisture contents of potato slices and potato chips were determined according to air oven method (AOAC 1975). The percent water removed from potato slices upon frying was determined as follows:

Water removed = $100^{*} (m_0-m_f)/(100-m_f)$. where m_0=Initial moisture, (%), m_f=Moisture after frying, (%).

The oil/fat contents of potato chips were estimated, using Soxhlet apparatus with hexane as solvent (AOAC 1975).

Packaging and storage : The fried potato chips were cooled to room temperature (~30°C) and packed in polypropylene pouches (100 gauge) for storage under ambient conditions (20 to 35°C, RH 30 to 65) for a period of 4 weeks.

Sensory evaluation : The freshly fried and stored potato chips were evaluated for texture, flavour and overall acceptability at weekly intervals for a period of 4 weeks by a panel of 6 scientific workers of this centre.

Results and Discussion

The data recorded on deep-fat-fried potato chips are presented in Table 2. As the initial oil content of potato slices was negligible, oil contents of chips and oil uptake by chips were treated as identical (Mottur 1989). Though oil uptake by chips is directly proportional to the amount of moisture lost (Gamble et al. 1987), there may occur deviations, especially when potato slices are subjected to treatments prior to frying (Pinthus et al. 1993). To alleviate this dependency of oil uptake on moisture, oil uptake ratio expressed as the weight ratio between the amount of oil absorbed and water removed (Pinthus et al. 1993) was used to explain the variation in oil uptake by potato chips under different frying conditions.

Effect of frying medium and temperature on oil uptake : The quality and type of oil/fat used for deep-fat-frying are reported to influence the extent

TABLE 2. EFFECT OF FRYING MEDIUM AND TEMPERATURE ON OIL UPTAKE IN POTATO CHIPS

Frying medium	Frying temperature, °C	Moisture,% Slices	Chips	Water removed, %	Oil uptake, %	Oil uptake ratio
Vanaspati	180	80.9	1.7	80.6	28.3	0.35
	190	80.9	1.7	80.6	28.9	0.36
Groundnut	180	81.4	1.5	81.1	27.9	0.34
oil	190	81.4	1.5	81.1	30.4	0.37
Cottonseed	180	81.6	1.3	81.4	28.3	0.35
oil	190	81.6	1.3	81.4	31.7	0.39
Sunflower	180	82.0	1.5	81.7	31.3	0.39
seed oil	190	82.0	1.5	81.7	32.7	0.40
Soybean oil	180	81.9	1.4	81.6	33.2	0.41
	190	81.9	1.4	81.6	32.5	0.40
Rapeseed oil	180	82.0	1.4	81.7	31.3	0.38
	190	82.0	1.4	81.7	29.8	0.36

Data based on triplicate samples

of oil pick up by food products (Blumenthal 1991). The oil uptake ratio varied between 0.34 and 0.41 when frying of potato chips was accomplished in different oils and fats, indicating differences in oil absorption (Table 2). Generally, oil pick up was more, when frying of chips was done at initial oil temperature of 190°C. However, samples fried in soybean oil and rapeseed oil picked up less oil at 190°C. On the other hand, it is reported that oil pick up by the food product is independent of the frying temperature (Gamble et al. 1987; Reddy and Das 1993). Published literature abounds with discrepancies as to the effect of process parameters on oil absorbed and water removed during deep-fat-frying of foods (Singh 1995).

Effect of carboxymethyl cellulose (CMC) on oil uptake : Powdered cellulose and its derivatives have been employed to reduce oil uptake by foods during frying (Ang 1993; Pinthus et al. 1993). In the present investigation, sodium salt of carboxymethyl cellulose was used. The potato slices were dipped in 0.1, 0.25% aqueous solutions of CMC for 20 min prior to frying. Potato chips obtained from CMC-treated potato slices fried in rapeseed oil at initial oil temperature of 180°C, picked up less oil compared to the untreated slices (Table 3). The chips were found to be more crunchy and possessed firm texture compared to the control sample. However, higher concentration of CMC in the steeping water imparted discernible hay/earthy taste to the chips. It was found that 0.1% CMC-treated potato slices yielded good quality chips both in respect of sensory quality and oil pick up. Upon heating, CMC forms a thin film, which affords partial barrier to oil penetration into the chips during frying (Saguy and Pinthus 1995).

TABLE 3. EFFECT OF CARBOXYMETHYL CELLULOSE (CMC) ON OIL UPTAKE IN POTATO CHIPS

CMC, %	Moisture,% Slices	Chips	Water removed, %	Oil uptake, %	Oil uptake ratio
0.00	82.8	0.8	82.6	31.7	0.38
0.10	82.8	0.9	82.7	29.9	0.36
0.25	82.0	0.8	81.9	29.5	0.36

Data based on triplicate samples

It would be worthwhile to examine other cellulose derivatives e.g., hydroxypropyl methylcellulose with optimum oil barrier properties to further reduce oil pick up by the chips without sacrificing the sensory quality.

Sensory quality : The potato chips generally develop rancid odour upon storage under ambient conditions. The type of oil/fat used in the deep-fat-frying process plays a decisive role. The triplicate samples of chips fried in the respective oil/fat were pooled, packed in PP pouches and stored under ambient conditions (20 to 35°C, 30 to 65% RH). The samples were examined for texture, colour, potato flavour and overall quality at weekly intervals for 4 weeks. The freshly fried potato chips in all the oils possessed crisp texture, pleasant golden colour, and appealing potato flavour. Upon storage, samples processed in vanaspati developed fatty odour at the end of third week, whereas those fried in soybean oil developed fishy rancid odour at the end of second week. The potato chips processed in groundnut oil developed slight unpleasant odour and became unacceptable at the end of fourth week. Banana chips processed in groundnut oil compared to other frying media, have also been reported to turn rancid during storage (Kutty et al. 1978). Potato chips fried in sunflowerseed oil, rapeseed oil and cottonseed oil possessed excellent sensory appeal during the entire period of storage. The samples processed in rapeseed oil were generally more crisp and were preferred by the taste panel. Sandhu and Bawa (1993), while comparing the frying properties of different oils and fats, also reported that potato chips fried in cottonseed oil were preferred most by the consumers, followed by those samples fried in sunflowerseed oil. Thakker et al (1987) too reported longer shelf life of snacks prepared in cottonseed oil.

The above results suggest that cottonseed oil would be preferred as frying medium for potato chips to pick up comparatively less oil and possess crisp texture and retain potato flavour without developing rancidity during storage. The treatment of potato slices in 0.1% carboxymethyl cellulose was found to be useful in reducing oil pick up by the chips without causing any discernible change in potato flavour.

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Protein Fractions and Characteristics of Lipids in Fresh Broiler and Layer Chicken Muscles and Penetration of Salt and Acid in Marinated Muscles

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Comparative characteristics such as protein solubility and muscle protein fractions and saponification and iodine values of lipids of pre - and post-rigor breast and thigh muscles from broiler and layer chickens and water holding capacity (WHC), acidity and salt content of marinated muscles have been studied. Myofibrillar protein content was lower and sarcoplasmic protein content was higher in breast than in thigh muscles. Saponification values were higher and iodine values were lower in broiler than in layer muscle lipids. Pre-rigor marinated muscles had higher WHC than post-rigor marinated muscles. Acidity was more and salt content was less in breast than in thigh muscles marinated for 30 min at ambient temperature (26±2°C) with solution of marinating mixture containing 20% (w/v) common salt, 2.5% (w/v) citric acid and 0.5% (v/v) lactic acid solution (pH 1.5).

Keywords : Broiler and layer chickens, Muscle proteins, Protein extractability, Saponification value, Iodine value, Marination.

Marinated chicken products such as tandoori chicken, barbecue, chilli chicken etc. are in increasing demand in India. The marinating process tenderizes muscles from spent layer hens (Kijowski and Mast 1993), ducklings (Smith et al. 1991), broiler chickens (Goodwin and Maness 1984) and beef (Hewat et al. 1983). Characteristics of muscle proteins and lipids could influence penetration of acid(s) and salts into muscles during marination and these factors have a marked effect on taste, flavour and acceptability of the products.

Broiler birds of 7-8 weeks of age and layer birds at the end of useful laying period, viz., 16-18 months, were slaughtered and dressed for the production of meat and further processing into culinary items. Systematic studies on comparative characteristics of pre - and post-rigor breast and thigh muscles from broiler and layer birds will help proper selection of birds/muscles for the preparation of marinated products such as tandoori chicken, barbecue, chilli chicken etc.

Studies on protein extractability and marination are generally reported for broiler birds, especially breast muscles and seldom for muscles from layer birds (Richardson and Jones 1987; Xiong and Brekke 1991). Further, marinating mixture with low salt concentration (up to 5%) and longer brining time (36-72 h) were generally used for the earlier studies (Janky et al. 1982; Kijowski and Mast 1993). There is a need for reducing brining time in view of increasing demand for fast foods in the market.

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In this context, the present work was undertaken to compare the characteristics of broiler and layer chicken muscles - breast and thigh muscle as well as pre - and post-rigor muscles. In addition, extractability and muscle protein fractions, saponification and iodine values of muscle lipids and penetration of salt and acid in muscles marinated with marinating mixture containing high salt concentration (20%) and short marinating time of only 30 min were studied.

Materials and Methods

Thirty each of Cobb broilers and White leghorn hens weighing in the range of 1.7-2.1 kg and 1.2-1.8 kg, respectively were procured from the local market, brought to the laboratory, starved overnight and dressed using standard procedure. The washed carcasses weighing 1.1-1.6 kg for broilers and 0.8-1.1 kg for layers were split vertically into two halves. The right halves were placed individually in polyethylene bags, tied, surrounded with crushed ice with draining facility, kept in chill room (4-5°C) for 24 h and then breast and thigh muscles dissected out were sampled as post - rigor muscles. The left halves were dissected out within 1 h of slaughter and sampled as pre-rigor muscles. Breast and thigh muscles (pre - and post-rigor) from 10 birds each were used for 3 sets of experiments as below :

Experiment I

Extraction and fractions of muscle proteins : The KCl-PO_4 buffer of ionic strength μ 0.55 and pH 7.2 was prepared by mixing 200 ml of 2 M KCl,

4.4 ml of 2M $NaH_2PO_4.2H_20$ and 23.6 ml of 2 M K_2HPO_4 solutions and diluting to 1 litre with distilled water. Breast and thigh muscles were separately minced twice, using Hobart Meat Mincer (Model 4812, USA). Muscle mince (2 g) was blended in a Waring blender with the buffer for 2 min and the extract was centrifuged at 3000 rpm. The centrifugate was diluted (1:10) to the ionic strength 0.05 to retain sarcoplasmic and non-protein nitrogen (NPN) in solution (Gracia et al. 1970). The protein in the buffer (ionic strength 0.55) extract (20 ml) was precipitated by adding equal volume of 15% trichloroacetic acid and centrifuged. All fractions of muscle nitrogen except stroma were extracted in 0.1N NaOH solution (McClain and Mullins 1969). Nitrogen contents in the muscle mince (TN) and in the extracts were determined using Kjeltec system (Model 1002, Tecator, Sweden). Fractions of sarcoplasmic, myofibrillar and stroma proteins were calculated as follows :

Sarcoplasmic N = N in buffer extract of ionic strength 0.05 - NPN

Myofibrillar N = N in 0.1 N NaOH extract - N in buffer extract of ionic strength 0.05

Stromal N = TN - N in 0.1 N NaOH extract.

Experiment II

Lipid characteristics : A known quantity (10 g) of muscle mince was ground using pestle and mortor with anhydrous Na_2SO_4 (50 g) and chloroform (100 ml). The mixture was held overnight at ambient temperature (26 ± 2°C) to extract lipids and filtered through Whatman filter paper No. 1. Saponification value and iodine value of lipids extracted in chloroform were determined (AOAC 1990).

Experiment III

Marination of chicken muscles : The marinating solution was prepared by mixing 2.5% (w/v) citric acid, 0.5% (v/v) lactic acid and 20% (w/v) common salt and adjusting its pH to 1.5. The marinating solution was prepared freshly for pre - and post-rigor muscles. The half carcass of each chicken was immersed in marinating solution (1 litre) for 30 min, breast and thigh muscles were dissected out and minced separately. The salt content and titrable acidity were determined according to AOAC (1990) methods. Muscle pH was measured using a Radiometer pH meter (Model 29, Copenhagen) by inserting the combined glass - calomel electrode directly into the muscle. A known quantity (0.5 g) of muscle mince was pressed on 9 cm dia Whatman filter paper No 1 at 500 psi for 2 min and % bound water was determined according to the procedure of Wierbicki and Deathrage (1958) and reported as water holding capacity (WHC).

Statistical analysis : The data were analysed according to completely randomised design with 10 birds in each experiment. The significance between treatment groups was determined by Duncan's new multiple range test (Duncan 1960).

Results and Discussion

Extractability and muscle protein fractions : Extractability of muscle nitrogen in KCl-PO_4 buffer of high ionic strength (0.55) was significantly ($P \leq 0.05$) higher in all the post-rigor than in the pre-rigor muscles (Table 1). Among the muscles, the extraction was more ($P \leq 0.05$) in breast than in thigh muscles and among the species of birds, the extraction was more ($P \leq 0.05$) in breast muscles of broiler than those of layer birds. On the contrary, with the buffer of low ionic strength (0.05), markedly greater ($P \leq 0.05$) extraction was observed only between pre - and post-rigor broiler breast muscles and between layer and broiler breast muscles.

TABLE 1. EXTRACTABILITY OF CHICKEN MUSCLE PROTEINS (SOLUBLE NITROGEN AS % TN)

	Extractability in		
	KCl-PO_4 Buffer		0.1 N NaOH
	μ = 0.55	μ = 0.05	
	Broiler		
Breast			
Pre-rigor	85.1^{a} ± 2.19	52.5^{a} ± 3.58	96.5^{ab} ± 1.20
Post-rigor	93.9^{b} ± 1.82	49.4^{c} ± 3.48	96.3^{abc} ± 1.66
Thigh			
Pre-rigor	71.5^{d} ± 2.58	39.5^{d} ± 2.36	93.1^{d} ± 2.74
Post-rigor	79.1^{e} ± 3.84	40.0^{d} ± 2.70	94.9^{bcd} ± 2.04
	Layer		
Breast			
Pre-rigor	79.9^{e} ± 3.20	56.3^{b} ± 2.48	96.4^{ab} ± 1.96
Post-rigor	92.0^{b} ± 1.99	55.7^{ab} ± 2.96	97.8^{a} ± 0.81
Thigh			
Pre-rigor	70.8^{d} ± 2.22	42.6^{d} ± 3.56	94.6^{cd} ± 1.87
Post-rigor	76.9^{e} ± 6.18	39.7^{d} ± 5.02	93.7^{d} ± 1.49
SEm (72 df)	± 1.04	± 1.06	± 0.57

Each values is a mean ± SD of 10 birds.
Means in the same column superscripted by different letters (a, b, c, d) differ significantly ($P \leq 0.05$)

Extractability in 0.1N NaOH solution, which extracts all nitrogen fractions except stroma (McClain and Mullins 1969), was in the range of 93 - 98% of total nitrogen (TN) in all the muscles with marginal ($P \geq 0.05$) variations due to rigor status (pre-and post-rigor), kind of muscles (breast and thigh) and species of birds (broiler and layer).

The above changes in extractabilities in buffer as well as in alkali have resulted into quantitative differences in muscle nitrogen fractions (Table 2). The extract of pre-rigor broiler breast muscle had significantly ($P \leq 0.05$) higher sarcoplasmic and lower myofibrillar nitrogen than post-rigor and the differences due to rigor state of muscles was marginal ($P \geq 0.05$) in all other cases.

Sarcoplasmic proteins were more ($P \leq 0.05$) in breast and less in thigh muscles, whereas myofibrillar proteins were more ($P \leq 0.05$) in thigh than in breast muscles. Among the species of birds, only broiler breast muscles had higher ($P \leq 0.05$) myofibrillar protein and lower sarcoplasmic protein contents than corresponding layer muscles. Stroma nitrogen content was higher ($P \leq 0.05$) in thigh muscles of both broiler and layer birds, whereas the non-protein nitrogen (NPN) content was higher in breast and lower in thigh muscles. The breast muscles (3.5-3.7%) had significantly ($P \leq 0.05$) higher TN than thigh muscles (3.2-3.3%) (Table 2).

Greater extraction of muscle nitrogen in buffer of higher ionic strength are reported in turkey muscles (Richardson and Jones 1987) and in beef (Bernthal Booren and Gray 1989). Xiong and Brekke (1991) reported greater protein extractabilities in post-rigor breast myofibrils and pre-rigor thigh myofibrils than pre-rigor breast and post-rigor thigh muscles. Samejima et al (1992) also observed increased extraction in post-rigor rabbit skeletal muscles and greater extraction in breast than in thigh muscles. Hay et al (1973) reported that Z-lines in breast myofibrils rupture after 48 h post-mortem, thus increasing protein solubility and this proteolytic change is not so appreciable for thigh muscles.

