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

Telephone : 521747, Telex 0846-241 FTRI IN, Cable : FOODSEARCH, MYSORE.
Fax : 521747, 27697, 24475 (Country code : 91, Mysore code: 821); E-mail afst @ nicfos. ernet. in

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## Editor :

## B.K. Lonsane

Fermentation Technology Department,
Central Food Technological Research Institute, Mysore-570 013, India

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## C O N T E N T S

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# Methods for Processing and Utilization of Low Cost Fishes : A Critical Appraisal

V. VENUGOPAL
Food Technology Division
Bhabha Atomic Research Centre, Trombay, Bombay-400 085, India.

Several fish species present in total catch are not commercially important and are therefore, not fully exploited for human consumption. From the economic as well as the nutritional view points, it is essential that these species are also used for food purposes. Development of food products from these fishes involve the processes generally based on flavourization, texturization and isolation of valuable edible components. This review examines various problems and possibilities in making use of these fishes as a source of human food.

**Keywords** : Under-utilized fish, Flavourization, Texturization, Product development, Value-addition.

India, which ranks seventh in the world in fish production, is on the threshold of a blue revolution as a consequence of steady growth of the fishery sector during the last several years (Anon 1994a). The marine fisheries sector has registered a growth of about 16% in volume and 41% in value in 1993-'94, the export of fishery items being worth about Rs. 2503 crores in foreign exchange (Anon 1994a). India has a coastal line of about 7500 kms, a shelf area of nearly 500,000 million sq. km., and an exclusive economic zone of 2 million sq. km., which provide several varieties of demersal and pelagic fishes (Kant 1991). Surveys have shown potentially good fishing grounds for demersal and pelagic fishery resources, the landings of which vary depending upon locations. These surveys have also shown the existence of diverse species such as anchovies, ribbon fishes, catfishes, silverbellies in abundant quantities in Western coasts up to a depth of 40 metres (Swamy 1994; Silas 1977). The country's fishery catch is about 4 million tonnes, while the projection of marine fish production during 1990-2000 has been estimated to range between 2.0 and 2.7 million tonnes (Silas 1977, Yogamoorthi and Sivashankar 1994). The fishery sector is bound to progress further with plans for collaborative ventures for deep-sea fishing and fish processing (Anon 1994b; Kant 1991).

In terms of consumer preference, a significant proportion of total available fish constitutes commercially unimportant and hence under-utilized species, most of which are landed as a by-catch of fishing operations aimed at targeted species (James 1986; Society of Fishery Technologists 1987). This has resulted in an industry, processing only a few selected items leaving several fish species not utilized to the full extent (Morrissey 1988; Santhana Krishnan 1987). This review discusses the various possibilities of value-addition to these fish species for the development of food products.

According to Whittle and Hardy (1992), fishery resources can be defined as under-utilized in a number of ways. These include : (1) species available, but difficult to catch, process or market (2) species caught in large quantities or as a by-catch and used for low value industrial products, but could be upgraded for human consumption (3) waste of edible flesh generated as a result of inefficient handling, processing or distribution of food products, and (4) simple loss of quality and value in the handling and sale of fishery products. The inherent problems in their use are their extreme heterogeneity of composition, bony structure, dark flesh, small size, unattractive appearance and texture, strong flavour and possible presence of toxic species (Connel and Hardy 1981; FAO 1981). Due to these reasons, it has become a common practice that such fish are discarded at sea in preference to high value species (Bell 1978). It is estimated that 5 to 20 million tonnes of shrimp trawling by-catch is discarded at sea worldwide, while 2 million tonnes of wet fish is lost for want of adequate icing and refrigeration facilities (James 1986).

The need for better utilization of total fish catch arises from several reasons, which include recognition of depletion of the commercially important fish stocks throughout the world, nutritional importance of fish as human food, and environmental significance. Current world fish landing is about 100 million tonnes (Anon 1994a). Table 1 shows the major groups of fish species that are landed annually (FAO 1990). It has been observed that several commercially important fish species are over-harvested, thereby leading to

TABLE 1. WORLD FISH PRODUCTION : MAJOR GROUPS OF SPECIES, 1978-1990 (IN 1000 METRIC TONNES).

| Fish Group | 1978 | 1984 | 1987 | 1990 |
|---|---|---|---|---|
| **Marine fish** | | | | |
| Herring, sardine, anchovy | 13,300 | 19,000 | 22,000 | 22,000 |
| Cod, hake, haddock | 10,300 | 12,000 | 13,700 | 11,900 |
| Jacks, mullets | 8,100 | 8,800 | 8,200 | 9,000 |
| Redfish, bass, conger | 5,700 | 5,500 | 5,700 | 5,500 |
| Mackerel, snock, cutlassfish | 5,300 | 4,200 | 3,600 | 3,500 |
| Tuna, bonito, billfish | 2,500 | 3,100 | 3,600 | 4,200 |
| Squid, cuttlefish, octopus | 1,300 | 1,700 | 2,300 | 2,300 |
| Flounder, sole, halibut | 1,200 | 1,200 | 1,200 | 1,200 |
| Sharks, skate, ray | 600 | 600 | 660 | 700 |
| **Marine shellfish** | | | | |
| Shrimps, prawns | 1,700 | 1,900 | 2,300 | 2,500 |
| Clams, cockles | 1,100 | 1,200 | 1,400 | 1,500 |
| Oysters | 900 | 1,000 | 1,100 | 1,000 |
| Scallops | 400 | 800 | 700 | 800 |
| Mussels | 600 | 900 | 1,100 | 1,300 |
| Lobsters | 200 | 200 | 200 | 200 |
| Krill | 100 | 100 | 375 | 380 |
| **Anadromous and diadromous fish** | | | | |
| Salmon, trout, smelt | 600 | 900 | 1,000 | 1,400 |
| Shad | 800 | 700 | 800 | 600 |
| Diadromous fish | 400 | 950 | 1,000 | 1,500 |
| **Freshwater fish** | | | | |
| Mis. freshwater fish | 4,800 | 4,800 | 5,300 | 6,000 |
| Carps, barbel, catfish | 600 | 2,660 | 4,400 | 5,000 |
| Tilapias, bream | 300 | 500 | 650 | 800 |

Adapted from FAO (1990)

depletion of stocks. The over-exploitation of world's most valuable fisheries is widespread, despite attempts to control it by efforts such as identification of maximum sustainable yields, and introduction of a quota system for fishing of several of these species (Bell 1978).

Fish, in general, is considered important from the nutritional point of view (James 1984; Kent 1987; Sikorski et al. 1990). It provides about 14% of the world's needs for animal proteins and 4-5% of the total protein requirements (Whittle 1984). Fish supplies a good balance of proteins, vitamins and minerals and relatively low calories (Sikorski et al. 1990). In addition, certain species of seafoods are excellent sources of n-3 polyunsaturated fatty acids, which have therapeutic value (Kinsella 1987). The role of fish in alleviation of malnutrition in India has been discussed in detail by Kent (1987). In several developing countries including India, where the population is dependent mainly on cereal-based diets, a small quantity of fish often provides a critical nutritional improvement for them (Whittle 1984). In many of these countries, the low income earners incur more of their household expenditure on fish than other protein foods (James 1984).

A third problem of wastage of fish is environmental. Under-utilization of fish leads to its neglect upon landing, which poses problems of environmental pollution. It is, therefore, important that the total available catch is put to maximum use as food for human consumption. Thus, a need has been realized to shift emphasis from increased production to increased utilization (Morrissey, 1988). The global trends in low cost fish catch have been extensively discussed (FAO 1981; Morrissey 1988; Burt et al. 1990; Hong et al. 1990; Whittle and Hardy 1992).

## Availability of under-utilized fish in India

In India, shrimp constitutes about 35% of marine export, while frozen fish consists of ribbon

TABLE 2. SOME LOW VALUE FISHES AND THEIR AVAILABILITY (IN TONNES) IN INDIA

| Fish | Potential | Annual average landing | |
|---|---|---|---|
| **Elasmobranchs** | 137,000 | 58,968 | |
| Indian dog shark | | | |
| Black tip shark | | | |
| Hammer head shark | | | |
| Guitar fish | | | |
| Ray | | | |
| **Sciaenids** | 198,000 | 106,622 | |
| Dhoma | | | |
| Croaker | | | |
| Jew fish | | | |
| Ghol | | | |
| **Carangids** | 200,000 | 54,491 | |
| Scad | | | |
| Horse mackerel | | | |
| Leather skin | | | |
| Naked breast trevally | | | |
| Ribbon fish | 258,000 | 62,037 | |
| Cat fish | 170,000 | 54,034 | |
| Anchovy | 240,000 | 124,057 | |
| Flat fish | 47,000 | 34,011 | |
| Sole | - | 25,114 | (1988) |
| Japanese threadfin bream | - | 53,241 | (1988) |
| Barracuda | 5,000 | 2,715 | |
| Bombay duck | 100,000 | 100,000 | |
| Oil sardine | 240,000 | 158,031 | |

Source : Indian Fishery Handbook, Marine Products Export Development Authority (MPEDA) Cochin.

fish, reef cod, seer, pomfret and mackerel (Swamy 1994). It is estimated that India exploits only 7.7% catfish resources, 13.3% cephalopods, 25% perch and 11.5% coastal tuna resources (Kant 1991). The Indian fish industry is mainly export-oriented, and processes only a few selected items to cater to the needs of foreign market. Several landed fish species viz., elasmobranchs, sciaenids, carangids, anchovies, ribbon fish, catfish, flat fish and a number of other items are under-utilized. Some of the low value fishes and their availability in India are shown in Table 2 (MPEDA Cochin). It may be noted that certain cephalopods such as squid and cuttle fish, which do not attract much internal market value are commercially processed due to their high overseas demand (Swamy 1994).