Chicken muscle lipids : Lipid contents (chloroform extract) in broiler muscles were markedly ($P \leq 0.05$) lower compared to corresponding layer muscles, as also breast muscles compared to thigh muscles (Table 3). Saponification values were markedly ($P \leq 0.05$) higher for broiler breast than layer breast muscles and the differences in other cases were marginal ($P \leq 0.05$). The lipids of broiler muscles were significantly ($P \leq 0.05$) more saturated

TABLE 2. NITROGEN FRACTIONS IN CHICKEN MUSCLES

Muscle	Total nitrogen (TN) g/100 g muscle	Non-protein nitrogen as % TN	Sarcoplasmic nitrogen as % TN	Myofibrillar nitrogen as % TN	Stroma nitrogen as % TN
Breast		**Broiler**			
Pre-rigor	3.7^{a} ± 0.19	15.3^{a} ± 0.74	36.4^{a} ± 3.12	45.6^{a} ± 4.20	2.9^{a} ± 1.08
Post-rigor	3.6^{a} ± 0.20	15.4^{a} ± 1.39	33.8^{b} ± 3.55	49.6^{b} ± 3.23	2.6^{a} ± 1.13
Thigh					
Pre-rigor	3.2^{c} ± 0.21	13.1^{b} ± 1.26	26.6^{c} ± 1.93	54.3^{c} ± 3.06	5.3^{b} ± 2.27
Post-rigor	3.2^{c} ± 0.14	13.0^{b} ± 0.91	28.5^{c} ± 1.93	51.9^{bc} ± 4.86	5.8^{b} ± 1.63
Breast		**Layer**			
Pre-rigor	3.6^{ab} ± 0.14	16.7^{a} ± 0.86	39.6^{b} ± 2.12	41.5^{d} ± 2.94	3.0^{a} ± 0.42
Post-rigor	3.5^{b} ± 0.16	16.8^{a} ± 1.04	38.9^{ab} ± 2.66	42.1^{d} ± 2.97	2.7^{a} ± 0.99
Thigh					
Pre-rigor	3.3^{c} ± 0.10	12.4^{b} ± 0.54	28.6^{c} ± 2.62	53.9^{c} ± 4.26	4.5^{b} ± 1.52
Post-rigor	3.2^{c} ± 0.12	12.2^{b} ± 0.71	28.9^{c} ± 2.79	52.7^{bc} ± 3.84	4.9^{b} ± 1.46
SEm (72 df)	± 0.05	± 0.31	± 0.84	± 1.18	± 0.44

Each values is mean ± SD of 10 birds
Means in the same column superscripted by different letters (a, b, c, d) differ significantly ($P \leq 0.05$)

TABLE 3. EXTRACTABILITY, SAPONIFICATION AND IODINE VALUES OF LIPIDS IN CHICKEN MUSCLES

Muscle	Extractability in chloroform, g lipid/100 g muscle	Saponification value, mg KOH/g fat	Iodine value, g Iodine/100 g fat
		Broiler	
Breast			
Pre-rigor	3.4^{a} ± 0.78	214.7^{a} ± 4.67	65.3^{a} ± 8.34
Post-rigor	4.7^{ab} ± 1.64	227.3^{d} ± 21.14	68.9^{ab} ± 4.66
Thigh			
Pre-rigor	6.5^{bc} ± 1.58	205.6^{ab} ± 8.04	66.6^{a} ± 9.83
Post-rigor	6.7^{bc} ± 1.17	199.1^{b} ± 21.48	70.1^{abc} ± 3.95
		Layer	
Breast			
Pre-rigor	6.3^{bc} ± 3.74	186.1^{c} ± 7.40	74.1^{bcd} ± 4.87
Post-rigor	7.7^{cd} ± 2.94	196.2^{bc} ± 3.12	77.3^{d} ± 1.94
Thigh			
Pre-rigor	10.0^{d} ± 4.21	199.8^{b} ± 2.56	75.9^{cd} ± 6.41
Post-rigor	9.0^{cd} ± 3.76	202.4^{b} ± 2.83	77.7^{d} ± 3.99
SEm (72 df)	± 0.88	± 3.59	± 1.90

Each values is a mean ± SD of 10 birds
Means in the same column superscripted by different letters (a, b, c, d) differ significantly ($P \leq 0.05$)

(low iodine values) than the layer muscles (high iodine value). Similar iodine and saponification values for lipids in chicken muscles were reported by Brito et al (1987) and Tawfeek et al (1992).

Marinated muscles : Results presented in Table 4 reveal that the pH of marinated muscles were in the narrow range of 5.3-5.5 possibly due to the buffering capacity of muscles. However, titrable acidity of breast muscles was markedly higher ($P \leq 0.05$) compared to thigh muscles, indicating clearly better penetration of acid during marination in breast than in thigh muscles. This may be attributed to greater ($P \leq 0.05$) lipid content in thigh muscles than in breast muscles (Table 1) because lipids hinder migration of polar molecules such as citric acid used in the marinating mixture. However, 0.3-0.5% higher salt content of thigh muscles compared to breast muscles, although satistically significant ($P \leq 0.05$), is not likely to have considerable effect on shelf life, health aspects and consumer tastes of marinated products. The WHC values were markedly higher ($P \leq 0.05$) in pre-rigor than post-rigor (except for broiler thigh muscles) and in thigh muscles of layer than those of broiler birds (Table 4).

The differences in protein solubilities (resulting into different concentrations of sarcoplasmic and myofibrillar proteins), lipid contents, length of fatty acid chain (saponification value), unsaturation of fatty acids (iodine value), WHC, penetration of acid between breast and thigh muscles observed could be due to the fact that the two muscles are entirely different in physiological functions. For example, power development in breast (white) muscles is swift but intermittent by anaerobic mechanism as opposed to slow, but continuous by aerobic mechanisms in thigh (red) muscles. White muscles are rich in glycolytic enzymes, whereas red muscles are rich in oxidative enzymes. White muscle fibres are of larger diameter than red muscles. White muscles have a lower concentration of mitochondria and myoglobin compared to red muscles (Addis 1986).

Greater concentration of myofibrillar proteins and lower concentration of sarcoplasmic proteins in thigh than in breast muscles, higher saponification value and lower iodine value of lipids of broiler than layer, higher water holding capacity of pre-rigor than post-rigor muscles, greater penetration of acid

TABLE 4. WATER HOLDING CAPACITY (WHC), ACIDITY AND SALT CONTENT OF MARINATED CHICKEN MUSCLES

Muscle	WHC % bound water	pH	Titrable acidity, mg NaOH/g muscle	Salt content, g/100 g muscle
		Broiler		
Breast				
Pre-rigor	80.9^{c} ± 2.93	5.3^{a} ± 0.06	2.3^{a} ± 0.32	1.3^{a} ± 0.22
Post-rigor	74.7^{a} ± 3.32	5.3^{a} ± 0.03	2.5^{a} ± 0.33	1.3^{a} ± 0.29
Thigh				
Pre-rigor	67.5^{b} ± 1.79	5.4^{b} ± 0.06	1.3^{b} ± 0.14	1.8^{cd} ± 0.19
Post-rigor	66.6^{b} ± 2.80	5.4^{b} ± 0.05	1.5^{b} ± 0.32	1.6^{abc} ± 0.32
		Layer		
Breast				
Pre-rigor	80.5^{c} ± 5.96	5.5^{bc} ± 0.08	2.5^{a} ± 0.27	2.6^{bcd} ± 0.34
Post-rigor	73.4^{a} ± 6.61	5.3^{a} ± 0.07	2.6^{a} ± 0.36	1.4^{ab} ± 0.30
Thigh				
Pre-rigor	87.5^{d} ± 6.35	5.5^{c} ± 0.09	1.4^{b} ± 0.20	1.9^{e} ± 0.26
Post-rigor	71.8^{a} ± 2.77	5.4^{b} ± 0.07	1.6^{b} ± 0.21	1.8^{de} ± 0.22
SEm (72 df)	± 1.40	± 0.02	± 0.088	± 0.086

Each values is a mean ± SD of 10 birds
Means in the same column superscripted by different letters (a, b, c, d, e) differ significantly ($P \leq 0.05$)

and poorer penetration of salt in breast than in thigh muscles were observed. This information is expected to be very useful in process optimization for the preparation of marinated products such as tandoori chicken, barbecue, chilli chicken etc. from breast/thigh muscles of broiler/layer birds.

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Thermal Stability and Texture of Emulsions from Pre- and Post-rigor Chicken Muscles

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Comparative characteristics such as composition, pH and water holding capacity (WHC) of muscles, thermal stability and textural quality of thermally set emulsions from pre - and post-rigor breast and thigh muscles of broiler and layer chicken have been studied. Protein and water contents were higher ($P<0.05$) and fat content was lower ($P<0.05$ for thigh and $P>0.05$ for breast muscles) in muscles from broiler than those from layer birds. Higher pH of pre-rigor muscles was associated with higher ($P<0.05$) WHC compared to post-rigor muscles from both broiler and layer birds. Emulsions from pre-rigor breast and post-rigor thigh muscles were distinctly ($P<0.05$) more stable as compared to those from post-rigor breast and pre-rigor thigh muscles from both broiler and layer birds. Layer muscles produced more firm and gummy ($P<0.05$ for breast and $P<0.05$ for thigh muscles) emulsions (textural quality) than muscles from broiler birds.

Keywords : Broiler and layer chicken, pH, Water holding capacity, Emulsions, Thermal stability, Textural quality.

With 525 million broiler and 150 million layer chickens (Chawla 1998), India has a large potential for the preparation of value-added emulsion type products such as sausages, loaves, pastes etc. The demand for such convenience pre-processed products is growing in institutional trades and fast food restaurants. In comminuted meat products, gelation of muscle proteins is one of the most important functional properties contributing to the textural characteristics. During heating, gelation occurs due to denaturation of proteins, causing the firmness in the emulsion products. The various factors affecting the gel characteristics are muscle type, rigor state and pH (Froning and Neelakantan 1971; Asghar et al. 1984, Foedeging 1987; Xiong and Brekke 1991). Heat-induced gelling properties of salt soluble proteins are largely responsible for the stabilization of fat and water (Smyth et al. 1998) in the thermally processed comminuted meat products. Broiler birds of 7-8 weeks of age and layer birds after completion of laying period (spent hens of 16-18 months age) are slaughtered and dressed for the production of meat and further processing into culinary items. Most of the work concerning the factors responsible for quality of meat emulsion has been done on broiler muscles. Systematic studies on comparative characteristics of emulsions from pre - and post-rigor breast and thigh muscles from broiler and layer chicken are lacking. However, this information is essential for efficient selection of birds/muscles for the preparation of emulsion type products such as sausages, loaves, pastes etc. from chicken. Hence the present investigation was undertaken.

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Ten each of Cobb broiler and White leg horn hens with live weights in the ranges of 1.35 - 2.18 kg and 1.32 - 1.68 kg, respectively were procured from the local market and were starved overnight. Water was supplied *ad lib.* The birds were dressed following standard procedure. The dressed birds weighing 1.1 - 1.6 kg for broiler and 0.7 - 1.0 kg for layer were washed and split into two halves. The right half was placed in low density polyethylene bag, tied to prevent moisture ingress, surrounded by slushed ice with draining facility and was kept in chill room (3 - 4 °C) for 24 h. The left half of the bird was sampled soon after dressing (pre-rigor), while the right half was sampled after 24 h chilling in slushed ice (post-rigor).

Muscle pH and water holding capacity (WHC) : Combined glass-calomel electrode (Radiometer pH meter, Model 29, Copenhagen) was inserted directly into the muscle and the pH was measured. The breast and thigh muscles were minced separately. The moisture, protein and fat contents of minced meat were determined according to AOAC (1990) methods, while the WHC was determined according to Wierbicki and Deathrage (1958).

Meat emulsion : One hundred grams of muscle mince were homogenized in a laboratory mixer for 1 min with 150 g ice, 50 g chilled water and 9 g NaCl (3%). To this mix was added 100 g refined sunflower oil and homogenized for another 1 min to obtain the emulsion in the form of fine paste. The temperature of the emulsion soon after the preparation was in the range of 6 - 11°C. The emulsion was transferred to a beaker, covered with a lid and held at 6 - 8°C for 2 h for conditioning.

Thermal stability : An aliquot (25 g) of the emulsion was weighed in a test tube and the test tube was placed in water bath, heated to 70°C and held at this temperature for 30 min followed by holding at 45°C for 10 min for tempering. The tubes were then centrifuged at 5000 rpm for 20 min. The juice was separated from solids. There was no separation of oil and water phase in the centrifugate. The thermal stability of the emulsion was expressed as ml juice per 100 g emulsion (Townsend et al. 1968).

Texture of emulsion : About 50 g of conditioned emulsion was weighed in a metallic can, covered with lid, placed in water bath, heated to 90°C and held at this temperature for 30 min. The thermally set emulsion was then allowed to cool and cored to obtain 10 mm thick and 40 mm dia discs. The circular discs were then compressed in General Foods Texturometer (Model GTX - 2, Zenken Company, Japan) under the operating conditions, viz., 50 mm dia flat plunger, 0.5 volt, 1 mm clearance, 6 times/min plunged speed and 750 mm/min chart speed. Two peaks were recorded for each sample. The texture parameters, viz., firmness, cohesiveness, gumminess and fracturability were determined according to the procedure of Friedman et al (1963).

Statistical analysis : The data were analyzed according to completely randomized design with 10 birds per group. The significance between the treatment groups were determined by Duncan's new multiple range test (Duncan 1960).

Composition, pH and WHC : Broiler muscles contained more water ($P<0.05$) and less ether extractives ($P<0.05$ for thigh and $P>0.05$ for breast muscles) than corresponding muscles from layer birds (Table 1). Higher ($P<0.05$) protein content in breast than in thigh muscles was also noticed. Higher pH of pre-rigor muscles was associated with higher WHC, compared to post-rigor muscles and the differences were significant ($P<0.05$) in layer muscles and marginal ($P<0.05$) in broiler muscles (Table 1). These findings are in accordance with our earlier observations for marinated chicken muscles (Rathina Raj et al. 1999) as well as for pre - and post-rigor ovine muscles (Bouton et al. 1972; Mahendrakar et al. 1988).

Thermal stability and texture quality of emulsions: Emulsions from pre-rigor breast muscles were more ($P<0.05$) stale (lower amount of juice separation on centrifugation of emulsions) than the corresponding post-rigor broiler and layer muscles, while in the case of thigh muscles, the reverse was true, i.e., more ($P<0.05$) stable emulsions were produced from post-rigor than pre-rigor muscles (Table 2). Similar trends in emulsion stability were reported for broiler muscles (Asghar et al. 1985, Xiong and Brekke 1991). With regard to texture of thermally set emulsions, the layer muscles produced more ($P<0.05$) firm and gummy emulsions than those from broiler muscles (Table 2). These differences could be due to the variations in muscle protein fractions between pre - and post-rigor breast and thigh muscles from broiler and layer birds. Sarcoplasmic protein content was more ($P<0.05$) in breast than in thigh muscles, while the myofibrillar and stroma protein contents were higher ($P<0.05$) in thigh than in breast muscles. The broiler muscles had higher ($P<0.05$) myofibrillar proteins and lower sarcoplasmic proteins than the corresponding layer muscles as reported earlier (Rathina Raj et al. 1999). Myosin and actomyosin played a significant role in stabilizing meat batter

TABLE 1. COMPOSITION, pH AND WATER HOLDING CAPACITY (WHC) OF CHICKEN MUSCLES

Muscle	Composition, %			pH		WHC (% Bound water)	
	Water	Ether extract	Protein	Pre-rigor	Post-rigor	Pre-rigor	Post-rigor
			Broiler				
Breast	74.4^{a} ± 2.16	6.3^{a} ± 2.16	21.8^{b} ± 1.50	5.7^{ax} ± 0.16	5.5^{ax} ± 0.14	71.0^{ax} ± 5.33	66.0^{abx} ± 5.17
Thigh	74.8^{a} ± 2.75	6.2^{a} ± 1.70	18.6^{a} ± 1.35	6.1^{cx} ± 0.15	5.9^{bcx} ± 0.29	67.0^{abx} ± 4.63	63.7^{bx} ± 6.48
			Layer				
Breast	69.5^{b} ± 1.77	6.6^{a} ± 2.26	24.0^{b} ± 1.31	6.1^{bcx} ± 0.20	5.7^{ay} ± 0.24	79.9^{ex} ± 7.67	71.3^{ay} ± 5.63
Thigh	68.9^{b} ± 3.63	9.8^{b} ± 3.83	19.9^{a} ± 2.15	6.4^{dx} ± 0.23	5.9^{by} ± 0.24	69.0^{abx} ± 6.25	65.5^{abx} ± 6.25
SEm	± 0.84 (36 df)	± 0.83 (36 df)	± 0.51 (36 df)	± 0.067 (72 df)	± 1.86 (72 df)		

Each value is mean ± SD of 10 birds
Means in the same column (a, b, c, d) and row (x, y) superscripted by different letters differ significantly ($P \leq 0.05$)

TABLE 2. STABILITY AND TEXTURE OF THERMALLY SET EMULSIONS OF CHICKEN MUSCLES

Muscle	Thermal stability of emulsion, ml juice released/ 100g emulsion	Firmness, kg/volt (F)	Cohesiveness, °A2/A1 (C)	Gumminess, F x C	Fracturability, kg/volt
Broiler					
Breast					
Pre-rigor	16.3^{a} ± 2.59	5.6^{ab} ± 1.06	0.59^{ab} ± 0.053	3.3^{a} ± 0.85	3.0^{a} ± 0.90
Post-rigor	31.4^{cd} ± 3.85	6.0^{ad} ± 1.06	0.58^{b} ± 0,048	3.4^{a} ± 0.66	2.9^{a} ± 0.43
Thigh					
Pre-rigor	24.1^{e} ± 3.35	4.7^{bc} ± 0.74	0.56^{b} ± 0.067	2.7^{d} ± 0.67	2.8^{a} ± 0.56
Post-rigor	17.9^{ab} ± 4.51	4.5^{c} ± 0.75	0.56^{b} ± 0.067	2.4^{d} ± 0.55	2.4^{a} ± 0.33
Layer					
Breast					
Pre-rigor	22.5^{b} ± 2.67	6.1^{adc} ± 0.90	0.60^{ab} ± 0.050	3.6^{ab} ± 0.68	3.0^{a} ± 0.27
Post-rigor	27.1^{cde} ± 3.76	6.9^{ef} ± 1.30	0.60^{ab} ± 0.031	4.2^{bc} ± 0.90	2.9^{a} ± 0.60
Thigh					
Pre-rigor	33.9^{f} ± 1.85	7.2^{f} ± 1.0	0.63^{a} ± 0.017	4.5^{c} ± 0.68	2.9^{a} ± 0.66
Post-rigor	27.3^{de} ± 3.08	6.6^{def} ± 0.90	0.59^{ab} ± 0.033	3.9^{abc} ± 0.57	3.0^{a} ± 0.51
SEm (72 df)	± 1.41	± 0.31	± 0.015	± 0.22	± 0.18

Each value is mean ± SD of 10 birds
Means in the same column superscripted by different letters (a, b, c, d, e, f) differ significantly ($P<0.05$)
*Area of second and first peaks

emulsion by forming an interfacial film between the water and oil interface (Gardon and Barbutt 1992), while the heat-induced gelling properties of salt soluble proteins were largely responsible for the stabilization of fat and water (Smyth et al. 1998). Myofibrillar hardening causes an increase in the gel strength (Cheng and Parrish 1979 ; Dawson et al. 1991 ; Voller et al. 1996). The differences in gel strength (firmness and gumminess) observed in various muscles could be due to variable quantities of extractable myofibrillar proteins (Rathina Raj et al. 1999).

It may be concluded that pre-rigor breast and post-rigor thigh muscles produced more stable emulsions. Thermally set emulsions from breast and thigh muscles of broiler birds were more firm and gummy in texture than those from layer birds.

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Luminous Enteric Bacteria as Spoilage Indicator of Tropical Fish, *Sardinella gibbosa*

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Luminous enteric bacteria in the marine fish, *Sardinella gibbosa* collected from retail fish markets were quantified. The market fish samples were characterised by high bacterial counts. Luminous bacterial counts per gram of samples ranged as follows: 1 - 2230 x 10^4 in gills, 1.27 - 28.3 x 10^4 in muscle with skin and 2.18 - 24 x 10^7 in digestive tract. Five different species of luminous bacteria, viz., *Vibrio fischeri, V. harveyi, V. orientalis, V. splendidus* 1 and *Photobacterium leiognathi*, were isolated from the samples of gill, muscle and digestive tract. *V. harveyi* was the most abundant species, comprising 61.07% of the total luminous bacteria. Presence of high counts of luminous enteric bacteria in the fish muscle could be taken as an indication of spoilage of fish.

Keywords : *Sardinella gibbosa*, Luminous enteric bacteria, *Vibrio harveyi*, Spoilage indicator.

Luminous bacteria (LB) belong to ubiquitous and ecologically versatile group, found as saprophytes and parasites of marine animals. They also live symbiotically in specialized organs of fish (Nealson and Hastings 1991) or in enteric tracts of fish and shrimp (Ruby and Morin 1979; Abraham et al. 1998). Their bioluminescence, being extremely sensitive to toxicants, has been employed in water quality testing (Bulich 1982) and coastal pollution surveillance (Ramaiah and Chandramohan 1993). Bacterial bioluminescence has also been proposed as an early indication of marine fish spoilage (Barak and Ulitzur 1980) and penaeid shrimp diseases (Abraham et al. 1998). This communication reports the results of a bacteriological study conducted on marine fish from retail fish markets with the main objective of quantifying the abundance of luminous enteric bacteria.

The marine fish, *Sardinella gibbosa*, caught in the Tuticorin bay, were collected from the retail fish markets in Ambasamudram and Pottalpudur, Tirunelveli District, Tamil Nadu and brought to the laboratory in iced condition within an hour of collection. The fishes were washed in seawater to remove sand and dirt before sampling. Each fish was dissected aseptically and the digestive tract was removed. The gills and muscles alongwith skin were also removed aseptically. Known weights of these samples were homogenized separately in 75% sterile seawater and serially diluted by 10-fold dilution. Bacteria were enumerated on seawater complex (SWC) agar at 30°C as per Ramaiah and Chandramohan (1993). Luminous bacterial counts (LBC) and total heterotrophic counts (THC) were recorded after 16 and 48 h of incubation, respectively. Luminous colonies were aseptically picked, purified on SWC agar and identified, following the taxonomic key of Abraham et al (1999).

Results of the plate counts of bacteria on fish are presented in Table 1. High bacterial counts were recorded in all the samples. Unhygienic handling and improper icing of retail fish were implicated from this data. All the 32 samples of gill, muscle and enteric tract from *S. gibbosa* yielded luminous bacterial colonies, indicating that they were the normal flora of fish, occurring as saprophytes during spoilage (Karthiayani and Iyer 1975). They were more common in digestive tract with counts ranging from 2.18 x 10^7 to 2.40 x 10^8/g, comprising 9.54 ± 1.21% of the THC. The muscle with skin had a mean THC of 3.74 x 10^6 ± 2.22 x 10^6/g, which exceeded the limit (10^6/g) set for conditionally acceptable fish (ICMSF 1988). These samples showed a mean LBC of 1.63 x 10^5 ± 1.07 x 10^5/g, which accounted for 2.93 - 5.48% of the THC. Morii et al (1988) also observed higher counts of THC and LBC in the skin of mackeral stored at 10°C than in iced fish. In their study, LB were not detected in the fish muscle and THC were extremely low. A perusal of the available literature revealed that LB, the most intensively studied groups among the marine bacteria, are often related to terrestrial enteric bacteria (Nealson and Hastings 1991) because of their gut symbiotic association in marine animals. In the intestines of many fishes and crustaceans, LBC upto 10^8/g of gut contents were recorded (Ruby and Morin 1979; Abraham et al. 1998) coinciding with the present study.

In recent years, there have been several

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TABLE 1. PLATE COUNTS OF HETEROTROPHIC BACTERIA (THC) AND LUMINOUS BACTERIA (LBC) IN *SARDINELLA GIBBOSA* COLLECTED FROM RETAIL FISH MARKETS

Samples	Number of samples	THC/g		LBC/g		Proportion of LB, %	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Gills	11	9.85×10^5 - 5.56×10^8	$1.257 \times 10^8 \pm 1.77 \times 10^8$	1.00×10^4 - 2.23×10^7	$5.48 \times 10^6 \pm 6.99 \times 10^6$	4.01 - 10.15	5.55 ± 1.97
Muscle with skin	11	4.32×10^5 - 6.32×10^6	$3.74 \times 10^6 \pm 2.22 \times 10^6$	1.27×10^4 - 2.83×10^5	$1.63 \times 10^5 \pm 1.07 \times 10^5$	2.93 - 5.48	4.10 ± 0.77
Digestive tract	10	2.11×10^8 - 2.32×10^9	$1.13 \times 10^9 \pm 0.93 \times 10^9$	2.18×10^7 - 2.40×10^8	$1.036 \times 10^8 \pm 0.87 \times 10^8$	7.86 - 10.95	9.54 ± 1.21

taxonomic studies on vibrios and LB, which led to recognition of a number of new species of bacteria including LB (Holt et al. 1994). This necessitated the quantification of individual species among the LB in line with recent advancements in the classification of marine bacteria. Species-wise composition of LB isolated from *S. gibbosa* is presented in Table 2. The data were pooled, as there was only a little variation in the species composition of LB from different sources. This could probably be due to the penetration of luminous enteric bacteria into the muscles possibly through the gills via vascular system or damaged digestive tract at elevated storage temperature. Five different species of LB were encountered in the gut, gill and muscle samples. These results are in agreement with those reported by Abraham et al (1998). The results also revealed dominance of *Vibrio harveyi* (61.07%) among the LB, followed by *V. fischeri* (13.21%). *V. orientalis* (11.79%), *V. splenddus I* (11.09%) and *Photobacterium leiognathi* (2.86%). Earlier study on LB in the gut contents of *Mugil cephalus*, however, indicated presence of only two luminous species, viz., *V. harveyi* and *V. fischeri*, with the former accounting for the majority isolates (Ramesh and Venugobalan 1988).

Results of the present study, in general, indicated contamination of *S. gibbosa* muscle by luminous enteric bacteria. LB were reported to produce histamine in fish at elevated storage temperature (Morii et al. 1988). These bacteria, accounting for about 12.99% of the total viable bacterial population of *Sardinella* sp (Ramaiah and Chandramohan 1993), together with non-luminous contaminants from enteric and other sources may aggregate the bacterial spoilage of fish under improper storage conditions. Based on these results, it may be inferred that presence of high numbers of luminous bacteria in fish muscle could be an indication of spoilage of tropical marine fish, as was the case for temperate fish (Barak and Ulitzur 1980).

TABLE 2. SPECIES-WISE COMPOSITION OF LUMINOUS BACTERIA OF *SARDINELLA GIBBOSA*

Species	Number of isolates (N=560)	Percentage
Vibrio fischeri	74	13.21
V. harveyi	342	61.07
V. orientalis	66	11.79
V. splendidus I	62	11.07
Photobacterium leiognathi	16	2.86

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Effect of Germination on Inorganic, Labile and Phytate Phosphorus of Some Legumes

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Changes in inorganic, labile, stable and phytate phosphorus were monitored in six legumes of different stages of germination. Phytic acid decreased with germination in all six beans studied. Maximum fall was seen in peas (98.47%) followed by *Bengal*gram (88.89%) after 96 h of germination with simultaneous increases in inorganic and labile phosphorus contents. Total, phytate and stable phosphorus contents decreased with germination. Moth bean showed maximum loss followed by Mung bean after 96 h.

Keywords : Germination, Inorganic phosphorus, Labile phosphorus, Stable phosphorus, Total phosphorus, Phytate phosphorus.

In dry legumes, phosphorus is present in organic forms like, phospholipids, hexose phosphates, phosphorus proteins and phytates. Phytate is the primary reserve phosphorus in the seed and is a complex salt of calcium and magnesium with myoinositol. The high amount of phytate found in beans makes the bioavailability of minerals and protein very low. The phytate molecule is negatively charged at physiological pH and chelates with mineral elements including iron, zinc, magnesium and calcium. This forms insoluble complexes, thereby making minerals unavailable for absorption. (Gustafsson et al. 1995).

Legumes undergo several processing such as steaming, pressure cooking, roasting, puffing (Khan et al. 1988), germination etc. Germination is a conventional process for legume consumption (Bapu 1976) and is beneficial in reducing some of the anti-nutritional factors in cereals and legumes (Deosthale 1982; Baber et al. 1988). Germination involves the breakdown of seed reserves and their utilization by the growing roots and shoots. The major metabolic processes associated with seed germination are the mobilisation of storage materials in the reserve tissue and their subsequent transfer to and utilization by the developing embryonic axis (Abdus Sultan 1989).

The metabolic activity of legumes increases with germination and for that, large amount of energy is required. This energy is supplied by the hydrolysis of phosphorus containing compounds like phytates, phospholipids, ATP and so on. Phosphorus is also a constituent of the high energy compound ATP and thus is necessary for cellular activities (Hall and Hodges 1966). The present study was, therefore, undertaken to study the changes occurring in different types of phosphorus during gemination.

Six legumes viz., *Bengal*gram (*Cicer arietinum* L.) moth bean (*Phaseolus aconitifolius* Jacq), lentil (*Lens esculenta* L.) greengram (*Pheseolus aureus* Roub), blackgram (*Phoseolus mungo*) and peas (*Pisum sativum* L.) were selected for the study. All the seeds were purchased from local market in bulk. After cleaning, the seeds were soaked in water for 6 h, followed by covering them in moist cloth for germination at room temperature, away from direct sunlight. The seeds were removed after 24, 48, 72 and 96 h. of germination.

Phytic acid was determined by the modified method of Makowar (1970).

A 4: 6 Fe/P atomic ratio was used to calculate phytic acid content. The values obtained for phytate were also compared with the values calculated with the equation given by Lolas and Markakis (1975).

$$Y = 0.141 + 0.273x$$

where Y is the percentage content in the total phosphorus and x is the percentage content in phytic acid. Phytic acid phosphorus was calculated by assuming 28.2% phosphorus in one molecule of phytic acid. Inorganic, labile and total phosphorus contents were estimated by the method of Fiske and Subbarow (1925).

Stable phosphorus was calculated by the following formula.

$$P \text{ stable} = P \text{ total} - P_{\Delta}10$$

Where $P_{\Delta}10$ = Labile phosphorus.

Inorganic phosphorus is represented by Pi.

Germinated legumes showed the concentration of different types of phosphorus viz., inorganic,

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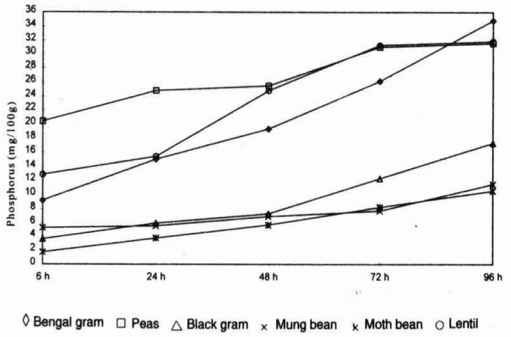

Fig. 1. Levels of inorganic phosphorus in germinated legumes

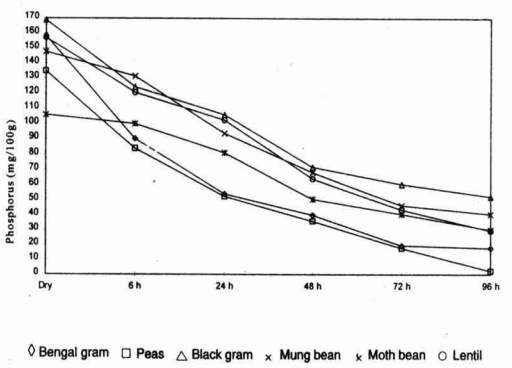

Fig. 3. Levels of phytate phosphorus in germinated legumes

labile, total and phytic acid phosphorus at different stages of germination (Fig. 1–4).

The percentage values for inorganic phosphorus were as follows: 18.61, 7.91, 14.08, 5.36, 4.72 and 14.13%, respectively for *Bengal*gram, blackgram, lentil, moth bean, mung bean and peas after 96 h. It is clear that maximum increase was found in *Bengal*gram compared to other legumes (Fig. 1). Lentil, on the contrary, showed maximum increase (23.62%) for labile phosphorus, followed by peas (20.58%). To quote other values, *Bengal*gram, blackgram, moth and mung showed 14.92, 6.45, 10.75 and 11.73% increases in the labile phosphorus contents after 96 h. After this period, inorganic as well as labile phosphorus were not estimated because of the onset of off odour in the seeds (Fig. 2).