**Problems in processing of under-utilized fishery resources**

Several problems exist in full exploitation of under-utilized fish resources as source of human food. These include : (1) seasonal nature or lack of steady supply of individual items, (2) mixed type catch where several species are present in a single lot, which requires its separation into individual species, (3) smaller size and compositional nature, e.g., small size of several pelagic, euphausids or mesopelagic species, which make cleaning operations, such as beheading and evisceration difficult. For example, krill is less than 5 cm in length. Several species have high fat content, while elasmobranchs have high urea contents affecting their flavour (4) high perishability of several species requiring immediate processing (5) insufficient availability of icing and cold distribution chains, which are, at present, intended only for commercially important species, and (6) lack of consumer interest, which makes value addition unprofitable.

These problems have been discussed in detail by several authors (Whittle and Hardy 1992; Morrissey 1988; James 1986; Connell and Hardy 1981).

**Conventional processing of under-utilized fishery resources**

The above mentioned problems give less scope for commercial processing of the fish by conventional techniques. While icing and refrigeration are essential in order to prolong the shelf-life of the items (Menabrito and Regenstein 1988), processes such as freezing, canning, radiation preservation or modified atmosphere storage are uneconomical. Conventional techniques may be advantageous, if used in conjunction with alternate processes, as will be discussed below. Currently, most of the species are processed by traditional methods such as curing, which does not involve high costs. Such products also do not have high market value. It has been estimated that only 42% of the total global catch of pelagic fish comprising herring, anchovies, mackerel, tuna and jacks, is used for human consumption, although, some amounts of species such as sardines, tuna and mackerel are processed by canning (Burt et al. 1990). Fatty species such as sardine and mackerel have poor shelf-life under frozen conditions. Thus, at -18°C, sardine having an oil content of 10-12% has a shelf-life of 20-40 weeks, while mackerel has a shelf-life of 16 weeks at -20°C, before rancid flavour and a hard texture developed. Swordfish discolour during frozen storage, with the appearance of a green colour beneath the skin (Ames and Poulter 1987). Large proportions of the fish species such as herring and menhaden is therefore, used for fish meal manufacture (Burt et al. 1990). About 400,000 tonnes of krill available in the Antarctic region are reduced to fish meal, while recovery of intact tail meat is most promising as human food. Connel and Hardy (1981) reported that the larger size or more familiar species will be utilized earlier than unusually small and oddly shaped fish. They described various under-utilized species available in the temperate waters and arranged them roughly in the order of probability of utilization as hake, Alaska pollock, Pacific cod, mackerel, sprat, pilchards, blue whiting and horse mackerel (Connell and Hardy 1981).

Curing (drying, salting and smoking), perhaps, is the oldest method of fish preservation. Generally, it involves salting before drying and operates through increase in the concentration of soluble substances in the fish either by abstracting water or by causing soluble substances to diffuse in (Horner 1992). In addition, concentrating the soluble substances by brining and dehydration reduces the water activity, inhibiting the growth of microorganisms. Depending on the fish composition and size, salting may be 'dry', where the fish are stacked in salt and the brine formed is allowed to drain, or 'wet', where they are immersed in a strong brine. While several dehydration techniques are currently available, the most common method employed for the under-utilized fish is sun-drying. The preservative effect of smoking on fishery produce is said to be due to a combination of factors, which include surface drying (providing a

physical barrier to the passage of microorganisms), salting (reducing water activity to control micro-organisms), deposition of phenolic anti-oxidants (which delay rancidity) and deposition of anti-microbial substances. Preservation of fish by curing has been recently discussed by Horner (1992).

**Technology for value-addition of under-utilized fish resources**

Two main technological problems need to be overcome in effective utilization of these fishes for human consumption. The first is concerned with the maintenance of quality from time of catch to that of purchase (Menabrito and Regenstein 1988) and the second is the conversion of the raw material into products desired by the consumer. The process selected should suit the individual fish species (FAO 1981; Grantham 1981; Flick et al. 1990; Bell 1978). The success lies in careful application of the techniques through which flavour and texture of original fish are masked or modified to get enhanced consumer responses to the finished products. Pigott (1976) has classified three types of products that can be prepared from fish. These include protein foods, supplementary additives and non-edible products. The raw materials for the products include not only ground fish, which is unsuitable for direct human consumption, but also carcasses frames and trimmings from filleting operations.

Based on the technology employed for product formulation, several possibilities can be broadly grouped into three; namely:-

(1) Processes depending upon modification of flavour include smoking, fermentation processes, pickling and canning in special juices.

(2) Processes based on modification of texture of isolated meat include development of meat-based food analogues such as imitation shrimp, lobster or crab, conventional processes such as preparation of fish cakes, breaded fish items, fish pastes, fish sauces etc. The texturization could be through the gel forming or emulsification properties of the proteins and may involve incorporation of additives and use of sophisticated processes such as extrusion cooking, fiberization etc.

(3) Processes are developed for the isolation of edible components such as functionally active proteins and oils. These isolates can be used as food ingredients for enhancing the nutritive and functional value of human foods, in which such ingredients are incorporated.

These processes are discussed in detail in the following sections.

**Flavourization processes**

*Smoking :* It enhances flavour of treated products and therefore, provides comparatively an easy method of value addition to low cost fish species. Smoked fish is regarded as a delicacy in several countries and, therefore, offers great potential to increase the marketability of such fishery items, when applied carefully. While both hot and cold smoking can be employed, smoking at lower temperature (<33°C) is preferred, since it gives a smoke with more flavouring and preserving substances, while at higher temperature (>80°C), these compounds are easily oxidized and destroyed. Further, hot smoking may also result in deposition of carcinogenic substances such as 3, 4-benzopyrene, apart from adversely affecting the biological quality of the proteins. Combination of smoking along with salting can help to reduce the salt content in the product and improve flavour.

The flavour of the smoked process depends on the source of smoke, apart from the temperature. Modern smoke producers feed sawdust slowly onto a very hot surface, but, worldwide, most smoking is still done using a simple fire, which is contained in a fire box. Wood shavings burn fiercely and start the smouldering process in the saw dust. Wood preservatives may produce harmful smoke, which might make the smoked fish dangerous to eat.

Smoke is an emulsion of droplets in a continuous phase of air and vapour stabilized by electrostatic charges on the droplets. Over 200 components have been identified in the vapours, which consist of organic acids, phenols, carbonyls, alcohols and hydrocarbons. Smoking preserves by depositing bacteriostatic chemicals such as phenol and formaldehyde in the system. Control of smoke production parameters must be complimented by control of raw materials, if a standard quality product is to be obtained. Wet fish is preferred for better absorption of flavour components. Colour imparted to the fish by the smoking process is due to carbonyl-amino reactions. Considerable scope exists for the improvement for traditional smoking processes for development of acceptable products from under-used fish species. The process has been successfully applied to several species including mackerel, herring, sprat, cod, haddock, salmon, trout and eel (Horner 1992; Govindan 1985).

Smoked eel is popular in Germany and Netherlands. The process, generally, consists of brining dressed eel in 10% salt followed by washing and dipping in boiling water. The fish are then given a two-stage smoke treatment, a 10 min treatment at 120-129°C followed by a cold smoking at 40-50°C for 4 h. Smoked eels are sold chilled, frozen or canned (Lane 1978). Processing of smoked, dried and powdered sardine into instant soups has been discussed (Oh et al. 1988).