Percent losses of total and phytate phosphorus were maximum after 96 h (Fig. 3 and 4) Phytate phosphorus of moth showed the maximum loss of 66.02% after 96 h. When other legumes were compared with moth, mung bean was the second bean where loss in phytate phosphorus was 61.56%, accompanied by loss of total phosphorus upto 44.78%. *Bengal*gram, blackgram, lentil and peas showed percent losses between 42.51 and 60.59%. Khan et al (1988) reported percent loss of 22.73 of phytic acid in brown *Bengal*gram after soaking for 6 h and a loss of 12.5% of phytic acid in white *Bengal*gram after soaking for 4 h. In a similar study, Hall and Hodges (1966) found a rapid decrease in phytic acid phosphorus in the endosperm of germinating oat seeds. Percent losses of total phosphorus of peas and blackgram were 52.01 and 50.31%, while minimum loss was found in moth beans compared to other beans. A decreasing trend was found in total phosphorus content in germinating moth beans, till 72% of germination (Powar 1986). Lolas and Markakis (1975) reported that phytic acid reacted with protein to form complex products of varying composition and this had an inhibitory effect on the peptic digestion of ovalbumin and elastin. This effect is believed to be related to form insoluble complexes with phosphorus and minerals like Ca

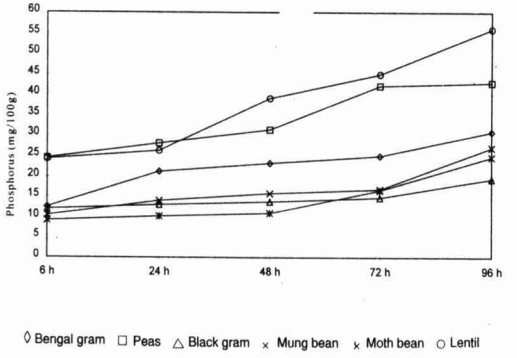

Fig. 2. Levels of labile phosphorus in germinated legumes

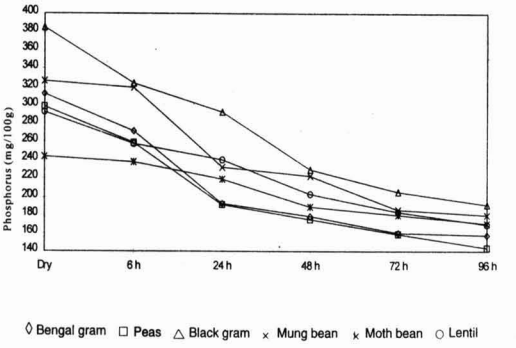

Fig. 4. Levels of total phosphorus in germinated legumes

and Zn in acid medium, which increases rackitogenic property. Germination increases the availability of minerals by decreasing the above mentioned property. Phytate phosphorus decreased in all germinated legumes and accordingly, rackitogenic property of the antinutritional factor decreased.

These results suggest that phosphorus containing compounds of seeds like phytate etc. get hydrolysed and converted to other forms like inorganic and labile phosphorus, which are more usable to plants and human beings. The energy released by the hydrolysis of stable and total phosphorus is utilized by the growing roots and shoots.

It may be that during germination, oxidation of the reserve food material occurs to yield energy in the form of ATP. Hall and Hodges (1966) found that protein phosphorus in the roots and shoots increases, while the entire plant exhibits an overall decrease.

Simultaneously, it was assumed that phytate phosphorus, being the stable one, hydrolysed maximum in all the germinating seeds, when compared with that of dry seeds. As stated earlier, phytic acid has an inhibitory effect on the peptic digestion of protein by forming insoluble compounds (Kumar and Chauhan 1993).

These results indicate that germination hydrolyses phytate phosphorus and free phosphorus, in turn, is utilized for various metabolic processes. Germination, therefore, can be recommended as a simple household process to enhance the nutritional quality of legumes.

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Role of Phenolics and Polyphenol Oxidizing Enzymes in Odour Generation in Pearl Millet Meal

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Water-soluble phenolics and peroxidase (POD) activity concentrated mostly in germ fraction of the grain appeared to be responsible for odour generation in stored pearl millet meal. The polyphenol oxidase (PPO) distributed uniformly in endosperm and germ was found relatively heat stable and not involved in odour generation. The heat sensitive POD activity was effectively inactivated by hot water blanching of seeds at 98°C for 40 sec or by steam heating of seeds at 15 psi for 5 min to arrest the odour generation in the meal.

Keywords : Pearl millet meal, Phenolics, Polyphenol oxidase, Peroxidase, Meal odour.

A rapid development of objectionable mousy, acidic odour in the pearl millet meal upon a short storage has been earlier attributed to the oxidative (Lai and Varriano-Marston 1980), or hydrolytic degradation of meal lipids (Kaced et al. 1984; Kadleg et al. 1995). Most of the lipids are located in germ of the seed and removal of the germ improved the shelf life of low-fat grits (Abdelrahman et al. 1983). Similarly, blanching of grains at 98°C for 10 sec in water before milling effectively retarded the development of fat acidity in the meal and enhanced its shelflife (Chavan and Kachare 1994; Palande et al. 1996).

Reddy et al (1986), however, reported that lipids were not involved, but the water-soluble phenolics from methanolic extract of meal were responsible for mousy, acidic odour of the stored meal. Hence, the enzymatic oxidation of phenolics has been proposed. Since a short heat treatment of grains eliminated the odour formation problem, it was speculated that phenolics and heat sensitive polyphenol oxidizing enzymes might also be located mainly in the germ fraction of the grain. Polyphenol oxidase (PPO) and peroxidase (POD) are the two polyphenol oxidizing enzymes commonly found in dry seeds. In this communication, PPO and POD activities of dry seeds on odour generation in pearl millet meal are reported.

Various meals were prepared as indicated in the footnotes for respective Tables and analysed for crude fat, fat acidity, acid value (AOAC 1990), water-soluble phenolics in methanolic extract (Reddy et al. 1986) by Folin-Denis reagent, PPO and POD activities using pyrogallol as substrate (Kumar and Khan 1982) and evaluated for odour generation by the method of Reddy et al (1986). The meals were mixed with 30% water (w/v), dried under cold air for 10 h, packed in sealed jars and the headspace was sniffed after 12 h by 5 semi-trained judges.

The whole grain meal and both undefatted and defatted germ meals exhibited a strong and extreme mousy odour, respectively, while the endosperm meals showed only fresh to mild odour (Table 1). Most of the fat acidity, water-soluble phenolics and POD activity were found to be concentrated in the germ, while the PPO activity was observed to be uniformly distributed throughout the grain. Removal of the fat from the germ meal eliminated only fat acidity, but not the phenolics and POD activity.

TABLE 1. FAT ACIDITY, WATER-SOLUBLE PHENOLICS, PPO, POD AND ODOUR GENERATION IN DIFFERENT GRAIN COMPONENTS

Grain component[a]	Fat acidity, mg KOH /100 g	Water-soluble phenolics, mg /100g	POD, units/ g/min	PPO, units/ g/h	Odour of meal[b]
Whole grain	43.0	80.1	159	89	Strongly mousy
Endosperm					
Undefatted meal	20.0	71.5	68	89	Fresh to mild
Defatted meal	1.2	66.2	68	85	Fresh to mild
Germ					
Undefatted meal	11.0	121.6	376	89	Extremely mousy
Defatted meal	1.8	136.7	386	92	Extremely mousy

a. The grains were manually separated into endosperm and germ fraction using razor blade and milled. The endosperm and germ meals were extracted in hexane to remoe lipids and analyzed. These meals contained 9.5% moisture

b. The odour generation was evaluated by the method of Reddy et al (1986)

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These results indicated that the water-soluble phenolics and POD activity mainly concentrated in the germ were contributing to the odour generation.

When a fresh whole meal was separately extracted by hexane to remove fat, by methanol to lower the phenolics and by both hexane followed by methanol to remove fat and phenolics, only whole and defatted meals generated a strong mousy odour, while the methanol extracted or both hexane - and methanol-extracted meals exhibited normal fresh odours (Table 2). In whole meal with 6.2% fat, fat degradation was marked, pH of water extract was acidic and it contained 160 units of POD, while in the defatted meal with only 1.3% fat, fat degradation was meagre, but water-soluble phenolics and POD activity were similar to those in whole meal. The methanol-extracted meal contained 5.9% fat, but the fat degradation was negligible and a significant decrease in water-soluble phenolics and POD activity was observed. The hexane - and methanol-extracted meal with 1.4% fat exhibited changes similar to methanol-extracted meal. The PPO activity in various meals, however, remained unaffected. These results further confirm the observation that water-soluble phenolics and POD activity are involved in odour generation of pearl millet meal. Reddy et al (1986) indicated that meal phenolics were enzymatically oxidized to apigenin-like phenolic aglycones that exhibited bitter taste and mousy odour. The results of present studies clearly indicated the involvement of germ peroxidase in this process.

TABLE 2. EFFECTS OF HEXANE AND METHANOL EXTRACTIONS OF PEARL MILLET MEAL ON CHANGES IN LIPIDS, PHENOLICS, PPO AND POD ACTIVITIES AND ODOUR GENERATION

Parameters[a]	Un-extracted meal	Hexane-extracted meal	Methanol-extracted meal	Hexane and methanol-extracted meal
Chemical analyses				
Crude fat, %	6.2	1.3	5.9	1.4
Acid value, mg KOH per g of oil	37.9	3.5	3.5	3.1
Fat acidity, mg KOH per 100 g meal	288.9	28.1	28.1	19.6
pH of meal water extract (1:10 w/v)	5.7	6.2	6.8	6.9
Water-soluble phenolics, mg/100 g meal	80.1	75.1	50.0	42.9
Enzyme assays				
Polyphenol oxidase units/g/h (PPO)	85	75	85	78
Peroxidase (POD) units/g/min	160	160	57	56
Odour of meal[b]	Strongly mousy	Strongly mousy	Normal fresh	Normal fresh

a. Fresh meals were separately extracted by hexane, twice, to remove fat; by methanol, thrice, to remove phenolics or by hexane followed by methanol to remove both fat and phenolics. All meals contained about 9.5% moisture

b. All analyses were done on 10 day stored meals (27±3°C, 70–80% RH) while enzyme assays and odour generation (Reddy et al. 1986) on freshly treated meals

TABLE 3. EFFECTS OF HEAT TREATMENTS OF SEEDS ON PPO, POD AND ODOUR GENERATION IN MEAL

Type of meal	PPO units/g/h	POD units/g/min	Odour generation after days[c] 1	5	10
Unheated grains	84	155	mousy	extremely mousy	unbearable off odour
Blanched grains[a]					
10 sec	68	65	fresh	mild	moderate off odour
20 sec	66	38	fresh	fresh	mild
40 sec	64	n.d.	fresh	fresh	fresh
Steamed grains[b]					
1 min	67	28	fresh	fresh	mild
5 min	50	18	fresh	fresh	fresh
10 min	16	n.d.	fresh	fresh	fresh

a. The grains were tied in muslin cloth and held in boiling water at 98°C for 10–40 sec, drained, dried and milled

b. The grains were autoclaved at 15 psi for 1–10 min, dried and milled

c. Odour evaluation by the method of Reddy et al (1986)

Blanching of seeds at 98°C for 40 sec or steam heating of seeds at 15 psi for 5 min eliminated the POD activity (Table 3). The meals obtained from such heat-treated grains remained fresh and did not show any mousy odour during storage upto 10 days even after odour acceleration treatment. These results indicated that simple heat treatments of seeds were effective in eliminating the POD activity and odour generation problem in pearl millet meal. The development of new cultivars with lower POD activity may be beneficial in this respect.

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Emulsification Capacity of Raw and Autoclaved Faba Bean Flour (*Vicia faba* L.)

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Emulsification capacity (EC) of raw autoclaved faba bean flour over 1–13 pH range and in three dispersion media (water, 0.1 M NaCl, 1.0 M NaCl), indicated increase below and above the isoelectric pH 7.0. Autoclaving decreased EC at all the pHs studied. High salt concentration (1.0 M NaCl) reduced emulsification properties. EC followed the trend of nitrogen solubility curve.

Keywords : Faba bean, Emulsification capacity, Raw, Autoclaved.

The functional properties of legumes and oil seeds have specific food applications. Emulsification capacity is one of the important functional properties in a particular food system (Kinsella 1976; Bera and Mukherjee 1988; Prakash and Ramanatham 1995). The present communication deals with results of a study on the emulsification properties of raw and autoclaved faba bean flour in various dispersion media to predict its relationship with other functional properties.

Faba bean (*Vicia faba L.*) variety "JV–1" was obtained from the Department of Plant Breeding and Genetics, J.N. Krishi Vishwa Vidyalaya, Jabalpur, India. Cleaned seeds (10.5% d.b. moisture content) were ground (for proper chemical analysis and better result of emulsification) and passed through a 40 mesh sieve (domestic type) and packed in polyethylene bags. A portion of raw faba bean flour was autoclaved at 121°C for 30 min and packed separately in polyethylene bags. The proximate composition of the sample was determined by AOAC (1975) procedures.

Emulsification Capacities (EC) of both raw (RF) and autoclaved (AUF) flours in various dispersion media were determined by a modified method of Beuchat et al (1975). Sample (2 g) in 23 ml of distilled water of different molarities of NaCl solutions were added in a domestic mixer (Bajaj Jar Blender). The pH of the dispersion was blended for 30 sec. at low speed for dispersing the material completely. After dispersion, refind groundnut oil was added from a burette and mixed thoroughly. The addition of oil was continued, until there was a phase separation. This was determined visually and recorded. Emulsification capacity was expressed as ml of oil emulsified by 1g of protein.

The emulsification capacity profiles of the RF and AUF as functions of pH and salt concentration are shown in Fig. 1 and 2.

Effect of pH on emulsification capacity : Both RF and AUF samples showed minimum values of 85.5 and 41.7 ml/g of protein at pH 7.0 (Isoelectric pH) and maximum EC of 218.0 and 129.0 ml/g of protein were obtained at pH 13.0. Both the sample showed positive correlations with pH. Emulsification capacity curve followed the trend of nitrogen solubility curve (Gour et al. 1992).

Effect of autoclaving on emulsification capacity : Emulsification capacity of AUF sample decreased and this might be due to protein denaturation. At pH 10.0, there was a substantial decrease in emulsification capacity of AUF as compared to the raw flour. Maximum decrease in emulsification capacity was observed in 30 min. autoclaved sample. The decreasing trends of emulsifying capacity for autoclaved peanut flour and winged bean flour were reported by Rahma and Mostafa (1988). Narayana and Narasingha Rao (1982), respectively. Heating

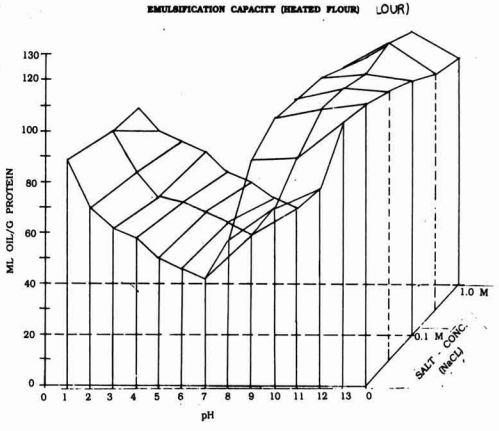

Fig. 1. Effect of pH and salt concentration on emulsification capacity of autoclaved flour of faba bean

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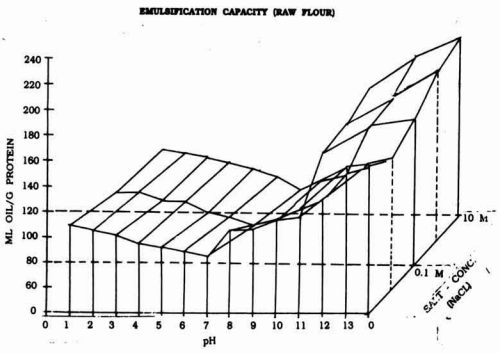

Fig. 2. Effect of pH and salt concentration on emulsification capacity of raw flour of faba bean

time was the primary determinant in the reduction of emulsifying capacity. Nakai (1983) reported that emulsifying capacity not only depended on the protein solubility, but also on hydrophilic lipophilic balance (HLB) of the particular protein.

Effect of salt concentration on emulsifying capacity : At a low salt concentration (0.1 M NaCl), the emulsification capacity increased but at a high salt concentration (1.0 M NaCl), EC decreased at each level of pH. The decrease in EC was due to "salting out" effect on the complex protein system. No shift in isoelectric pH was noticed in the AUF samples and in dispersion medium containing salt. EC was highly influenced by change in pH and salt concentration.

High emulsification capacity value can be obtained from raw faba bean flour at both acidic and alkaline pH ranges. Hence, it is expected, that other functional properties, i.e., nitrogen solubility index, foaming capacity and gelation would also be improved and may find application as a protein ingredient in food formulations.

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Standardization of A Method of Juice Extraction from Ber Fruit

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Mature golden yellow coloured fruits of ber cv. 'Umran' were obtained and washed thoroughly in running cold water and destoned. Such destoned fruits were crushed to get the pulp. Different methods of extraction such as cold extraction, blanching and enzymatic viz., use of Pectinex 3XL, and Trizyme P-50 at 0.2, 0.4, 0.6, 0.8 and 1% concentrations were employed for getting the highest recovery of raw and clarified juices. Among various concentrations tried, it was found that Pectinex 3XL, at 0.6% and Trizyme P-50 at 0.4% gave higher recovery of raw and clarified juices. In general, the enzymatic methods of extraction gave the highest TSS and sugars as compared to control. Among the enzymes, Trizyme P-50 was found to be superior over Pectinex 3XL, in yielding maximum raw and clarified juices recovery irrespective of concentrations.

Keywords : Ber fruit, Cold extraction, Blanching, Pectinex, Trizyme, Recovery, Enzyme.

The ber (*Zizyphus mauritiana* Lamk) is an ancient and common fruit growing in wild, semi-wild and cultivated form throughout India (Pareek 1983). Because of its potential for high yields, excellent economic returns to the growers, cultivation of this hardy and highly nutritive fruit is expanding at a faster rate. Ber is eaten mostly fresh, but it can be processed into declicious products. Since information on the extraction of juice from ber fruits is scanty, the present investigation was undertaken to standardize a method of extraction of juice from ber fruits.