*Fermentation :* It enhances the flavour and stability of treated fish. Fermented products are highly popular in South-east Asia, where they add variety to the diet and contribute to general nutrition (Beddows, 1985). Fermented products can be classified broadly into two groups. The first group consists of fish and salt formulations, whereas the second consists of fish, salt and carbohydrates (Owens and Mendoza 1985). In the former category, fermentation results from the action of autolytic enzymes present in the tissue, while high levels of salt (>20%) prevent microbial deterioration of the meat. Fish sauce is an example of a product prepared by this method. The long fermentation time for fish sauce (5 to 12 months) may be reduced by the addition of exogenous proteases. Depending upon aerobic or anaerobic fermentation, the product aroma could be modified (Sanceda et al. 1992).

Lactic acid fermentation of fish-carbohydrate mixtures in the presence of small amounts of salt (6-10%) provides possibilities for developing a number of products from under-utilized fish species. The principal source of carbohydrate used in such fermentation is cooked rice followed by cassava flour or cooked millet. The organisms used for fermentation generally belong to the genera of *Lactobacillus, Streptococcus, Pediococcus or Leuconostoc* (Owens and Mendoza 1985). Fermentation by different lactobacilli gives a sour taste to the product, which can also help in the inhibition of pathogens (Aryanta et al. 1991).

Fermentation in combination with other processes can be advantageous to modify flavour. These include possibilities for preparation of fermentated sausages (Hwang et al. 1989), fish pastes (Beddows 1985; Niwa et al. 1991) and surimi-based items (Kanooh et al. 1992). The status of fish fermentation technology in South-east Asia has been reviewed by Lee et al (1989).

*Pickling :* It can mask natural flavour and improve consumer appeal. Pickled fish products are popular in some parts of India. In the process of pickling, the fish is immersed in concentrated brine along with spices for long periods. The process is also applicable for long term preservation of fatty fish. Spice salting gives a pleasant flavour to the product. However, in spice salting, because of the lower concentration of salt used, the product may have to be supplemented with stronger preservatives such as sodium benzoate. About 20 spices have been used, giving a wide range of flavours in a wide range of combinations. The economics of fish pickling in India has been examined (Annamalai et al. 1987). A process for pickled eels preserved in gelatin has been described by Lane (1978).

Preparation of marinades involves treatment of fish with acetic acid. Marinades may be divided into two types viz., cold salt and warm marinades. The latter is prepared from pre-cooked or pre-smoked fish. Cold marinades are, however, popular. For marinading, fish are kept in solutions containing 6 to 18% salt and 0.2 to 4% acetic acid. Generally, the products are kept at 0°C for 10 to 30 days for maturing, when flavour improves (Govindan 1985).

*Canning :* It involves preparation of the fish after evisceration and beheading, which is then filled in appropriate cans. The cans are filled with brine, exhausted, seamed and sterilized. Packing in brine or oil is usually practised for commercially important fish species. Possibilities exist for flavour modification of canned under-utilized fish by either subjecting it to initial smoking, prior to canning or using filling media such as tomato juice. Thus, in the case of oil sardine, the fish is brined, smoked at 45°C and then canned in refined oil (Govindan 1985). Procedures for canning of fish species such as eel, hilsa, seer, lactarius, tilapia and threadfins have also been described (Govindan 1985).

**Texturization processes**

Development of texturized products involve initial isolation of the meat from the fish, which is further processed into products having modified texture and flavour. Protein texturization has been elaborately discussed by Kinsella (1978) and Lilliford (1986).

*Recovery of minced fish meat and its properties :* Recovery of flesh by mechanical means is the first step in development of texturized products from under-utilized fish species (Grantham 1981). Bone separation processes can be applied to most fish species, crustaceans such as krill and molluscs as well as fish frames obtained from filleting operations.

A wide range of machines is available for the purpose, a common type being the belt and drum model. The beheaded and gutted fish pass between a counter rotating belt and a perforated drum. The fish muscle, fat and blood are squeezed through a drum having an orifice size of 3 to 5 mm. Fish of approximately 20 to 50 cm in length can be easily deboned. While eviscerated fish is preferred, deboning of whole fish may give mince, which is contaminated with viscera and skin portions and consequently may require refining before use.

The colour, flavour and functional properties of the mince depend upon the initial nature and quality of fish, apart from the conditions of deboning. Prolonged ice storage of fish or frozen storage of mince prior to processing affects protein solubility, emulsification capacity, water binding capacity, cooking loss, drip loss and texture (Reddy and Srikar 1991; Reddy et al. 1992). The yield of mince obtained ranges from 50 to 75% having a content of few mg% of bone. A low bone content is desirable for better functional properties. The minced meat obtained after deboning is less stable than the intact muscle. The disruption of tissue membranes and exposure of the meat to air accelerate oxidative processes during storage. Further, the treatment also enhances microbial proliferation in the product. Trimethylamine oxide present in gadoid fish undergoes enzymatic cleavage to dimethylamine and formaldehyde, the latter influencing protein denaturation and toughening. Refrigerated storage may affect the lipid stability of mince from fatty fish species (Hwang and Regenstein 1993). Ideally, the mince should be frozen at -40°C immediately after preparation and stored at -20°C, until used. Glazing of blocks with chilled water and oxygen impermeable packaging can prevent freeze-burn on the surface.

The frozen mince has an average storage life of about 6 months depending upon the fish. Longer storage may affect the protein quality, with significant loss in functionality. Colour and odour of minces from several pelagic fish species can be improved by washing. Protein denaturation during frozen storage may be prevented by the addition of cryoprotectants such as polyphosphates or hydrocolloids such as alginate and carrageenan. Other additives include carboxymethyl cellulose, guar gum, xanthan gum and sorbitol (MacDonald and Lanier 1991; Grantham 1981).

The technology of fish mince production has been discussed by several authors (Babbit, 1986; FAO 1981; Grantham 1981; Regenstein and Regenstein 1990; Mackie 1983; Suzuki 1981; Venugopal and Shahidi 1994a). Currently, about 550,000 metric tonnes of fish mince are produced from a variety of fish species. FAO/WHO (1983) has provided a code of practice for minced fish production.

The fish mince can be used for the development of a number of value-added products. The important parameter which determines the use of the mince is its gel forming ability (Asghar et al. 1986). The myosin and actomyosin, the major structural proteins of fish muscle, have the capacity to form gel under appropriate conditions. The process involves partial denaturation of the proteins followed by aggregation due to molecular associations. During the association step, water, oil and flavouring compounds are entrapped in the gel, which in turn, determines the sensory attributes of the final product. Incorporation of ingredients such as starch, polyphosphate or salt helps to modify the texture of the finished product. Washing of the fish mince removes the sarcoplasmic proteins and other soluble components and enhances the gel formation capacity of the structural proteins. Gel forming capacity of fish varies from one species to another. Of the white-flesh varieties, species having the highest gel strength consist of croaker, barracuda, threadfin bream, lizard fish, seabream, mackerel and red bigeye, Alaska pollock, cuttlefish and hoki have poor gel-forming capacity. Among the dark-flesh species, blue marlin has the highest gel-forming capacity followed by flying fish, horse mackerel, yellowfin tuna and skipjack tuna. The proteins in the minces influence the functional properties, which include swelling, solubility, viscosity, water-holding capacity, fat binding and emulsification capacity. Ingredients that can modify the properties are vegetable oil, polyphosphates, milk proteins, gluten, sugars, salt and preservatives. A number of ingredients can be useful for the development of seafood products (Anon 1988).

*Traditional products :* Several products can be manufactured using the whole fish mince. One popular item is the composite fillet, where the fish mince is shaped to get fillets of popular species (Connel and Hardy 1981). Other traditional products include sausages, pastes, patties, balls, wafers, loaves, burgers, fish fingers, fish fritters and pickles (Grantham 1981). Several breaded items such as fish sticks and fingers have received commercial acceptance in several countries. In these, the fish

portions are usually pre-dusted with flour or a dry batter mix, coated with batter and pre-fried to set the batter as well as to enhance the texture quality of the product. Machinery for automation of the process is also available (Chand et al. 1991; Flick et al. 1990). A variety of fish cakes, nuggets, chowders, scalloped fish and stew have been prepared from minced fish by Cornell University (Regenstein 1986). The acceptability of fish cake prepared from shrimp by-catch has been discussed by Poulter and Poulter (1984). Several traditional products prepared in India and elsewhere, using low cost fish species are given in Table 3.