Mature, golden yellow colour fruits of ber cv. 'Umran' were obtained from the Experimental Orchard, Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri and brought to the Post-harvest Technology Laboratory. The fruits were washed thoroughly in running cold water and destoned. The destroned fruits were crushed to get the pulp. To obtain the maximum recovery of ber juice with better quality and clarity, the following methods of extraction were tried.

(i) Cold extraction (control), (ii) Blanching (dipping the fruits in boiling water for 4 min) and (iii) Enzymatic method by using Pectinex 3XL and Trizyme P-50 enzymes of various concentrations viz., 0.2, 0.4, 0.6, 0.8 and 1%.

The pulp was squeezed and filtered to get raw juice in cold extraction and blanching methods. In enzymatic extraction method, after addition of chemical preservative (potassium metabisulphite 600 ppm) in the pulp, the pulp was treated with various concentrations of the respective enzyme separately and the mixture was kept for 16 h. Later on, it was squeezed, filtered and allowed to sediment by adding gelatin (2 g/lit) in the raw juice for another 8 h. The clarified juice was syphoned off, pasteurised and bottled. The juice obtained from various methods was analyzed for its physico-chemical composition. The total soluble solids (TSS) were determined by Hand refractometer and sugars were determined by the method given by Lane and Eynon (1923). The titratable acidity of juice was determined and expressed in terms of per cent (AOAC 1975). The observations of juice recovery were also recorded. The experiment was conducted in completely randomised block design with twelve treatments and three replications.

Juice recovery (%) : The data in respect of juice recovery and chemical composition are presented in Table 1. It was obvious from the data that the yield of raw and clarified juice varied, depending upon the method of extraction from 53 to 75.2% and 37.1 to 52.6%, respectively. The highest recovery of juice was noticed in enzymatic method of extraction compared to other methods. The enzyme, Trizyme P-50 at 0.4% concentration showed the highest recovery of raw (77.00%) and clarified (54.7%) juice followed by Pectinex 3XL at 0.6% concentration showed recovery of raw (73.50%) and clarified (52.00%) juice. The lowest pomace (11%) was noticed in Trizyme P-50 at 0.4% concentration, whereas the highest (19%) pomace was by cold extraction method. Enzyme treatment also lowered the viscosity of ber juice considerably as compared to other methods. Similar results were reported by Prabhu Desai (1991) in ber fruit. However, the juice recovery obtained in the present

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TABLE 1. JUICE RECOVERY AND CHEMICAL COMPOSITION OF BER JUICE EXTRACTED BY DIFFERENT METHODS

Particulars	Raw juice yield, %	Clarified juice yield, %	Pomace, %	TSS %	pH	Acidity, %	Reducing sugars, %	Total sugars, %
Cold extraction	53.0	37.1	19.0	18.2	4.52	0.30	4.46	8.17
Blanching	64.0	44.8	16.0	18.8	4.08	0.40	4.10	7.82
Pectinex 3XL, %								
i. 0.2	69.0	48.3	16.3	18.6	4.67	0.38	5.32	10.78
ii. 0.4	70.0	49.0	16.0	19.0	4.12	0.40	5.27	11.77
iii. 0.6	73.5	52.0	16.0	20.0	4.14	0.43	5.94	10.86
iv. 0.8	72.5	50.7	16.0	20.0	4.12	0.48	5.34	11.47
v. 1.0	70.0	49.3	16.0	20.0	4.00	0.48	5.79	9.36
Mean	71.0	49.9	16.06	19.5	4.21	0.43	5.53	10.80
Trizyme P-50, %								
i. 0.2	74.0	51.8	13.0	19.0	4.57	0.34	4.03	10.50
ii. 0.4	77.0	54.7	11.0	20.0	4.41	0.40	4.86	9.36
iii. 0.6	75.0	52.5	13.6	19.0	4.40	0.46	4.63	10.22
iv. 0.8	75.0	51.2	14.0	19.4	4.26	0.49	4.62	11.62
v. 1.0	75.0	52.8	13.0	20.0	4.29	0.48	4.06	10.53
Mean	75.2	52.6	12.9	19.5	4.39	0.43	4.44	10.44
S.Em ±	0.39	0.49	0.63	0.37	0.10	0.019	0.16	0.33
CD at 5%	1.15	1.42	1.83	1.83	0.31	0.050	0.48	0.97

investigation was found to be the highest as compared to the methods reported by Prabhu-Desai (1991), who obtained 68.90% and 34.40% raw and clarified juice by using Pectinex enzyme.

Chemical constituents : The chemical composition of clarified juice as influenced by method of extraction showed that enzymatic method of juice extraction recorded the highest TSS and sugars content as compared to control and blanching methods. The highest percentage of acidity was found in the enzymatic, whereas the lowest was noticed in control. Among the enzymatic extraction, the lowest acidity (0.34%) was recorded by using Trizyme P-50 at 0.2% whereas, the highest acidity (0.49%) was noticed by Trizyme P-50 at 0.8% (Table 1). The increase in acidity of the ber juice after enzyme treatment could probably be due to the release of carboxyl groups from the pectin compounds during hydrolysis of pectin by the enzyme (Sreekantiah et al. 1963).

Colour of the juice : The raw juice obtained was cloudy and colloidal in nature with lots of suspended material. After sedimentation, the clarified juice obtained was found to be clear and transparent. The colour of raw juice appeared yellow to dark yellow by visual observation. Blanching treatment induced slight browning of raw juice, while clarified juice had lemon yellow colour. Similar results were also reported by Prabhu Desai (1991), while working with the methods of juice extraction from ber fruit.

From this study, it may be concluded that the enzymatic method of juice extraction from ber fruits was the best. Among the enzymes, Trizyme P-50 was found to be superior than Pectinex 3XL in yielding higher raw and clarified juice from ber fruit.

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Studies on the Nutritional Composition of *Sterculia* Species

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Apart from industrial uses of *Sterculia* spp., the seeds are used for human food. Hence, the nutritional composition of seeds of *Sterculia foetida and S. urens* from 4 localities was studied. The results showed that carbohydrates ranged from 18–48%, crude protein from 9.63–30.81%, ascorbic acid from 2.3–5.2 mg/100g and fat from 24–32.5%. Appreciable amounts of minerals were also present. Tannin content was found to be appreciably low.

Keywords : *Sterculia urens, S. foetida,* Nutrients, Nutrition.

Forest food products are significant components of the diets of rural people (Campbell–Platt 1980). They are not generally dietary staples. However, they can make significant contributions to the overall diversity and nutritional quality of the diet adding calories, oil, protein and minerals (Becker 1983). At present, only a fraction of the total potential is being tapped, signifying wastage of large quantities of these resources. Better and fuller utilization of these resources is necessary to improve the nutritional status of the population.

Sterculia is a genus of trees distributed throughout India in the Himalayas and sub–Himalayan tract, Gujarat, Rajasthan, Deccan Plateau and on the West coast in Konkan region. Species like *S. urens* and *S. foetida,* widely distributed in Central India yield edible seeds rich in fat, minor gums and bast fibres. Seeds of *S. urens* (Kullu) are consumed as a pulse (Wealth of India 1989). The oil is reported to be suitable for edible purposes and soap making. It is the source of Karaya gum also known as Indian tragacanth, as it resembles true tragacanth in properties and uses and has long been used as its substitute and adulterant. It is of considerable commercial and industrial importance. The leaves possess nutritive value as any fodder and are rich in vitamin A. Similarly, the seeds of *S. foetida* commonly known as 'jangli badam' sometimes called 'Java' olives or stinking beans are eaten raw or roasted. People from low socio–economic group consume these seeds (Jain 1991). They are reported to be used to adulterate cocoa. As very little information is available on the nutritive value of these seeds, the present investigation was undertaken to estimate the nutrients in the seeds of *S. urens* and *S. foetida.*

Seeds of *S. urens* and *S. foetida* were collected from different regions of Madhya Pradesh viz., Barha, Bargi, Kundwara and Jabalpur and analysed for their chemical composition. Total carbohydrates were estimated by the anthrone method (Sadasivam and Manickam 1991). Total nitrogen in the samples was determined by micro–kjeldahl method and the crude protein content was calculated by multiplying N X 6.25 (AOAC 1970). Total soluble proteins were estimated as per the procedure of Lowry et al (1951). Fat was extracted from the samples following standard methods (AOAC 1970). Ascorbic acid was estimated by visual titration method based on the reduction of 2, 6 dichloroindophenol dye (Mahadevan and Sridhar 1982). Calcium, magnesium, sodium, potassium and phosphorus were determined in aqueous solutions of ashed sample. Calcium and magnesium were determined by EDTA titration method, phosphorus was estimated with vanado–molybdate reagent and sodium and potassium by flame photometry (Jackson 1973). Tannins, an antinitritional factor was estimated by using Folin Denis reagent method (Sadasivam and Manickam 1991).

The nutrient composition of *Sterculia urens* and *S. foetida* seeds in both raw as well as deoiled form is given in Table 1. The data indicate that there are significant differences in their nutrient contents. The crude protein contents of deoiled seeds of *S. urens,* Bargi (30.81%) and *S. foetida,* Jabalpur (28.89%) were found higher as compared to raw seeds. The crude protein content of raw seeds of *S. urens* from different regions varied from 9.63 to 21.11%, the highest being in *S. urens* seeds from Kundwara. Total nitrogen content was maximum in deoiled seeds of *S. urens,* Bargi (4.93%) followed by *S. foetida* (4.62%) and minimum (1.84%) in raw seeds. Nitrogen content was also highest in the raw seeds of *S. urens* (3.69%)

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TABLE 1. NUTRIENT CONTENTS IN SEEDS OF *STERCULIA SPECIES*

Species	Locality	Total carbo-hydrate %	Soluble protein %	Crude Protein %	Total nitrogen %	Ca %	Mg %	Na %	K %	P %	Fatty oil %	Ascorbic acid mg/100g
Sterculia urens (raw)	Bargi	41.0	2.10	9.63	1.54	0.48	0.24	0.16	0.67	0.49	26.0	3.4
S. urens (raw)	Barha	48.0	2.09	11.55	1.84	0.40	0.24	0.18	0.56	0.38	24.0	2.3
S. urens (raw)	Kundwara	41.0	2.00	23.10	3.69	0.56	0.28	0.19	0.39	0.50	29.0	3.1
S. foetida (raw)	Jabalpur	18.0	1.30	11.55	1.84	0.40	0.28	0.21	0.71	0.30	32.5	5.2
S. foetida (deoiled)	Jabalpur	-	3.11	28.89	4.62	0.40	0.32	0.25	0.60	0.45	-	-
S. urens (deoiled)	Bargi	-	3.20	30.81	4.93	0.45	0.26	0.20	0.63	0.40	-	-

collected from Kundwara, while the seeds of Bargi, Barha and Jabalpur showed 1.54, 1.84 and 1.84%, respectively. Total soluble protein content was also found to be maximum in deoiled seeds of S. *urens* (3.20%) of Bargi, followed by 3.11% in *S. foetida*, Jabalpur, whereas the average observed in raw seeds of all three localities was 1.87%. The total mineral contents ranged from 1.76% in raw seeds of *S. urens* (Barha) to 2.10% in raw *S. foetida* seeds.

S. urens seeds, Barha had the lowest fatty oil content (24.0%) and *S. foetida* seeds the highest (32.5%), with an average value of 27.8%. The calcium content varied from 0.40 to 0.56% and the phosphorus content from 0.38 to 0.50%. The variability for sodium, potassium and magnesium was almost negligible. Total carbohydrates of *S. urens* obtained from all the three localities varied from 41.0 to 48.0%, whereas *S. foetida* showed the lowest value (18.0%). Ascorbic acid ranged from 2.3 to 5.2 mg/100g.

The data on tannin contents are shown in Table 2. The contents varied from 0.34 - 1.08%, highest being in deoiled *S. urens*, seeds of Bargi and lowest in *S. foetida* seeds of Jabalpur.

Overall, the results have shown that the raw and deoiled seeds are rich sources of protein and fat. The contents of crude protein and crude fat reported in the present study for the seeds of *Sterculia species* are higher than those of the most common pulses, greengram, blackgram, pigeonpea and chickpea, consumed in India (Jambunathan 1980). The fatty acid composition of the oil showed presence of myristic, palmitic, stearic, lignoceric, oleic and linoleic acid content as reported by earlier workers (Wealth of India 1989). The seeds contained appreciable amounts of mineral matter and low levels of tannins.

TABLE 2. TANNIN CONTENT OF SEEDS OF *STERCULIA SPECIES*

Species	Locality	Tannin, %
S. urens (raw)	Bargi	0.60
S. urens (raw)	Barha	0.49
S. urens (raw)	Kundwara	0.49
S. foetida (raw)	Jabalpur	0.34
S. foetida (deoiled)	Jabalpur	0.58
S. urens (deoiled)	Bargi	1.08

The usefulness of forest food products is uncommon because of the presence of toxic/ antinutritional compounds present in them. However, these can be eliminated by several treatments. Tannins detected in the present study are heat labile (Liener 1980) and its potential hazards can be overcome by moist heat treatment or autoclaving. In addition to these treatments, soaking of the seeds in dilute alkali might reduce the tannin content to a large extent. While these forest resources are thought to be of comparatively minor importance, available evidence suggests that its nutritional contribution can be important for agriculturists and pastoralists as well as for forest dwellers and hunters/gatherers.

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TABLE 2. EFFECT OF YEAST FERMENTATION ON TOTAL DIALYSABLE ZINC(mg/100 g DRY MATTER) AND % DIALYSABILITY IN VARIOUS BREADS

Sample	Total zinc	Zinc dialysability: Unfermented Total	Unfermented %	Naturally fermented Total	Naturally fermented %	Yeast fermented Total	Yeast fermented %
Greengram	2.8	0.20	7.1	0.24	8.7	0.37	13.2
Blackgram	3.0	0.28	9.3	0.33	11.1	0.53	17.7
Redgram	0.9	0.05	5.6	0.06	6.4	0.07	7.6
*Bengal*gram	1.7	0.15	8.9	0.19	11.3	0.26	15.0
Rice	1.3	0.15	11.2	0.18	14.3	0.26	20.3
Rice + blackgram	1.9	0.20	10.5	0.25	13.2	0.33	17.9
Rice + greengram	1.8	0.17	9.7	0.20	11.3	0.29	16.3
Rice + *Bengal*gram	1.4	0.14	9.8	0.17	11.9	0.24	16.9
Rice + redgram	1.2	0.11	9.2	0.12	10.5	0.14	11.8
Wheat	2.2	0.13	5.9	0.14	6.3	0.18	8.2
Wheat + blackgram	2.5	0.16	6.7	0.22	9.1	0.32	12.8
Wheat + greengram	2.4	0.16	6.5	0.17	7.3	0.25	10.4
Wheat + *Bengal*gram	2.0	0.13	6.3	0.18	8.7	0.29	14.1
Wheat + redgram	1.8	0.09	5.4	0.10	5.7	0.12	7.0
Sorghum	1.6	0.12	7.5	0.13	7.9	0.18	11.1
Sorghum + blackgram	2.1	0.19	9.0	0.20	9.9	0.29	14.3
Sorghum + greengram	2.0	0.18	8.8	0.19	9.4	0.25	12.7
Sorghum + *Bengal*gram	1.6	0.14	8.6	0.15	8.9	0.21	12.9
Sorghum + redgram	1.4	0.09	6.9	0.10	7.3	0.13	9.5
Pearl Millet	3.1	0.25	8.2	0.27	8.7	0.41	13.3
Pearl Millet + blackgram	3.1	0.27	8.8	0.29	9.4	0.45	14.4
Pearl Millet + greengram	3.0	0.24	7.9	0.25	8.4	0.39	13.1
Pearl Millet + *Bengal*gram	2.6	0.22	8.2	0.23	8.7	0.36	13.7
Pearl Millet + redgram	2.4	0.19	8.1	0.20	8.5	0.22	9.5
Refined wheat flour	0.6	0.04	7.1	0.05	8.2	0.10	17.1
Mean	2.0	0.16	8.05	0.18	9.2	0.27	13.2
S.D.	0.68	0.06	1.50	0.68	2.0	0.11	3.3
% increase with respect to unfermented					14.9		64.5

Values represent average of triplicate estimations for each type of bread and variation between replicate estimations was 1.8–4.5%

($p<0.05$), indicating considerable variability between the kind of flour used for bread making (Table 2). Total dialysable zinc was significantly ($p<0.05$) higher in bajra breads than the other three cereals, which were comparable in this respect.

Phytate degradation : In comparison to unfermented breads, change in % phytate as IP6 + IP5 was also higher in yeast breads than the naturally fermented breads (12.9–6.9) and the differences were significant in case of yeast-raised breads ($p<0.05$) (Table 3).

Addition of legume : Addition of legumes significantly increased the bioavailable amounts of iron in rice, wheat and sorghum breads ($p<0.05$), but there was no significant effect on bioavailable amounts of zinc as also for iron and zinc in case of bajra breads. Blackgram, greengram and *Bengal*gram but not redgram increased the bioavailable amounts of iron and zinc as compared to cereal alone breads of rice, wheat and sorghum to variable extents (50–150%).