TABLE 3. SOME VALUE-ADDED PRODUCTS DEVELOPED FROM UNDER-UTILIZED FISH

| Fish | Product | Reference |
|---|---|---|
| Anchovies | Minced muscle blocks | Ghadi and Lewis (1977) |
| Bombay duck | Dried laminates | Doke etal (1978) |
| Barracuda | Surimi | Putro 1989 |
| Capelin | Spray dried protein | Venugopal et al (1994b) |
| Cat fish | Dehydrated salt mince | Young et al (1979) |
| | Breaded patties | Burgin et al (1985) |
| Croaker | Sausage | Setty (1987) |
| | Surimi | Putro (1989) |
| | Fish fingers | Reddy et al (1990) |
| Flying fish | Meat sol | Kitabatake et al (1988) |
| Jew fish | Cutlet | Joseph et al (1984) |
| Lizard fish | Cutlet | Joseph et al (1984) |
| | Surimi | Putro (1989) |
| | Dehydrated salt mince | Young et al (1979) |
| Miscellaneous fish | Creamy fish bites | Regenstein (1986) |
| | Salt dried minces | Young et al (1979) |
| Perch | Fish fingers | Reddy et al (1990) |
| Ribbon fish | Fish balls | Jantawat and Yamprayoon (1990) |
| | Sausage | Setty (1987) |
| | Fish fingers | Santhana Krishnan (1986) |
| Sardine | Surimi | Roussel and Cheftal (1988) |
| | Marinbeef | Suzuki (1981) |
| | Salted dry mince | Sudhakaran and Sudhakara (1985) |
| Shark | Spray dried powder | Venugopal et al (1994c) |
| Silver belly | Fish sauce | Velayudham et al (1987) |
| Sole | Minced muscle blocks | Ghadi and Lewis (1977) |
| Treadfin bream | Cutlet | Joseph et al (1984) |
| | Hydrolysate | Venugopal (1994) |
| | Surimi | Putro (1989) |
| | Spray dried powder | Venugopal et al (unpublished) |
| | Salted dry mince | Sudhakaran and Sudhakara (1985) |
| Mixed by-catch (Containing pelagic as well as demersal fish) | Fish ball | Jantawat and Yamprayoon (1990) |
| | Composite fillets from mince | Connel and Hardy (1981) |

Salted mince is another product, which has been prepared from several fishes. The process involves heat treatment of the salt/fish mix at 70°C for 2 h prior to low temperature dehydration. The product had high acceptability, when incorporated into local dishes and served to Mexican communities (Young, et al. 1979). Such products can also be stabilized for room temperature storage, after mixing with tapioca starch, soy fibre and salt and drying at 71°C for 10 h, which could be easily rehydrated (Bello and Pigott 1979). Development of salted and dried minces from threadfin bream and oil sardine has also been described (Sudhakaran and Sudhakara 1985).

A popular product developed in Japan from fish mince is known as marinbeef. The product is prepared by kneading and extruding of salted mackerel mince into alcohol to get grains of denatured proteins, which could be used as a protein source (Suzuki 1981).

*Surimi-based products :* Surimi is a myofibrillar protein concentrate produced by repeated washing of fish mince, which gives enhanced gel forming capacity to the proteins. Surimi can be kept frozen at -20°C for up to six months without significant loss of quality. About 2 to 4% of sugar or sorbitol and 0.3% of polyphosphates are added to the washed mince as cryoprotectants before freezing. Characteristics of surimi, apart from its gelling ability, are its bland taste, appreciable emulsification and water-holding capacities. Surimi generally contains 75% water, 16% proteins and less than 1% fat (Lee 1986). The development of surimi has been described by Grantham (1981), Lee (1994, 1986) and Miyake et al (1983) and Lanier and Lee (1992).

Although initially, surimi was produced on a commercial scale from Alaska pollock, several other species have been recognized to give good quality surimi. These include croaker, jack mackerel, threadfin bream, blue whiting, sardine, lizard fish, eel, barracuda and leather jacket (Flick et al. 1990; Martin and Collette 1990; Lee 1994, 1986). The technical problems associated with dark-flesh fish species are high lipid content, lipid hydrolysis, large proportions of dark muscle and water-soluble proteins and rapid protein deterioration, all of

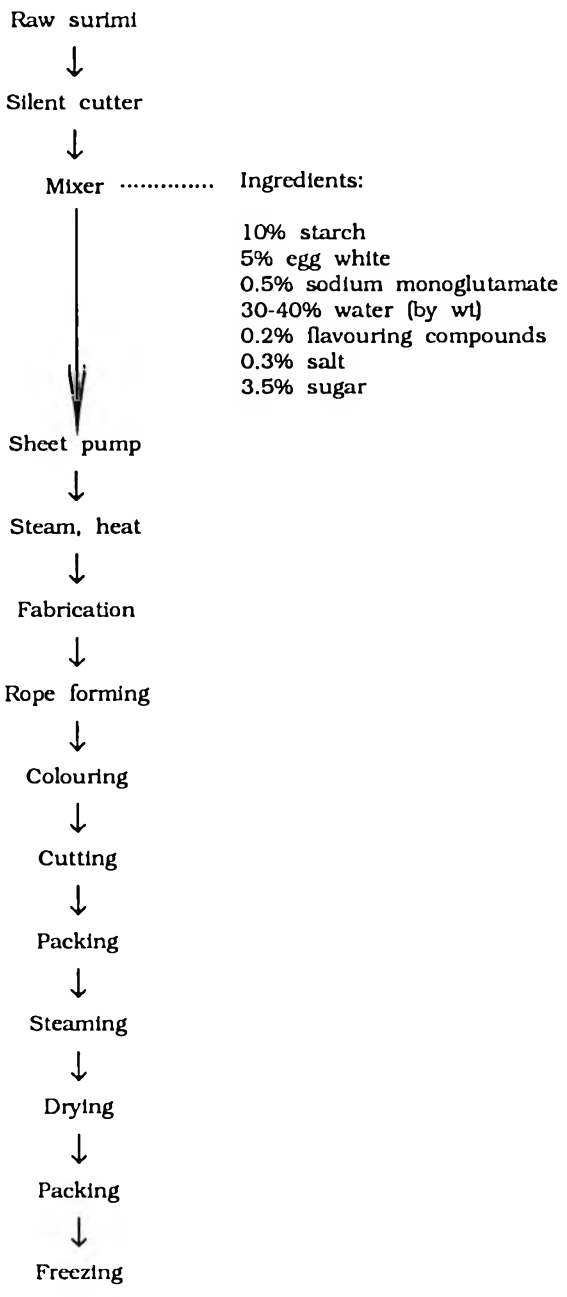


Fig.1. Processing of imitation crab legs and shrimp
*Source : INFOFISH Technical Handbook 2.*

which influence the surimi quality (Hastings et al. 1990). These problems may be overcome by removal of dark meat, washing with bicarbonate solutions, use of nitrogen-purged water for washing and cryoprotectants, and use of an improved leaching process (Hultin 1993; Putro 1989, Suzuki 1981).

Quality standards to differentiate surimi into various grades are available. These include colour, viscosity and gel forming ability. Moisture content and folding test are also used for classification of surimi (Flick et al. 1990, Suzuki 1981.)

*Fabricated foods :* Surimi is a unique protein preparation that can be texturized, flavoured and formulated to develop a wide variety of foods, including seafood analogues such as shrimp, crab legs, scallop and lobster tail, which possess the accepted texture and flavour and appearance of the authentic products (Pigott 1986). Surimi forms a gel under mild heating conditions, to give rigid matrices entrapping the water, oil or flavour components (Lanier and Lee 1992). The technology of development of Japanese 'kamaboko' products including fish sausages has been described in detail by Miyake et al (1983). Depending upon the process of preparation, these products are referred to as "kamaboko" (steamed), "chikuwa" (broiled), fried "kamaboko", fish ham and sausage. For preparation of products, surimi is mixed with binding agents such as salt, soy protein, starch, egg white and alginate in order to modify the texture. Synthetic or seafood extracts may be incorporated to enhance the flavour (Miyake et al. 1983).

Chopped surimi paste may be moulded into desired shapes which are then allowed to set and form an elastic gel. The fiberized sheets are extruded into rectangular nozzle into ropes and then developed into imitation products. Fig. 1 illustrates the flow diagram for preparing imitation crab legs and shrimp. Preparation of sausage-type products is another method, which makes use of composite moulding of emulsions (Flick et al. 1990; Lanier and Lee 1992, Pigott 1986). Finger size portions can also be cut easily from the heat-set sheets to make conventional battered products (Connell and Hardy 1981).

Several texturized products such as snack foods and breakfast cereals incorporating pasta, cereal proteins and fish proteins can be developed through extrusion cooking. Food extruders are considered as high-temperature-short-time (HTST) reactors. The process involves forcing a mixture of starch and other ingredients, usually at 15-40% moisture, through a barrel under variable conditions of temperature and pressure. This results in gelatinization of starch, which binds itself to other ingredients. The movement of the material through the barrel can be affected by single, twin or multiple screw conveyors, which help to give sufficient shear to the raw material and to get the desired product (Kinsella 1978; Harper 1981; Harper and Jansen 1985). Product development using extrusion cooking has received poor attention in the case of protein foods unlike starch products. Texturization of soya protein is the only commercial success, which processes approximately 70 million tonnes of raw

material annually (Areas 1992). There is wide scope for development of extruded products incorporating fish proteins, which could be developed by extrusion cooking at higher moisture levels. The ideal conditions for protein extrusion consist of complete disaggregation of the flour through mechanical mixing, formation of homogeneous suspension and alignment of the protein in the direction of flow (Areas 1992). Extrusion cooking for seafood industries has been discussed recently by Jayasekharan and Shetty (1992).

Development of products incorporating proteins from soybean and surimi has been attempted. Extrusion at 140°C of the mix containing 70 to 80% surimi and 30 or 20% soy concentrate or gluten at 80% moisture level, facilitated 'plastification' of the proteins apart from emulsification, gelation, restructuring, microcoagulation and/or fiberization of specific constituents. An extruded crab analogue prepared from Alaska pollock surimi is already under commercial production in Japan (Cheftel et al. 1992). Noguchi (1989) described texturization of sardine meat, where the fish meat and defatted soy flour in the ratio of 7:3 at 50% moisture level were extruded in a twin screw extruder equipped with a long cooling die. The product had the texture of animal meat and differed from fish products. Similarly, development of a low temperature extruded product from flying fish, which had texture similar to that of "kamaboko" gels, has been reported (Kitabatake et al. 1988). Extrusion cooking of surimi to get products comparable to the texture of lobster, crab and squid has also been described by Nicklason and Pigott (1989). The use of the extrusion technology for development of feeds for aquaculture has also been recognized (Durairaj et al. 1987). Development of spun fibres is another texturization method for proteins, which has been described by Lilliford (1986). The process has wide scope for extension to surimi.

As observed by Areas (1992), the enormous potential of extrusion cooking to produce texturized protein from seafoods is yet to be exploited. The process, under appropriate conditions, can improve functional characteristics of proteins without affecting the nutritional quality. The process also helps in reversing the unacceptable characteristics of several under-utilized fish species, thereby offering better possibilities for their use.

*Sausages :* These are complex mixtures of proteins, binders, fat, spices, salt and water, which are stabilized by an emulsion formation of a protein film around fat globules. The resulting product is stuffed into casings and is either steamed or smoked. Since fish contains more unsaturated lipids than red meats, the packaging material should be less permeable to light and oxygen in order to control off-flavour development. At present, polyvinylidene chloride casings are generally used in Japan. Chemical preservatives used in formulation include nitrofurylamide, nitrofurazone and sorbic acid. Fish species such as tuna, marlin and shark are being used in Japan for sausage making. Surimi, because of its high functionality and low content of unsaturated lipids, is ideal for sausage manufacture (Miyake et al. 1983).

**Isolated edible components**

The under-utilized fish species are also rich sources of edible components such as functional proteins, protein hydrolysate and unsaturated fatty acids apart from being the raw material for secondary by-products such as chitin, chitosan, enzymes, gelatin, etc. The edible items recoverable from fish are discussed below.

*Fish protein concentrates and hydrolysates :* Fish protein concentrate (FPC) was one of the earlier products developed from low cost fish for human consumption. FPC type A is a colourless and odourless product, having less than 1% lipid and is produced by solvent extraction of fish meat, while FPC type B is prepared by drying and grinding of fish mince. FPC type B has shown some success as a dietary supplement in several countries (Tagle et al. 1977). The problem of poor functionality of FPC type A, associated with high cost of production led to lack of commercial success in the process (Finch 1977), although the functionality of the product could be improved by enzymatic treatment. Production of fish protein hydrolysates from fish mince has been discussed by several workers (Mackie 1982; Venugopal 1994). The product is prepared by controlled hydrolysis of fish proteins by proteases, followed by inactivation of the enzymes and spray-drying of the digest.

*Thermostable water dispersions and functional proteins :* Fish structural proteins are insoluble in water and consequently pose problems in their use as ingredients. The problem has been overcome recently with the development of thermostable water dispersions of fish muscle. The process involves washing of the fish mince, similar to that used for surimi processing, followed by its homogenization in cold water, and reduction in

viscosity of the prepared homogenates. In case of fish species, such as Atlantic herring and mackerel, the viscosity reduction was effected by lowering of pH by acetic acid (Venugopal and Shahidi 1994a, b; Shahidi and Venugopal 1994), while in case of capelin, the viscosity was reduced by mild heating (Venugopal et al. 1994a). The dispersions having protein contents upto 5% were stable upto 100°C. Such dispersion has also been prepared from threadfin bream (Chawla et al. unpublished data).

The dispersions can have several applications as a protein ingredient. The immediate application could be for the development of functional protein powder by spray-drying. Such products have been prepared from capelin (Venugopal et al. 1994b) and threadfin bream (Venugopal, unpublished data). Uses of the powders as protein supplements have been discussed recently (Venugopal and Shahidi 1994a).

*Fish liver oil :* Shark liver oil has attracted commercial interest for the last 40-50 years because of its high contents of vitamins A and D. Further, it contains significant amounts of total hydrocarbons (squalene and pristane) and diacyl glyceryl ether (Summers and Wong 1992). The recovery of oil from shark consists of natural decomposition of liver, acid ensilage in presence of formic acid, alkali digestion and steam rendering (90°C for 30 min). Traces of antioxidants such as TBHQ (tertiary butyl hydroxyquinone) protects the unsaturated fatty acids against oxidation. The liver oil recovered is degummed, bleached and deodourised.

The liver oils from deep water sharks are important to many industries. Crude shark liver oil is processed in Japan for the preparation of cosmetic products. The squalene present in shark oil is used in the manufacture of lubricants, bacteriocides, pharmaceuticals and cosmetics. In cosmetic products, it is incorporated to enhance skin permeability for the passage of active ingredients. Squalene is also useful to prevent formation of nitrosamines, to synthesize steroid hormones and as a stimulant to enhance the synthetic capacity of hormones by the body. The hydrogenated squalene, namely, squalane is an excellent moisturizer and carrier of fragrances. Diacyl glyceryl ethers have bacteriostatic action, protect against radiation, aid in the healing of wounds and inhibit tumour growth (Summers and Wong 1992).

Several other ingredients useful to human can also be isolated from fish species. These include collagen and gelatin from elasmobranchs, chitin and chitosan from crustaceans and carotenoid pigments and enzymes such as proteases. These are discussed elsewhere (Society of Fisheries Technologists, 1987).

**Industrial prospects**

In India, in spite of availability of a large amount of under-utilized fish species, development of value-added products for human consumption has not reached commercial level, although some attempts are currently being made in this direction. The problems in this respect are many. These include lack of steady supply of raw material of high quality, higher cost of production and lack of steady market for the products in comparison with well recognized commercial items. However, it should be noted that development of these products provides dual opportunities of diversification of the fish processing industry and the utilization of low value fish for human consumption. Table 3 summarizes products and processes developed, most of them from several under-utilized fish available in India. One of the major potential areas is manufacture of surimi-based products. The value-added products developed from low cost fish such as ribbon fish, anchovies, sardines, threadfin bream, sardines etc., can have both internal and external markets (Santhana Krishnan 1987). Economic feasibility aspects of some new products are also available (Annamalai et al. 1987). In Thailand, fish ball preparation has grown to an industrial scale requiring about 35 tonnes per day of fish species like Trichiurdae, Synodontidae and Cynoglossidae (Jantawat and Yamprayoon 1990). The increase in value-added production capabilities allows year-round seafood processing instead of a seasonal or traditional operation, as pointed out by Devanter and Swientek (1986). For example, flexible breading line can produce a variety of products and enable to move into new product areas (Devanter and Swientek 1986). Successful production of value-added items from under-utilized fish species depends upon continuous availability of high quality raw material, selection of the right process for individual fish species as well as suitable packaging systems for the products, adherence to rigorous quality control and identification of potential markets.

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# Drying Characteristics of a Tropical Marine Fish Slab

D. KANNAN AND S. BANDYOPADHYAY*

Aquacultural Engineering Section, Agricultural Engineering Department,
Indian Institute of Technology, Kharagpur - 721 302, India.

A study was conducted on drying rates of a tropical marine fish, Bombay duck *(Harpodon nehereus)*, in the form of a rectangular slab, at dry bulb temperatures in the range of 40° to 70°C, air velocities from 0.818 to 2.128 m²/s, and relative humidities from 30 to 60%. Drying occurred at constant rate and falling-rate, when the drying rate was low, but otherwise only falling-rate period was observed. Prediction of drying time for moisture changes in the body of the fish, with the help of a diffusion model, showed that it was fairly accurate for a limited period, and that a deviation occurred at the last stage of the second falling-rate period. Liquid diffusion may not, therefore, be the only mechanism of moisture movement in drying fish.