Nutritional iron deficiency is a common and serious problem in many developing countries

TABLE 3. % REDUCTION IN IP5 + IP6 FRACTION OF PHYTIC ACID DUE TO YEAST FERMENTATION AS AGAINST UNFERMENTED PRODUCT

Sample	Alone	+ Blackgram	+ Greengram	+ Bengalgram	+ Redgram
Rice	22.6	26.6	24.0	30.9	29.5
Wheat	30.9	18.9	17.7	42.9	31.2
Sorghum	20.9	25.3	25.4	39.7	46.9
Pearl Millet	12.9	13.4	16.9	34.4	46.6

% Reduction in IP5 + IP6 fraction of phytic acid due to yeast fermentation as against naturally fermented product

Values represent the differences between average duplicate analysis for yeast fermented breads and unfermented breads. Paired t indicates the differences due to yeast fermentation to be highly significant ($p<0.01$).

including India. This fact has been evident through several studies in the developing world (De Maeyers and Adiels-Tegman 1985, MacPhail and Bothwell 1992). Sub-normal intakes of bioavailable iron in vegetarian whole cereal-based meals has been considered as a major factor for poor iron status (Hallberg et al. 1992). Although the status of zinc in predominantly vegetarian populations has been predicted to be poor, information supporting this view is very limited (Ayengar 1996). Nevertheless, it has been reported that reducing the levels of phytates helps to improve bioavailability of both iron and zinc (Sandberg 1991). In the present study, unfermented and naturally fermented breads were baked under conditions identical to yeast-raised breads for meaningful comparison. However, methods of preparation for some of the traditional foods like *roti, tandoori roti, handwo* used in different regions of India are fairly close to the method used in this study. Present results, thus, show that instead of making *roti* with unfermented whole cereal flours as has been traditionally used, if the flours batters are treated with baker's yeast or cereals other than bajra are mixed with legumes, will help to improve the micro-nutrient quality with respect to iron as well as zinc.

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Browning and Carotenoid Oxidation in Some High Moisture Fruit Slices Prepared by Hurdle Technique as Compared with Intermediate Moisture Fruits During Storage

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Shelf stable high moisture fruit slices (moisture content 50-60%) were prepared from some fruits using hurdle technique (HT) and compared with the semi-moist slices prepared using intermediate moisture (IM) technique (moisture content 30-40%) during storage in flexible polymeric pouches under ambient condition (19-33°C). They were evaluated with respect to non-enzymatic browning (NEB) and pigment (carotene) oxidation besides component transfer. The results showed that high moisture HT fruits were significantly less susceptible to NEB and carotenoid oxidation during storage as compared to IM fruits. Component transfer data and sensory evaluation showed that the IM fruits exhibited higher weight and volume reduction and solute gain compared to HT fruits. The latter were less sweet due to lower solute gain and softer due to higher moisture.

Keywords : Hurdle technique, Intermediate moisture technique, Non-enzymatic browning, Carotenoid oxidation, Component transfer, Fruit slices.

Discolouration is one of the major symptoms of deterioration in fruit products. For an average consumer, the concept of quality is mainly based on colour and appearance. Among the chemical phenomena of causing deterioration of fruits, the Maillard reaction, which is non-enzymatic, is of prime importance. The interaction of amino group and reducing sugar moiety in the early stages and further complex reactions in later stages, yielding volatiles and water soluble substances give brown pigments, melonoidins. Model studies by Eichner (1975) described a maximum in the browning rate at water contents in the intermediate moisture range.

As regards its effect on major food pigments, water influences the oxidation of carotenoids by interaction with free radicals produced during their oxidation and reducing their contents (Labuza 1975). Even though carotenoid oxidation takes place in non-aqueous phase, the control of mobility in aqueous phase may influence carotenoid oxidation rate. Loss of carotenoid by oxidation in several fruit and vegetable products containing them is a major factor, affecting their quality and stability, as it brings about a number of other undesirable reactions involving free radicals and carbonyls with proteins (Jayaraman 1995). The determinants of the rate and degree of browning are comparable in complexity with those of carotenoid oxidation. Both browning and carotenoid oxidation are subjected to change due to processing and storage, all of which are influenced by water.

Intermediate moisture (IM) fruits developed earlier (Jayaraman 1988) had a drawback due to the high solute (sugar) concentration needed to depress a_w to below 0.90 and are more susceptible to browning and carotenoid oxidation besides making them too sweet. Hurdle technology (HT) fruits enables shelf stability to be achieved at higher a_w levels by combining with mild heat treatment and pH besides addition of antimicrobials. This allows less solute concentration and high moisture content (Jayaraman et al. 1997).

This communication reports the results of experiments on the effect of hurdle technique on non-enzymatic browning and carotenoid oxidation (besides component transfer) in comparison to IM technique during processing and storage of some fruits.

Preparation of HT and IM fruits : The fruits guava, pear, papaya and mango of optimum maturity and ripeness were selected and HT fruits were prepared using sugar syrup as per procedure described by Jayaraman et al (1997). IM fruits were prepared as described by Ramanuja and Jayaraman (1980). Citric acid was added to bring down the pH in case of HT fruits only where the natural pH was above 4.5.

Analytical methods : Moisture, ascorbic acid and beta carotene contents were determined as described by Ranganna (1986), pH using a standard digital pH meter (Toshniwal Model CL 54), total SO_2 by the iodimetric method (Pearson 1973), equilibrium relative humidity by the modified graphical interpolation technique of Jayaraman et

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TABLE 1. COMPARISON DATA ON COMPONENT TRANSFER DURING PROCESSING OF HURDLE TECHNOLOGY FRUITS (HTF) AND INTERMEDIATE MOISTURE FRUITS (IMF)

	Solid gain, %[a]		Weight loss, %[b]		Moisture loss, %[c]	
Fruits	HTF	IMF	HTF	IMF	HTF	IMF
Guava	9.2	11.4	50.0	52.0	68.0	80.7
Pear	8.4	10.6	48.6	67.6	65.5	83.8
Papaya	5.1	11.3	45.7	57.1	60.1	81.1
Mango (Badami)	5.2	15.6	44.0	52.7	61.1	82.1

[a]Solid gain = $\frac{\text{Final solid} - \text{Initial solid} \times 100}{\text{Initial gross wt.}}$

[b]Weight loss = $\frac{\text{Initial gross wt} - \text{Final wt} \times 100}{\text{Initial gross wt.}}$

[c]Moisture loss = $\frac{\text{Initial moisture} - \text{Final moisture} \times 100}{\text{Initial moisture}}$

al (1977) to get water activity and component transfer as described by Bolin et al (1983). Non-enzymic browning during storage was periodically analyzed by extraction, using aqueous ethanol and measuring extract OD at 420 nm in photoelectric colorimeter (Baush and Lomb, Sprectronic 20, USA) and also as diffuse reflectance of ground sample in a reflectance meter (Elico, Hyderabad). Overall acceptability was evaluated by 10 semi-trained staff members and final score was expressed as the average on a 9-point Hedonic scale.

Component transfer : Component transfer data during soaking of HT and IM fruit slices in sucrose syrup are given in Table 1. Different fruit slices exhibited different degrees of osmotic effect, when soaked in sugar solution. Component transfer data showed that the IM fruit slices exhibited higher weight and volume reduction and solute gain compared to HT fruits.

Physico-chemical data : Moisture content of IM fruit slices varied between 27.2 and 37.4, °Brix 58 to 61° and ERH between 79.2 and 88.5. In case of HT fruit slices, moisture varied from 53.8 to 59.0, °Brix 37 to 41° and ERH between 94.2 and 95.0. Retentions of ascorbic acid and SO_2 contents were better in IM fruit slices compared to those of HT fruits (Table 2).

Storage studies : Characteristics of HT and IM fruit slices during storage are given in Table 3. Non-enzymatic browning was more in all the samples of IM fruits as compared to HT fruits during storage. Similarly, fading of colour due to carotenoid oxidation at the later stage of storage was more pronounced and rapid in case of IM mango and papaya as compared to HT fruits. It was apparent that at higher water contents, the dilution of reactants by water was predominant and this slowed down the development of browning and carotenoid oxidation. The results showed that high moisture HT fruits were significantly less susceptible to browning and carotenoid oxidation during storage as compared to IM fruits. This is in confirmity with the earlier findings of Labuza and Saltmarch (1981) who found that browning rate generally increased with increasing a_w at low moisture contents, reached maxima at a_w 0.4–0.8 and decreased with further increase in a_w. According to Williams (1976), reaction rates of lipid oxidation and non-enzymic browning are near or maxima at IM range of a_w 0.7–0.9 and decreases only at higher on lower a_w. Thus, browning and carotenoid oxidation strongly influenced by a_w and water content and are closely related to the mobility of the reacting species.

The susceptibility to browning during storage indicated the necessity to incorporate sufficient SO_2 in the product to ensure satisfactory shelf life. This confirms with the earlier finding of Lopez Malo et al (1991) who found a direct relationship between sulfite loss and browning induction period in apple preserved by combined methods. Sulfurous acid prevents aldose-amine interaction by blocking the carbonyl group of the reducing sugars. It was

TABLE 2. PHYSICO-CHEMICAL PROPERTIES OF HURDLE TECHNOLOGY FRUITS (HTF) AND INTERMEDIATE MOISTURE FRUITS (IMF)

Paremeters	Guava		Pear		Papaya		Mango (Badami)	
	HTF	IMF	HTF	IMF	HTF	IMF	HTF	IMF
Moisture, %	53.8	31.4	58.4	27.2	59.0	37.4	55.8	36.5
ERH, %	95.0	88.5	94.2	84.8	95.0	88.1	95.0	79.2
pH	4.0	4.3	4.0	4.2	4.5	5.4	3.9	4.4
Brix°	37.0	61.0	40.0	60.0	37.0	60.0	41.0	58.0
Ascorbic acid, mg/100g MFB	77.0	91.0	9.4	12.5	18.0	21.0	28.0	31.0
Beta-carotene, mg/100g MFB	-	-	-	-	2.0	2.1	9.7	9.9
SO_2, ppm	350	460	510	550	401	500	340	420

TABLE 3. CHARACTERISTICS OF HURDLE TECHNOLOGY FRUITS (HTF) AND INTERMEDIATE MOISTURE FRUITS (IMF) DURING STORAGE AT AMBIENT TEMPERATURE (19-33°C)

Fruit	Period, months	Non-enzymatic browning				Beta-carotene Retained, mg/100g MFB		SO_2 retained, %		Organoleptic score overall acceptability	
		Extract OD (420 nm)		Reflectance, %							
		HTF	IMF	HTF	IMF	HTF	IMF	HTF	IMF	HTF	IMF
Guava	0	0.08	0.09	44	41	-	-	100.0	100.0	8.0	8.0
	6	0.18	0.23	31	25	-	-	28.3	16.1	6.4	5.5
Pear	0	0.02	0.03	30	26	-	-	100.0	100.0	8.0	8.0
	6	0.05	0.06	23	17	-	-	32.6	15.1	6.4	5.8
Papaya	0	0.04	0.05	24	22	2.0	2.1	100.0	100.0	8.0	8.0
	8	0.09	0.11	15	13	0.9	0.3	43.2	30.5	6.5	5.6
Mango, (Badami)	0	-	-	32	30	9.7	9.9	100.0	100.0	8.0	8.0
	12	-	-	22	18	5.1	2.9	21.8	12.5	6.5	5.6

observed in the present studies that SO_2 content slowly decreased during storage both in HT and IM fruits (Table 3). However, HT fruit slices were less susceptible to browning and carotenoid oxidation. Although SO_2 is effective in reducing the browning, there is a limit of food regulation tending to reduce or prevent its use. The appropriate alternative inhibition is yet to be tried out. In the present studies, optimum level of SO_2 achieved by the addition of 0.3% potassium metabisulphite to soak solution to bring the level to 400-600 ppm in the product. In addition to prevent browning, SO_2 also acts as an antioxidant in retention of carotenoids. These properties are reflected in the present studies, as manifested in retention of β-carotene in case of HT mango and papaya during storage.

Sensory evaluation data showed that the HT fruits with better organoleptic scores, were less sweet due to lower solute gain and softer due to higher moisture content compared to IM fruit slices.

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Iodine Content in Water of Tripura in North Eastern India

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Iodine content in water of Tripura in North Eastern India is reported for the first time. In 22 representative localities throughout the State, 103 water samples were collected from deep tubewells, shallow tubewells, shallow wells, ponds and rivers/streams for determining iodine content. The results showed that the overall level of iodine in deep tubewell water was in the range of 0.1–0.8, shallow tubewell water in the range 0.2–2.2, shallow well water in the range of 0.4–8.9, pond water in the range of 0.6–9.5 and river/stream water in the range of 0.3–5.0 μg/L. When the iodine levels from different sources were compared, it was observed that these levels were significantly lower in the subterranean water than in the surface water ($p<0.0005$), in the deep tubewell water than shallow tubewell water ($p<0.0005$), in the surface flowing water than pond water ($p<0.05$) and shallow well water ($p<0.025$). The analytical data showed that subterranean water was severely deficient in iodine, while the surface water was mildly deficient. Thus, the State of Tripura can be regarded as an iodine deficient zone geologically.

Keywords : Iodine, Goitre, Endemic, Water.

In the iodine deficient environment, the people do not get enough iodine from food and water for thyroid hormone synthesis and the deficiency of iodine causes several health problems. The major consequences are goitre (enlargement of thyroid gland), weakness and paralysis of muscles, mental defect, deaf mutism, still birth and miscarriages as well as lesser degree of physical and mental function. Iodine deficiency also affects the socio-economic development of a community. The entire North Eastern India including the State of Tripura is in the conventional goitre zone in India (Pandav and Kochupillai 1982). Recent goitre surveys in the State and the Capital City Agartala have shown that endemic goitre is still prevalent in the region (Chandra and Ghosh 1993; Chandra 1994; Chandra et al. 1997 a, b). The environmental iodine deficiency as the underlying cause of Himalayan endemic goitre was established and it was also found that the level of iodine in drinking water was extremely low in the endemic zones with no values higher than 3 μg/L (Kochupillai et al. 1980). Iodine content of the soil can be estimated by the iodine levels in drinking water and in general, the deficient areas have iodine levels below 2 μg/L (Hetzel 1989). Zeltser et al (1992) have categorised the iodine deficient zones - as the severe dificient zone having iodine less than 4 μg/L of water; moderate deficient zone with iodine level 4-10 μg/L of water and the relative deficient zone having iodine level 20 μg/L water. Therefore, in order to understand the environmental iodine status in the region, water samples from different sources throughout the State were analysed and the results are reported in this communication.

For the proposed study, 22 representative localities (17 from the rural blocks and 5 from the urban notified areas) were selected by purposive sampling method (Cochran 1977) to get proper representation. In each study area, at least 4 water samples of about 100 ml were collected at random from the available sources in the marked wide mouth screw capped plastic bottles for the determination of iodine. The collected water samples were brought to the laboratory and kept at 4°C until analysis. On the day of analysis, the samples were brought to room temperature.

During analysis, all the collected samples from the same area and same source were mixed properly and the iodine contents of water samples were measured following the method of Karmarkar et al (1986). Iodine in water was measured by its catalytic action on the reduction of the ceric ion (Ce^{4+}) to the cerous ion (Ce^{3+}) coupled to the oxidation of arsenite, As^{3+} to As^{5+}. The ceric ion (Ce^{4+}) had a yellow colour, while the cerous ion (Ce^{3+}) was colourless. Thus, the course of reaction was followed by disappearance of yellow colour. Water sample (7 ml) was taken in the test tube in which 1 ml of 20% sodium chloride, 0.5 ml 60% sulphuric acid and 0.5 ml 0.1N arsenous acid were added separately. All the test tubes were then vortexed and kept in a water bath at 30°C. Then 1 ml of ceric ammonium sulphate was added to all the tubes. Exactly after 20 min, 1 ml of ferrous ammonium sulphate was added in each test tube to stop the reaction. Then, 0.5 ml of potassium thiocyanate (4% KCN) was added to each test tube

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and the reading was taken in a spectrophotometer at 550 mμ. The standard iodine solutions with known strength were processed similarly and the readings were taken in the spectrophotometer. A standard curve was drawn and the concentration of iodine in water sample was determined using this as reference.

In all, water samples from 22 localities were collected and analysed. The iodine levels of water are presented in Table 1. The overall iodine content of subterranean water was in the range of 0.1–2.2 μg/L, while that of surface water was 0.3–9.5 μg/L. Comparison of iodine contents of water of different sources was made and the data (Table 2) showed that iodine levels were significantly lower in subterranean water than in surface water ($p<0.0005$), in deep tubewell water (i.e., water of about 400-500 feet depth) than shallow tubewell water (i.e., water of about 150-200 feet depth) ($p<0.0005$), in surface flowing water than pond water ($p<0.01$) and than shallow well water ($p<0.025$).