**Keywords** : Drying characteristics, Fish slab, Tropical marine fish, Bombay duck, Diffusion model

Although drying of marine fish is still in vogue as a processing technique in India (Mukherjee et al 1990), and many other developing countries, published information on drying characteristics of tropical fish is lacking. There is also a great paucity of data on drying rate, and the basic mechanism of moisture movement in the body of the fish during drying. Such data are essential for process design and improvement of the drying system. The present paper incorporates the results of drying characteristics of a tropical fish variety, in the form of a rectangular slab. The objective was to get the basic information on mechanism of fish drying.

*Diffusion model* : In the design of dryers, the time required for drying the material under the conditions existing in the dryer, decides the size of the equipment for a given capacity (Treybal 1981). For this purpose, rates of drying curves are constructed, and they give the drying time directly (Treybal 1981). In the case of fish, which is a colloidal-gel type of material, it is assumed that moisture is transported within the material by the mechanism of liquid diffusion (Brakel 1980). Thus, Fick's second law of diffusion (Treybal 1981) can be applied to predict the average drying time during the falling-rate period. The following equation

$$\frac{\delta c}{\delta t} = D \frac{\delta^2 c}{\delta x^2} \qquad \ldots\ldots (1)$$

has long been used as a basis for calculating the rate of drying of various solids (Jason 1965; McCabe and Smith 1967; Brakel 1980). Jason (1965) found that diffusion was practically isotropic in fish muscle and therefore, he used the solution of diffusion equation in three-dimensional rectangular coordinates as follows :

$$\frac{\delta c}{\delta t} = D\left(\frac{\delta^2 c}{\delta x^2} + \frac{\delta^2 c}{\delta y^2} + \frac{\delta^2 c}{\delta z^2}\right) \qquad \ldots\ldots (2)$$

for a slab of dimensions 2a, 2b, 2c with boundary conditions :

$c = c_0$, when $t = 0$

$c = c_e$ at $x = -a, a$; $y = -b, b$; $z = -c, c$, when $t > 0$... (3)

All the terms, c in equation (2) denote moisture content, kg/kg (db), with subscripts o and e for initial and equilibrium values, respectively. D is the moisture diffusivity (m²/s), while a, b, and c are the dimensions (m). It is assumed that the moisture content at the surface attains its equilibrium value once drying commences, and that drying takes place throughout the surface of the slab (Jason 1965).

The solution of equation (2), when expressed in terms of weight loss, (Carslaw and Jaeger 1959) becomes :

$$M^* = \frac{M-M_e}{M_0-M_e} = \left(\frac{8}{\pi^2}\right)^3 \exp-\left[\frac{\pi^2 D}{4}\left(a^{-2}+b^{-2}+c^{-2}\right) t\right] \qquad \ldots (4)$$

where all but the first term are neglected. In equation (4), M denotes total moisture (kg) and $M^* = (M-M_e/M_0-M_e)$ is dimensionless.

Equation (4) was simplified by introducing a quantity $\tau$ termed as drying-time constant (Jason 1965) as follows :

$$M^* = \frac{M-M_e}{M_0-M_e} = \left(\frac{8}{\pi^2}\right)^3 \exp-t/\tau \qquad \ldots (5)$$

where $\tau = 4/\pi^2 D(a^{-2}+b^{-2}+c^{-2})$ for a particular slab. ... (6)

Equation (5) reduces to the straight-line equation :

$$\ln M^* = \ln(8/\pi^2)^3 - t/\tau \qquad \ldots\ldots (7)$$

A plot of ln M* against t can be constructed

* Corresponding Author

TABLE 1. RESULTS OF FISH DRYING UNDER DIFFERENT CONDITIONS

| Air velocity, m/s | Dry bulb temp, °C | Relative humidity, % | Moisture content, kg/kg (db) | | | | Constant rate, kg/kg.h $m^2$ | Moisture diffusivity $(m^2/s)10^{10}$ |
|---|---|---|---|---|---|---|---|---|
| | | | Initial | Equilibrium | First critical | Second critical | | |
| 0.818 | 40 | 60 | 8.843 | 0.601 | 5.50 | 2.5 | 510 | 0.99 |
| | 50 | 60 | 7.327 | 0.319 | 5.25 | 2.5 | 590 | 1.21 |
| | 60 | 60 | 7.327 | 0.086 | 4.65 | 1.0 | 615 | 2.04 |
| 1.965 | 40 | 60 | 6.729 | 0.137 | 5.00 | 2.25 | 800 | 1.75 |
| | 50 | 60 | 8.230 | 0.257 | 6.00 | 2.125 | 1130 | 2.21 |
| | 60 | 30 | 8.834 | 0.386 | - | 3.85 | - | 3.52 |
| | | 40 | 8.864 | 0.605 | - | 3.90 | - | 3.07 |
| | | 60 | 8.850 | 0.601 | - | 4.40 | - | 2.53 |
| | 70 | 60 | 8.855 | 0.701 | - | 2.25 | - | 2.85 |
| 2.128 | 60 | 60 | 8.850 | 0.458 | - | 3.80 | - | 3.26 |

- : Absence of data.

to calculate the diffusivity from the slope, if the data fall on a straight line. Equation (7) can, therefore, be used to predict the drying time.

## Materials and Methods

*Fish sample :* The tropical marine fish, Bombay duck *(Harpodon nehereus)*, was selected for the drying study. It is one of the most important species landed in Indian coasts, and one that is preferred for dry-cured products (Talwar and Kacker 1984). Fish procured from the local market was deheaded, gutted, cleaned and stored in deep freezer, for use in drying experiments.

*Experiments on drying :* The experimental set-up consisted of a constant temperature cabinet for drying and an air saturator for supply of air at saturated condition into the cabinet. The saturation temperature was determined from the desired relative humidity and the drying air temperature, and was controlled to the set point within ±1°C. Thin rectangular slabs of dimensions 37.44 mm length x 21.14 mm breadth x 7.21 mm thickness were cut from fish fillet for use in experiments. All these dimensions were the average of 9 rectangular slabs used in the experiments. Measurements of the thickness of a particular slab were made at the corners, and at the centre of a slab. The fish slab was suspended inside the cabinet on a basket from a precision balance and air, at a constant dry bulb temperature, velocity, and relative humidity, was passed in cross-flow over the slab. Changes in weight as a function of time were recorded under various experimental conditions are given in Table 1. A value of relative humidity of 60% was chosen for studying the effects of air temperature and velocity on drying rate, primarily to reduce shrinkage during drying (McCabe and Smith 1967). Considerable shrinkage can occur in fish slabs during drying at lower humidity, as has been observed during fish drying at 35% relative humidity and 24.5°C. Shrinkage is also known to be less along the major axes, than along the other two dimensions (Balaban and Pigott 1986).

## Results and Discussion

Fig.1 shows the drying curves plotted as moisture ratio M* versus time at dry bulb temperatures 40°, 50° and 60°C, with 0.818 m/s air velocity and 60% relative humidity. The data revealed the expected result of drying due to difference in drying potential with temperature (Treybal 1981). Effect of other variables on drying behaviour - such as (a) air velocities 0.818, 1.965 and 2.128 m/s at 60°C dry bulb temperature and 60% relative humidity, and (b) 30, 40 and 60% relative humidities at 60°C dry bulb temperature and 1.965 m/s air velocity - showed a similar trend.

Drying rates expressed as kg/kg (db).h.$m^2$ and plotted against free moisture content (Figs.2 and

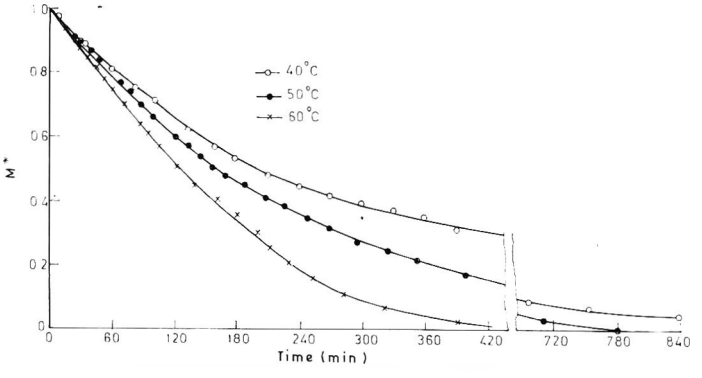


Fig. 1. Drying curves at 0.818 m/s air velocity and 60% relative humidity ($M^*=M-M_e/M_0-M_e$).

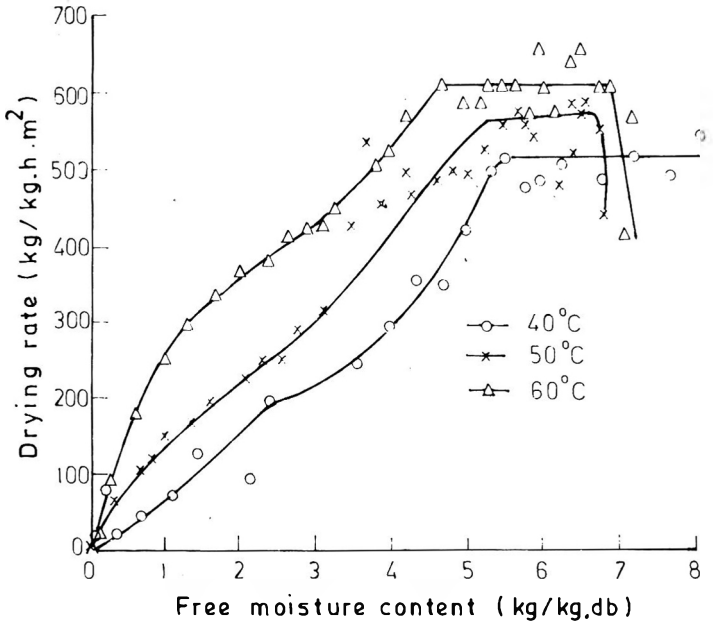


Fig. 2. Drying rate curves at 0.818 m/s air velocity and 60% relative humidity.

3) show that constant rate period is observed at air temperatures in the range of 40° to 60°C, with 0.818 m/s air velocity, but at 40° and 50°C air temperatures only with 1.965 m/s air velocity. All other results show that drying takes place only in the falling rate period. Results of fish drying under different conditions are shown in Table 1.

Constant-rate periods occurred probably due to low rate of drying at the conditions mentioned above, when fish surface behaved truly saturated (Jason 1965). The constant-rate increased with dry bulb temperature of air and the critical moisture content varied within the range of 4.65 and 6.00 kg/kg (db). However, duration of the constant-rate period was very short, i.e., about 1-1.5 h. Several workers (Jason 1965; Brakel 1980) have reported that a strictly constant-rate period may not be observed, although careful experimentation may be helpful in this regard. Irrespective of whether constant-rate period was observed or not, falling-rate period showed two distinct phases, with the first phase terminating at the so-called second critical moisture content (McCabe and Smith 1967), which ranged between 1.0 and 4.4 kg/kg (db) under the conditions of drying (Table 1). It has been found that the second falling-rate period constituted the major portion of the total falling-rate period i.e. 67-89%. Since the constant-rate period is of very short duration and overall drying takes place in the falling-rate period, where drying rate is governed by the rate of internal moisture movement by diffusion, the liquid diffusion theory (Treybal 1981) may be applicable in fish drying.

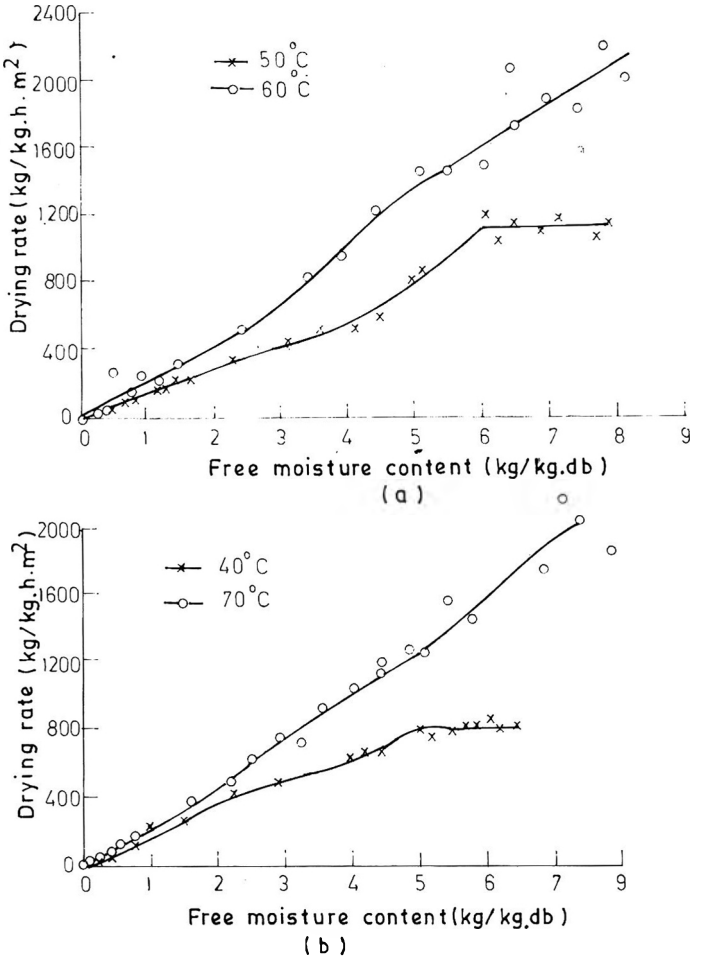


Fig. 3. Drying rate curves at 1.965 m/s air velocity and 60% relative humidity.

Fig. 4 shows the linear representation of the experimental moisture changes, and those predicted by equation (7) for the entire range of temperatures and air velocities studied. For prediction, experimental values of moisture diffusivity (D) were obtained from the slopes of the experimental straight lines ln M* versus time. Two discrepancies appeared in these plots : (a) the whole range of data did not fit the straight line relationship, except that at 40°C and 0.818 m/s air velocity; and (b) the values of intercept obtained were higher (0.96-1.0) than predicted by equation (7). Furthermore, the initial curvature predicted by equation (7) has not been observed. In spite of these discrepancies, the prediction of moisture changes is fairly accurate up to the period of drying in the second falling-rate period. There is, however, one exception with the data of 60°C at 0.818 m/s air velocity, which deviated from the predicted line, before the second falling-rate period commenced. All these discrepancies showed that liquid diffusion may not be the only mechanism for moisture movement in fish drying. This has been further reflected by the

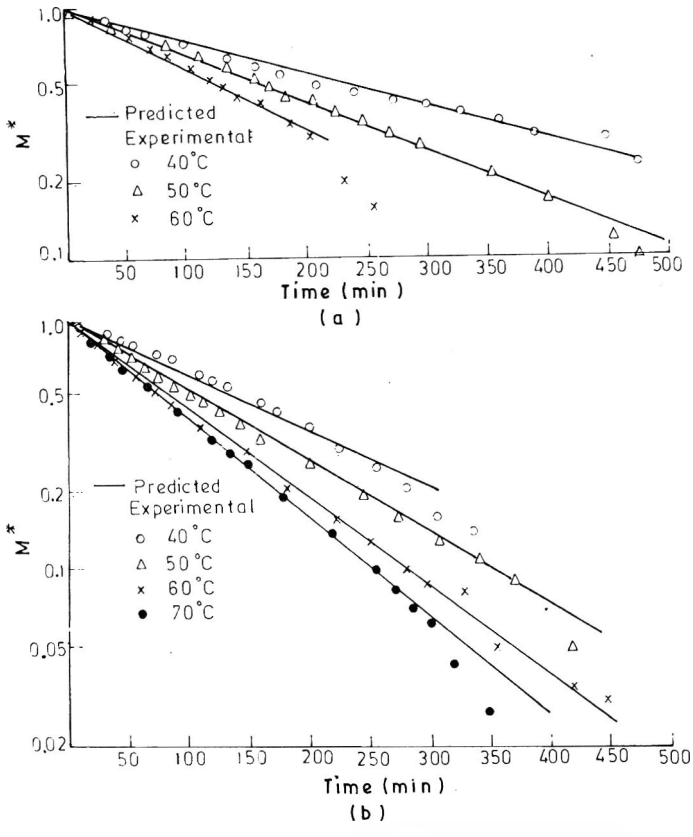


Fig. 4. Comparison of experimental and predicted moisture changes by diffusion model. Air velocity (a) 0.818 m/s, (b) 1.965 m/s at 60% relative humidity.

results of temperature dependency of moisture diffusivity.

In the diffusion model, D was assumed to be constant, but it was found to be linearly dependent on temperature. Fig. 5 shows such temperature dependency of the moisture diffusivity. The result was expressed in the form of Arrhenius equation:

$$D = D_0 \exp(-E/RT) \quad \ldots (8)$$

where $D_0$ = diffusion constant, $m^2/s$, and E=energy

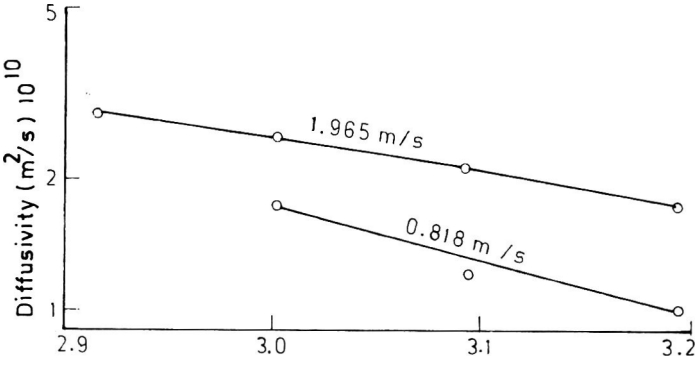


Fig. 5. Temperature dependence of moisture diffusivity.

of activation for diffusion, J/mole. The values of E for 0.818 m/s and 1.965 m/s air velocities are 9927 J/mole and 6152 J/mole, respectively. The variation of D was not found to be very strong within the temperature range studied.