TABLE 1. IODINE CONTENT OF WATER FROM DIFFERENT SOURCES IN 22 STUDY AREAS OF TRIPURA

	Iodine content (μg/L) of water from various sources				
	Subterranean water		Surface water		
Study areas	Deep tubewell water	Shallow tubewell water	Shallow well water	Pond water	River/ Stream water
Taranagar	–	2.2	8.3	4.6	4.9
R.K. Nagar	–	0.7	3.3	4.1	2.5
Agartala	0.4	1.7	4.9	3.5	1.5
Madhupur	0.6	1.2	0.7	3.5	2.2
Kalitila	0.3	0.2	6.0	0.9	2.5
Kunjaban	0.5	0.4	1.9	2.5	1.7
Purba Ramchandraghat	–	1.2	4.5	5.3	2.0
Uttar Durganagar	0.3	0.9	3.5	2.0	2.8
N.C. Nagar	0.2	1.0	4.1	4.2	4.8
Mohanbhog	–	0.3	1.0	6.6	5.0
Bhuratali	–	1.3	1.2	9.1	2.9
Kathalchari	–	0.4	4.6	2.7	1.9
Jolaibari	0.8	1.3	5.9	9.5	0.3
Arya Colony	0.8	1.0	4.6	3.0	4.4
Kalabaria	0.6	1.0	5.3	9.3	1.8
Dakshin Chandrapur	–	0.9	3.9	3.5	1.7
Rajkang – Rangkang	0.3	0.9	0.4	0.6	1.4
Manik Bhander	0.4	1.8	5.0	6.8	3.3
Manughat	0.1	0.5	1.2	2.9	1.3
Sonaimuri	0.1	0.9	0.4	3.4	4.5
Ragna	0.6	1.0	7.4	4.9	3.0
Vidyanagar	0.1	1.4	8.9	1.1	0.8
Ranges	0.1-0.8	0.2-2.2	0.4-8.9	0.6-9.5	0.3-5.0
Subterranean water : 0.1-2.2			Surface water : 0.3-9.5		

TABLE 2. COMPARISON OF IODINE CONTENTS OF WATER FROM DIFFERENT SOURCES

Type of compared water	Sample No.	Median iodine level, μg/dL	Level of significance of difference one tail t test
Surface water and	66	3.5	$p < 0.0005$
Subterranean water	37	0.8	
Shallow tubewell water and	22	1.0	$p < 0.0005$
Deep tubewell water	15	0.4	
Pond water and	22	3.5	$p < 0.05$
Shallow well water	22	4.3	
Pond water and	22	3.5	$p < 0.01$
Surface flowing water (River and/Stream)	22	2.5	
Shallow well water and	22	4.3	$p < 0.025$
Surface flowing water (River and/Stream)	22	2.5	

The results indicated that iodine content of water varied from source to source and from locality to locality. Overall, it was found that subterranean water was severely deficient in iodine, while the surface water was mildly deficient. All these data suggest that the State of Tripura is geologically an iodine deficient zone.

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Chemical and Microbiological Properties of *Mehiawah* - A Popular Fish Sauce in the Gulf

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Poor handling of raw fish at ambient temperature can easily lead to contamination with pathogens like *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* and there was concern that *mehiawah*, a spiced, fermented fish sauce popular in the Gulf States, could pose a health hazard to consumers. An extensive examination of home-made and commercial samples of *mehiawah* revealed that samples were free from vegetative pathogens even after storage for several months at 20-25°C; *Bacillus cereus* was identified in one sample. The inhibitory nature of the product was confirmed, when fresh *mehiawah* was inoculated with *Salmonella typhi*, *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *E. coli*. Only *V. parahaemolyticus* survived beyond 14 days and even this species could not be detected at 21 days. It was concluded that, during routine manufacture, the initial processing of the fish eliminated any serious contamination, while the combined effects of salt, acidity, spices and, perhaps fatty acids from the fish oil, arrested the growth of any pathogens that might enter the product during bottling.

Keywords : Fish sauce *(mehiawah)*, Chemical composition, Microbiological properties, Survival pathogens.

Countries of the Middle East are surrounded by sea and/or criss-crossed by rivers and hundreds of years ago, catching fish was the full-time job of the majority of the people in the region. Since modern methods of preservation were unknown, salting/drying and fermentation were the only ways to preserve fish and many types of fermented fish were produced (Dirar 1993; Musaiger et al. 1990; Salama et al. 1977).

Mehiawah is one fermented fish product that is still made today and it was probably brought to the Gulf States by immigrants from the Iranian coastal region many years ago. It can be consumed with bread at almost any meal, or may be used as a condiment. It is prepared mostly at home, but there is one licensed factory in Bahrain, which produces *mehiawah* commercially under the supervision of the Public Health Authorities. *Sardinella gibbosa* (Indian Sardine) is commonly used in the manufacture of *mehiawah*, and large quantities are caught in May and June mostly in the waters off Oman (Sivasubramanian and Ibrahim, 1982). While some fish are processed immediately into *mehiawah*, much of the catch is sun-dried for later use.

Equally important is the fact that *mehiawah* is made from whole fish that may have been exposed to high ambient temperatures during catching and landing and yet despite the fact that fish come from this potentially contaminated background, it has been reported that *mehiawah* has a shelf-life of two years at temperatures of > 20°C. Consequently, the aims of this study were to examine this microbial stability in more detail and specifically to (a) establish the typical chemical and microbiological characteristics of *mehiawah* and (b) determine whether selected pathogens could survive and/or grow in the retail product.

Typical procedures for producing *mehiawah* at home or on a commercial scale are shown in the Flow diagram. In both cases, the main ingredients are fish, salt, wheat and spices; dried lemon is used only in the home-made product. No specific steps are taken to avoid microbial contamination.

Chemical and microbiological analyses : Sealed bottles of fresh *mehiawah*, both home- and factory-made were collected from the State of Bahrain and two bottles from each source were analysed immediately. After shaking each bottle thoroughly to distribute the suspended solids, duplicate samples were tested for total solids, protein, fat, ash, salt (sodium chloride) and pH according to the methods of the Kirk and Sawyer (1991). Two bottles were also stored at room temperature (20-25°C) for 6 months and the analyses repeated.

In addition, the duplicate samples from two bottles of fresh *mehiawah* were examined for total colony counts (TCC), *coliforms*, *Staphylococcus aureus*, *Bacillus cereus*, yeasts and moulds, lactic acid bacteria, *Listeria monocytogenes* and *Salmonella* spp. As before, the bottles were shaken thoroughly to mix the sediment throughout the water phase, and serial dilutions from each sample were prepared

* *Corresponding Author*

Fresh fish	**Dried fish**
Wash the fresh fish	Grind the cleaned, dried fish (3 kg)
Add salt (20-25%, w/w and thyme, *Thymus capitatus* (1%, w/w)	Soak in water (3-4 h)
Fill into glass jars (5-10 l) or plastic tanks (75 l), and leave to stand in the sunlight for 2-3 months	Add salt (20-25%, w/w) and mash into a slurry
The 'fish paste' is then mashed into a slurry and coarse filtered through a stainless steel mesh	
Add 1 kg of fish paste to 8 l of warm water (30°C)	3 kg of slurry are added to 8 l or warm water (30°C)

Add 300 g of the ground/mixed spices[1]

(Ground, dried fish may be added along with the spices to enhance the flavour)

At Home :	**In Factory :**
Mix thoroughly and fill into clean glass bottles with light closures that allow for escape of gas.	Mix thoroughly in stainless steel vat, and boil for 1 min; cool *mehiawah* to room temperature and allow to remain in vat for 24 h. Boil for a second time and while hot, fill into clean, glass bottles (750 ml) with sealed closures.

Bottles of home-made *mehiawah* are stood in direct sun for one week, but the factory product is ready to consume immediately; unopened bottles from either source may be stored at ambient temperature for up to one year.

[1]The spices are a ground blend of cumin, *Cuminum cyminum* (500 g); coriander, *Coriandum sativum* (1000 g); mustard, *Brassica juncea* (1000 g); wheat, *Triticum aestivum* (1500 g); fennel seeds, *Foeniculum vulgare* (750 g); black pepper, *Piper nigrum* 100 g*; dried lemon, *Citrus limon* 20 pieces*

* Not included in factory-made *mehiawah*

Flow diagram of the general procedures employed for the manufacture of *mehiawah*

in peptone water (0.1%) before subsequent examination, using the procedures specified in AOAC (1995). The entire procedure was repeated again after storing a similar pair of bottles for 6 months at 20-25°C. On both occasions, mean counts were reported as colony-forming units (cfu)/ml of product, except for *Listeria* and *Salmonella* where the enrichment technique meant that only 'presence' or 'absence' could be recorded.

Survival of food-borne pathogens in Mehiawah: Suspesions of *Vibrio parahaemolyticus* (Dubai Public Health Laboratory, Dubai). *Staph. aureus* (Ref. No. 53, Pasteur Institute, France), *Escherichia coli* (Ref. No. 5157, Pasteur Institute, France) and *Salmonella typhi* (Ref. No. 6062, Pasteur Institute, France) were prepared in 0.1% peptone water (3% NaCl for *V. parahaemolyticus)* to match a turbidity of 4 on the McFarland Standard (approx. 90 x 10^7 cfu/ml) using a densitometer (API-BioMerieux, Marcy L'Etoile, Lyon, France).

Based upon these estimated counts, appropriate volumes of each suspension were added separately to four batches (1,500 ml)of home-made *mehiawah* to give counts of about 1.0 x 10^4 cfu/ml for *V. parahaemolyticus, Staph. aureus, E. coli* or *S. typhi,* respectively. The four batches of inoculated *mehiawah* were then thoroughly mixed to ensure even distribution of the inoculum and duplicate samples (25 ml) were removed for analysis. Serial dilutions of each sample were made in peptone water (0.1%) down to 10^{-8} and then, from the three highest dilutions (10^{-6}, 10^{-7}, 10^{-8}), the total colony counts for each species or presence/absence in the case of *V. parahaemolyticus* and *S. typhi,* were determined, using the procedures specified in AOAC (1995). The remaining volumes of inoculated *mehiawah* were then dispensed into bottles (2 x 750 ml) and stored at room temperature. The enumeration procedures were repeated at 7, 14, 21 and 28 days from inoculation.

The chemical composition date of typical samples of *mehiawah* are shown in Table 1. It is clear that the different procedures have altered the composition of the end-products. The higher protein and ash contents of the factory-made material are probably a reflection of the use of dried fish, but the modest pH of the same product suggests that the acidity would not inhibit microbial activity. The decline in pH with time is likely to be due to the production of lactic acid by the lactic acid bacteria present and/or a build-up of the breakdown products of proteolysis or lipolysis. The breakdown

TABLE 1. THE CHEMICAL COMPOSITION OF *MEHIAWAH* OF FACTORY AND HOME MAKES

Source of *mehiawah*	Water	Ash	Fat	Protein	Salt	pH
Home-made (initial)	83.1	4.5	3.8	4.6	3.4	4.5
(at 6 months)	81.9	5.0	2.5	3.8	4.6	3.8
Factory-made (initial)	73.9	7.4	2.8	9.6	5.3	5.7
(at 6 months)	73.9	7.3	2.0	9.4	5.9	4.9

* All figures (except pH) are expressed as g/100 ml and are averages of four readings; protein equals nitrogen x 6.25

of fat could be of significance for the liberation of short chain fatty acids could play an important role in enhancing both the microbial stability of the product and its flavour (Kabara 1993). However, the data confirm that *mehiawah* is not a uniform product and even the factory *mehiawah* was subject to variation. This problem is not unexpected, because *mehiawah* is, in effect, a coarse slurry of fish and spices and hence a precise distribution of water and solids would require more sophisticated filling equipment than is available locally at the present time.

The total colony counts (TCC) recorded in the home-made *mehiawah* was 1.5 x 10^6 cfu/ml and this figure only declined to 1.9 x 10^5 at 6 months even though the pH had dropped from 4.45 to 3.65, In contrast, Musaiger and Jaidah (1991) detected 1.3 x 10^9 cfu/ml in one sample of home-made *mehiawah* produced in Bahrain and the difference between the microbial loads may be because dried lemon was not used during the preparation of the samples studied by Musaiger and Jaidah (1991). The addition of dried lemon raised the titratable acidity (Kirk and Sawyer 1991) from 0.86% in the fish paste to 1.4% in the sample of home-made *mehiawah*.

The numbers of lactic acid bacteria followed the pattern of the TCC, in that the mean count was 1.3 x 10^6 cfu/ml for the first sample, before slowly declining to 2.0 x 10^5 cfu/ml at 6 months. A tentative examination (API *Lactobacillus* strips) of some typical colonies on the Rogosa Agar showed that *Lactobacillus brevis* was the dominant species and it may be that, in the acidic conditions of *mehiawah*, lactobacilli are the only micro-organisms to survive in any number. Presumably, many of the colonies recorded in the TCC were *Lactobacillus* spp.

One of the samples of home-made *mehiawah* examined shortly after production gave rise to peacock blue colonies of *B. cereus* on *Bacillus cereus* (BC) agar after 48 h of incubation at 35°C, but the counts (60 cfu/ml) were quite low and all subsequent tests were negative. Nevertheless, *B. cerus* was detected in some other fermented fish sauces, such as *patis* and *nampla*, seven months after preparation (Crisan and Sands 1975).

Despite the presence of coliforms in some of the ingredients of *mehiawah*, none were detected in the end-products. It is likely that the concentration of salt in the fish paste employed to make *mehiawah* (20%, w/w) inactivates many coliforms and even the concentration of salt in the *mehiawah* (3.34%) may inhibit the growth of some relevant species. Although *Staph. aureus* has a marked degree of salt tolerance, it was not detected in any of the samples of home-made *mehiawah*, and similar negative results were recorded for *L. monocytogenes*. Gohil et al (1996) suggested that the competitive ability of this latter species was lower at ambient temperatures than below 5°C, so that the storage of *mehiawah* at room temperature could operate against the survival of *Listeria*. *Salmonella* was also absent in all the samples, as were yeasts or moulds.

In the factory-made *mehiawah*, the initial microbial load was 1.4 x 10^8 cfu/ml, but this level had declined to 1.9 x 10^6 cfu/ml at 6 months. These TCCs were higher than those for the home-made *mehiawah*. *Lac. brevis* dominated the microflora with an initial count of 1.42 x 10^7 cfu/ml and a final figure of 2.5 x 10^6 cfu/ml (6 months). In spite of the fact that the pH of the factory-made products ranged from 4.90–5.70, no pathogens or yeasts or moulds were detected.

Table 2 shows the results of analyses of the inoculated *mehiawah* at zero time (immediately after inoculation) and then at 7-day intervals up to 28 days of storage at 25°C. After one week, *Staph. aureus*, *E. coli* and *S. typhi* could no longer be detected and it would appear that the anticipated 'hurdle effect' arising from the combined inhibitory action of salt, acidity, spices and/or fatty acids (Kabara 1993) was having an immediate effect. Over three weeks, these influences, along perhaps with a decline in nutrients, led to the elimination of *V. parahaemolyticus* as well. These results would suggest that *mehiawah* is an intrinsically safe

TABLE 2. SURVIVAL OF SOME COMMON FOOD PATHOGENS IN HOME-MADE *MEHIAWAH;* ALL FIGURES (CFU/ML) ARE MEANS OF DUPLICATE COUNTS FROM TWO BOTTLES OF PRODUCT

Period, days	*S. aureus*	*V. parahaemolyticus*	*S. typhi*	*E. coli*
0	1.45×10^4	1.75×10^4	Positive	1.3×10^4
7	7.0×10^3	Positive	Positive	2.5×10^3
14	ND	Positive	ND	ND
21	ND	ND	ND	ND
28	ND	ND	ND	ND

product, but as retail bottles may be sold and the product should be consumed within ten days of production, high standards of production hygiene remain essential.

The authors acknowledge with gratitude the generous assistance and cooperation of the Municipality of Dubai, United Arab Emirates and the Ministry of Public Health, Qatar in ensuring the successful completion of this study.

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FOOD CHEMISTRY : Edited by H.D. Belitz, W. Groch and translated by Peter Hessel, Christiane Sprinz, Dr. Sabine Jordan and Dr. Margaret Burghagen, Published by Springer Verlag, Berlin, Heidelberg, 1999, pp. 992. Price US $ 59.95.

The book entitled, "Food Chemistry" is a translated version of Fourth German edition of this text book. This book is comprised of 24 chapters starting with 'Water' as Zero chapter and ending with 'Drinking Water, Mineral and Table Water' as the 23rd chapter. It covers the basic knowledge of the chemistry of different constituents of foods such as water, proteins, amino acids, lipids, carbohydrates, minerals, vitamins, enzymes, aroma substances etc., relationship of their structures with functionality, changes that take place in these constituents and the impact of these changes on the quality of foods during processing. The interactions of these constituents during processing have also been presented by the authors. The book also gives basic as well as latest information about different food additives, food contaminants and toxicants. In the second half of the book, authors have presented different food groups alongwith their functional constituents and their role in processing of each food group.

There are 460 Figures and 531 Tables in the book. The book has been translated by 4 authors. There are more than 850 references in this book.

The Zero chapter describes the chemical and physical structure of water, water activity, their role in storage of foods alongwith nine Tables and seven Figures.

First chapter covers the full description of the chemistry of amino acids, peptides and proteins by presenting their chemical structure, classifications and reactions with different groups. It also describes the denaturation of proteins and its impact on food quality. The effects of individual amino acid on the quality of foods have been presented by the authors, processing of proteinaceous materials using texturization, hydrolysis, spinning. Extrusion cooking has also been highlighted to give the role of proteins in developing food products.

Chapter 2 describes general properties of enzymes, their classification, co-factors, theory and kinetics of enzymatic reactions, factors affecting rate of enzymatic reactions. In the end of the chapter, the authors have highlighted the enzymes analysis and their applications in food processing.

Chapter 3 deals with lipids, fatty acids and their physical and chemical properties. It also describes phospho and glycolipids with their structures. Lipoproteins have been described very well. It also covers the changes in acyllipids of foods alongwith unsaponifiable constituents.

Chapter 4 deals with chemical structure, properties and utilization of monosaccharides, oligosaccharides and polysaccharides. It also covers enzymatic degradation of carbohydrates, which would be very useful for biotechnological approach of carbohydrates processing.

In Chapter 5, the authors have tried to describe aroma substances, their threshold value and aroma value, off flavour, food taints. It also covers distillation, extraction and structural analysis of aroma compounds and the relationship of structure with odour.

Chapters 6 and 7 deal with vitamins and minerals, their classification, occurance, role and requirements.

Chapter 8 covers various types of food additives alongwith their structures and applications.

Chapter 9 deals with chemical structure of different toxicants and contaminants present in raw materials and processed foods.

Chapter 10 gives the description of milk and dairy products. It includes compositional constituents of milk, processing of dairy products, aroma of dairy and milk products.

Chapter 11 deals with structure, physical properties and composition of eggs. It also covers storage of eggs and egg products.

Chapter 12 describes in detail the structure composition and functions of muscles tissues. This chapter also covers various kinds of meat, storage and processing and different meat products. However, information on the chemistry of meat curing should have been included.

In Chapter 13, various types of marine foods including fish, whales, shrimps, crabs, lobsters etc, their composition and post-mortem changes have been described. It also deals with storage and processing of fish and fish products. Wherever required, chemistry part has been highlighted.

Chapter 14 deals with edible fats and oils including their origin, processing and

identification. It also describes detection of changes during processing and storage of fats and oils. However, this chapter lacks basic chemistry part of fats and oils.

Chapter 15 describes chemistry of cereals and cereal products including the composition and functions of individual constituents. This chapter also covers the influence of additives/minor ingredients on baking properties of wheat flour and baking process. In this chapter, emphasis has been given on wheat and wheat products only. This should have been either general or few more cereals like rice should have been included.

Chapter 16 deals with legumes and the authors have included sufficient data on the composition of constituents.

In Chapters 17 and 18, the author has described vegetables and their products and fruits and their products, respectively. These chapters cover chemistry of their constituents, alongwith chemical changes which take place during the manufacture of different products from fruits and vegetables.

Chapter 19 deals with chemistry, processing and chemical changes, which take place during the manufacture of sugar, sugar alcohols and honey.

In Chapter 20, authors have described chemistry of beer, wine and spirits along with their manufacturing process.

Chapter 21 deals with composition of coffee, tea, cocoa and chocolates. It also highlights major constituents chemically. Manufacture of chocolate has also been described.

Chapter 22 describes spices, salt and vinegar. The aromatic compounds present in spices have been highlighted very well. This chapter also deals with composition of salt and vinegar.

Last chapter of the book deals with drinking water, mineral and table water including their treatments, physical and chemical analysis.

To summarize, authors have presented useful information in depth on the chemistry of different food constituents alongwith their physical and chemical properties and their role in food processing and human nutrition. The authors have also covered composition of different food groups, their processing and chemical changes during processing. Thus, the information presented in 23 chapters makes this book most suitable for the graduates, post-graduates and researchers in the disciplines of Food Science, Food Technology, Plant Biochemistry, Post-harvest technology, Food Process and Engineering, Food Quality and Safety.

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SAFETY EVALUATION OF CERTAIN FOOD ADDITIVES : WHO Food Additives Series 42, Prepared by The Fifty-First meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Published by International Programme on Chemical Safety, World Health Organisation, Geneva, pp. 490, 1999; Price Sw fr. 90, Price in developing countries: Sw ft. 63/-

Joint FAO/WHO Expert Committee on Food Additives (JECFA) serves as a scientific advisory body to FAO, WHO, their member States and the Codex Alimentarius Commission by preparing reports of their meetings and publishing specifications and toxicological monographs. This volume was prepared at the 51st Meeting of the JECFA which met in Switzerland, 9-18 June 1998. The monographs in this volume summarise the safety data on selected food additives, viz.,

Flavouring agents : Trans-Anethole, Furfural, Menthol.

Food Colours : Riboflavin from genetically modified *B. subtilis*.

Preservatives : Sulphur dioxide and sulphites.

Sweetening agent : Stevioside.

Thickening agents : Carrageenan, *Eucheurna* seaweed, sodium carboxymethyl cellulose, enzymatically hydrolysed, and miscellaneous substances like γ-cyclodextrin, glucono-δ-lactone and calcium, magnesium, potassium, and sodium salts of gluconic acid and polyglycitol syrups.

The volume also covers safety evaluation of groups of related substances of flavouring agents and assessments of the intake of specific food additives, namely, benzoates, BHA, BHT, TBHQ and sulphites. Following Annexures are included in this publication :

Annexure 1 : Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives.

Annexure 2 : Abbreviations used in the monographs

Annexure 3 : Participants in the fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives

Annexure 4 : Acceptable daily intakes, other toxicological information and information on specifications

One of the monographs in this volume deals with Menthol - a widely used flavouring agent. The first draft prepared by Dr.G.J.A. Speijers of the Netherlands is given in detail with recommendations of the Committee. Menthol was first evaluated at the eleventh JECFA meeting in 1968, when it was allocated an unconditional ADI of 0-0.2 mg/kg b.w. and a conditional ADI of 0.2-2.0 mg/kg b.w. Since that time, new studies have become available, principally, two-year studies of carcinogenicity in mice and rats. At the 51st Meeting of JECFA, the Committee noted that the highest dose of ±Menthol (two optical isomers of menthol) tested in the long-term studies in mice and rats had no specific toxic effect. The Committee recommended an ADI of 0-4 mg/kg b.w. on the basis of the NOEL of 380 mg/kg b.w. per day in the long-term study in rats, applying a safety factor of 100.

Joint FAO/WHO Expert Committee's efforts to establish the scientific basis for assessment of the risk to human health due to intake of Food Additives, through a series of WHO publications is highly commendable. This Volume-42 in WHO Food Additives series is of immense value for Food industry Managers for effective use of food additives and for experts engaged in decision making in national and international committees for food standards.

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AFST (I) ANNOUNCEMENT

ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA)

Central Food Technological Research Institute Campus, Mysore - 570 013, India.

Invites Nominations for Fellows of AFST (I) for the year 1999

The Association has pleasure in inviting nominations from persons to be conferred as "Fellow of Association of Food Scientists and Technologists (India)" (FAFST) to honour those who have contributed significantly to the progress of Food Science and Technology.

General

1. The awardee will be called as Fellow of Association of Food Scientists and Technologists (India) and in an abbreviated form as FAFST.
2. The total number of Fellows of the Association will not exceed 5% of the total membership, including regular and life members of the Association, in any given year or 100, whichever is lower.
3. The title of Fellow has so far been awarded to 45 AFST(I) members and 6 non-members who have contributed to the progress of Food Science and Technology.

Eligibility

1. The aim is to honour persons of outstanding merit who have contributed significantly in the field of Food Science and Technology including R & D, Product/Project Development, Industry, Transfer of Technology and Marketing. The merit of contribution should be the main criterion.
2. Among the Fellows to be nominated every year, 70% will be from AFST(I) and remaining 30% may be from non-members who have contributed significantly for the development of Food Science and Technology.

Nominations

1. The nomination for Fellow should be proposed by 5 AFST(I) members of good standing for a minimum of 5 years or by 2 Fellows of the Association. This is applicable to AFST(I) members as well as non-members.
2. Any regular or life member of AFST(I), who has been continuously a member of the Association can sponsor the nomination for only one Fellow in a particular year.
3. The nomination shall be accompanied by acceptance of the person proposed.
4. The nomination shall be in the format given on the following page. A brief bio-data of the nominee, highlighting the Scientific or Technological achievements in the area of Food Science and Technology, supported by a list of publications not exceeding 10 important research papers or other supporting documents not **exceeding 20 pages,** must accompany the nominations.
5. Central Executive Committee Members of AFST(I) are not eligible to be nominated as Fellows.
6. The nomination duly proposed and agreed by the nominee shall be sent to the Hon. Executive Secretary, AFST(I) by **31st March 2000.**

Selection of Fellows

The nominations received will be placed before an Expert Committee, appointed by the CEC for suitable recommendations to CEC each year. CEC by majority decision will finalise the names of Fellows for each year. **The decision of CEC in this matter will be final.**

Privilege of a Fellow

The Fellow shall be entitled to the following rights:

1. The awardee will be entitled to add FAFST after his/her name as short title.
2. To be present and vote at all general body meetings.
3. To propose and recommend the candidates for Fellow of the Association.
4. To receive *gratis* copies of one of the publications of AFST(I).
5. To fill any office of AFST(I) duly elected.
6. To be nominated to any committee of AFST(I).
7. To offer papers and communications to be presented before the meeting of the Association.
8. The title will remain for life time of the member.

Cessation of Fellow

1. Any Fellow may withdraw his/her title of the Association by signifying his/her wish to do so by a letter addressed to the Hon. Executive Secretary, AFST(I), which will be placed before the CEC for acceptance.
2. If the Association comes to know of any activity prejudicial to the interest and well being of the Association, the CEC will have the right to withdraw the title.

Conferring as Fellows

The Fellow will be conferred with a Citation at the time of AGBM or at any other suitable function of the Association.

The Association may invite some Fellows, nominated each year, to deliver special lectures in the area of their specialization either at the AGBM or any other function arranged by the AFST(I).

Please forward your nominations duly filled as per the format given on the following page and mail it by Registered Post to the Hon. Executive Secretary, AFST(I), CFTRI Campus, Mysore-570 013, before **31st March 2000.**

The envelope containing the nomination along with the bio-data and contributions **(6 copies)** should be superscribed 'Nomination for Fellow AFST(I)'.

R. NARESH
HON. EXECUTIVE SECRETARY

ASSSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA)
CFTRI CAMPUS, MYSORE - 570 013
Nomination Form For Fellows

We, the following members of AFST(I) wish to propose

Full name and academic distinction

FULL NAME

DATE OF BIRTH

AREAS OF SPECIALIZATION

ACADEMIC QUALIFICATIONS

---

for election as the Fellow of AFST(I). We append below the statement of his/her claims for election as Fellow and certify that in our opinion he/she is fully qualified for that distinction. We also certify that he/she has been informed of the obligations attached to the fellowship of the AFST(I) and is agreeable to abide by them, if elected.

Statement of the proposer (not to exceed 100 words) setting out the discovery, invention or other contribution to newer processes/products or the industrial development of the knowledge made by the nominee.

.....

Seconder's name & signature

Date :

Station :

Proposer's name & signature

Date :

Station :

(Signature of supporters from their personal/general knowledge)

(1)

(2)

(3)

I agree for the above nomination

(Name & Signature)

.....

Note : (1) **Six copies** of the candidate's bio-data and list of important scientific publications not exceeding 10 pages and one set of reprints or supporting documents not exceeding 20 pages shall be attached to this form.

(2) Additional information that would be of assistance in considering the nomination may be supplied on separate sheet.

(3) Last date for receipt of nomination at the office is **31st March 2000.**

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

Nominations for AFST (I) Awards for 1999

Nominations for the following awards of the AFST(I) for the year 1999 are invited. All nominations should be sent by Registered Post, so as to reach Honorary Executive Secretary, Association of Food Scientists and Technologists (India), CFTRI Campus, Mysore-570 013, **before 31st March 2000.**

PROF. V. SUBRAHMANYAN INDUSTRIAL ACHIEVEMENT AWARD

The guidelines for the award are :

(i) Only Indian nationals with outstanding achievement in the field of Food Science and Technology will be considered for the award.

(ii) The nominee should have contributed significantly to the enrichment of Food Science and Technology and the development of agro-based food and allied industries in India.

(iii) The nominations duly proposed by a member of the Association must be accompanied by the bio-data of the nominee, highlighting the work done by him/her for which he/she is to be considered for the award.

(iv) The awardee will be selected by an expert panel constituted by the Central Executive Committee of the Association.

(v) Central Executive Committee Members of AFST(I) are not eligible to apply for the award during their tenure.

The envelope containing the nominations, along with bio-data and contributions **(six copies)** should be superscribed **"Nomination for Prof. V. Subrahmanyan Industrial Achievement Award-1999".**

LALJEE GODHOO SMARAK NIDHI AWARD

The guidelines for the award are :

(i) The R & D group/person eligible for the award should have contributed significantly in the area of Food Science and Technology in recent years, with a good standing in his/her field of specialization.

(ii) The nominee(s) should be duly sponsored by the Head of the respective Scientific Institution and the application for this award should highlight complete details of the contributions made by the nominees and their significance.

(iii) The nomination duly proposed by a Member of the Association must be accompanied by the bio-data of the nominee.

(iv) Central Executive Committee Members of AFST(I) are not eligible to apply for the award during their tenure.

The envelope containing the nominations along with bio-data and contributions **(six copies)** should be superscribed **"Nomination for Laljee Godhoo Smarak Nidhi Award 1999".**

BEST STUDENT AWARD

The award is to be given to a student having a distinguished academic record and undergoing the final year course in Food Science and Technology in any recognised University in India. The aim of the award is to recognize the best talent in the field and to encourage excellence amongst the student community.

The guidelines for the award are :

(i) The applicant must be an Indian national.

(ii) He/she must be a student of one of the following courses:

(a) M.Sc. (Food Sciences/Food Technology).

(b) B.Tech., B.Sc. (Tech), B.Sc. (Chem. Tech) with Food Technology specialization.

(iii) He/she should not have completed 25 years of age on 31st December 1999.

Heads of the Department of Food and Science and Technology in various Universities may sponsor the name of one student from each institution, supported by the candidate's bio-data, details starting from high school onwards, including date of birth and post-graduate performance to date **(six copies).**

The envelope containing the nomination should be superscribed **"Nomination for Best Student Award 1999".**

YOUNG SCIENTIST AWARD

The award is aimed at stimulating distinguished scientific and technological research in the field of Food Science and Technology amongst young scientists in their early life.

The guidelines for the award are :

(i) The candidate should be an Indian national below the age of 35 years on 31st December, 1999, working in the area of Food Science and Technology.

(ii) The candidate should furnish evidence of either.

(a) Original scientific research of high quality, primarily by way of published research papers and (especially if the papers are under joint authorship) the candidate's own contribution to the work.

OR

(b) Technological contributions of a high order, as reflected by accomplishments in process design etc., substantiated with documentary evidence.

The application along with details of contributions and bio-data **(six copies)** may be sent by registered post with the envelope superscribed **"Nomination for Young Scientist Award 1999".**

BEST PAPER AWARD AND BEST FEATURE ARTICLE AWARD

These awards are given by the AFST(I) Educational and Publication Trust to the author(s), who have contributed the best paper to the *Journal of Food Science and Technology* and best feature article to *Indian Food Industry* published in 1999. A panel of experts, constituted by the Central Executive Committee will scrutinize the issues and select the best paper/feature article for the award.

R. NARESH
HON. EXECUTIVE SECRETARY

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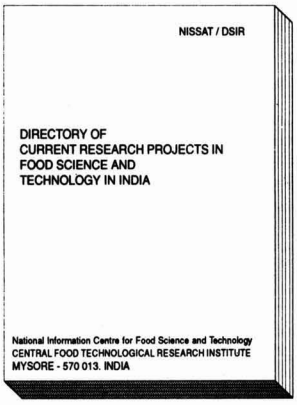

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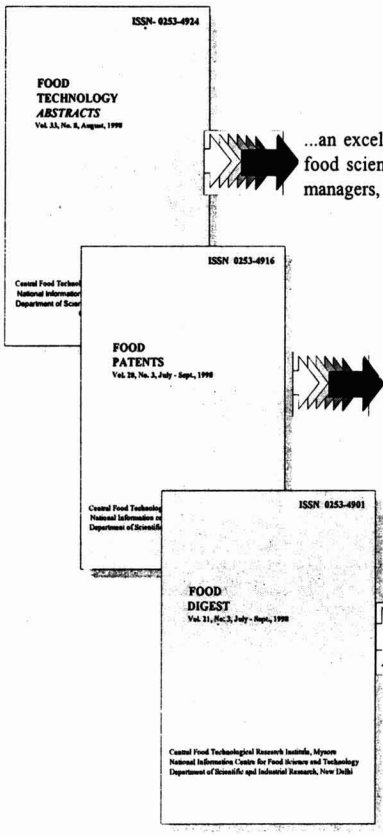

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