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# Changes in Properties of Parboiled Rice During Ageing

K.R. UNNIKRISHNAN AND K.R. BHATTACHARYA*[1]

Department of Grain Science and Technology,
Central Food Technological Research Institute, Mysore - 57 013, India.

Five non-waxy varieties of paddy were parboiled under two conditions, milled, stored for 40 months in the laboratory, and analyzed for various properties at intervals. A waxy variety was partially tested. Original raw paddy was also stored, and then parboiled at the same intervals as above and analyzed as control. In both sets, hydration, Brabender viscosity and slurry viscosity increased over a period of 6-10 months, and then decreased steadily until the end of the experiments. Solubility of amylose and mobility of alkaline gel decreased steadily, while firmness of cooked rice increased steadily. The changes with time were strikingly similar to those undergone by raw rice during ageing, thereby showing that ageing changes occurred in parboiled rice just as in raw rice. The results suggested that neither amylases, free fatty acids, nor carbonyl compounds are responsible for the storage changes in rice. Firmness of cooked rice was well correlated with water-insoluble amylose content in all samples, regardless of parboiling or ageing.

**Keywords** : Rice, Waxy and non-waxy varieties, Parboiled rice, Ageing, Storage, Insoluble amylose.

It is well known that raw rice undergoes ageing after harvest (Desikachar 1956). The stickiness of cooked rice and the loss of grain solids into the cooking water decrease, while the firmness of cooked rice and the extent to which the rice swells during cooking increase substantially after the rice has been stored for a few months after harvest (Desikachar 1956). Besides, steaming of paddy is also practised for the improved culinary, milling and storage properties (Desikachar et al. 1969). Simultaneously, many changes occur in the physico-chemical properties of rice, and these continue steadily for at least seven years - the maximum period for which this phenomenon has been studied (Bolling et al. 1977; Indudhara Swamy et al. 1978).

Occurrence of similar changes in parboiled rice during storage does not seem to have been investigated. To the Indian consumer, new parboiled rice is as unacceptable as new raw rice, and becomes acceptable only, when it has become old. Thus, the implication is that it too ages. But, there is a fallacy in this argument. Being seasonal, the harvested paddy crop is stored in bulk and then processed, over several months, to either raw milled rice or parboiled milled rice. The milled rice is stored only for the time it takes to reach the retail end. What Indian consumers consider as aged parboiled rice is thus usually nothing but rice milled from aged raw paddy, which has been parboiled recently. Knowledge relating to ageing changes in parboiled rice, if any, is of considerable theoretical importance for throwing light on the mechanism of rice ageing. It is also of some practical significance due to the practice of procuring huge quantities of both raw and parboiled milled rice and storing these for long periods as buffer stock. A study on this subject is reported in the present paper.

## Materials and Methods

*Materials :* Five varieties of non-waxy rice were used. The varieties (and their total and insoluble amylose contents, in % dry basis, d.b., respectively) were : 'Jaya' (28.9, 17.0), 'IR 20' (27.8, 14.1),'SR26B' (29.7, 11.0) 'Intan' (25.6, 8.7) and 'Tainan 3' (18.0, 7.0). In addition, a waxy variety, 'Purple puttu' (5.0, 4.0), was used in a few experiments.

Paddy was collected from the University of Agricultural Sciences, Experiment Station, Mandya, Karnataka, immediately after harvest. It was air-dried (about 13% moisture, wet basis), cleaned and fumigated. About half of each sample (treatment B) was immediately parboiled by soaking in warm water overnight, followed by steaming under 0 and 1 kg/cm$^2$ gauge pressure for 10 min each, to obtain relatively mild and severe parboiled rice, respectively (Unnikrishnan and Bhattacharya 1987). It was air-dried and milled in laboratory by standard methods (Indudhara Swamy et al. 1978). The balance of raw paddy (treatment A), and the above milled parboiled rice (B) were stored in rectangular tins in the laboratory at room temperature (23°-30°C) for 40 months. Samples were withdrawn at intervals, the untreated raw paddy (A) was parboiled, under exactly identical conditions, as the B lot, and then milled. Samples of both- i.e., the freshly parboiled rice and the rice from the B lot- were analyzed. The stored raw paddy was also directly milled to raw rice at the beginning and the end of the experiments.

* *Corresponding Author.* [1]*Present address : Supriti, 61, Fifth Main Road, Jayalakshmipuram, Mysore-570 012, India.*

In the above scheme, treatment A, i.e., paddy stored raw and then parboiled at intervals, was the control lot, where known ageing changes would have occurred in the rice during storage. The treatment simulated what happens in the trade, as explained above. Treatment B, i.e., paddy parboiled all at once after harvest and then milled and stored, was the experimental lot. It simulated the conditions of buffer stock mentioned above and also the variable period of trading-related storage of milled rice between milling and retailing. Stored raw paddy acted as the general control.

All preliminary preparations were completed within 3 weeks of harvest. For calculation of percentage changes, this was considered as zero time. Waxy rice became misshapen upon severe parboiling and was parboiled only mildly. Only a few selected analyses were done on these samples, and raw waxy rice too was excluded from the study.

*Analytical methods :* The following properties were determined by the methods described or quoted elsewhere (Unnikrishnan and Bhattacharya 1987, 1988) : 1) EMC-S (%, w.b.) : The equilibrium moisture content attained by whole-grain milled rice when soaked in water at room temperature; 2) $W_{96}$ (g/g, d.b.) : water uptake by rice when cooked for 1 h at 96°C; 3) $W_{LT}$ (g/g, d.b.) : water uptake by rice when soaked in water for 1 h at a lower temperature (LT = 60°C for samples showing a gelatinization temperature [GT] of 68°C or more, and 55°C for those having a GT less than 68°C); 4) slurry viscosity (cP) : apparent viscosity of a 20% (d.b.) aqueous rice-flour slurry determined with a Rheotest 2 coaxial cylinder viscometer at a shear rate of 145.8 $sec^{-1}$ at 27°C; 5) Brabender viscogram : determined using 10-15% (d.b.) slurry, as appropriate in different samples; 6) $sA_{96}$ (%, d.b.) : soluble amylose content extracted from rice flour by water at 96°C for 20 min; 7) $sA_{LT}$ (%, d.b.) : soluble amylose content of rice flour extracted with water for 1 h at a lower temperature (LT=50°C for varieties showing a GT of 68°C or above and 45°C for those with lower GT); 8) gel mobility (mm) : length of travel of 2 ml of alkali 4.4% (d.b.) rice-flour gel in 1 h in a horizontal 13x150 ml test tube at room temperature; 9) firmness (%) : thickness of replicate sets of 3 cooked rice grains each, after being compressed with a weight of 500 g for 35 sec, in an INRA-viscoelastograph, expressed as a ratio of the original thickness; and 10) elastic recovery (%): ratio of spring-back of the above 3 cooked grains, 20 sec after removal of the above weight, to their compression under the weight. Total amylose content and the gelatinization temperature of the samples, which are known not to change with storage (Indudhara Swamy et al. 1978), were determined in the original raw rice. Total amylose minus the $sA_{96}$ gave the amount of insoluble amylose in rice.

In view of the good reproducibility of these analyses, as confirmed in initial checks, the analyses were generally not replicated.

**Results and Discussion**

In view of the vast amount of data generated from six varieties, three processing treatments (raw, mild and severe parboiling), two storage conditions (A, B), five storage times (0, 4, 10, 20, 40 months) and ten properties analyzed, only the results of 'IR-20' variety are graphically presented in Fig.1 to illustrate the general trends. The average values of the other data are presented in Table 1.

The trends of changes in various properties of parboiled rice with time of storage were exactly the same, both for samples A and B, as was seen earlier for raw rice (Indudhara Swamy et al. 1978). As in raw rice, the values for hydration (EMC-S, $W_{96}$, $W_{LT}$), slurry viscosity and Brabender viscosity increased initially upto 6-10 months of storage and then steadily decreased (Figs. 1a-1e). Other properties showed a continuous change from 0 to 40 months. Amylose solubility ($sA_{96}$, $sA_{LT}$) and gel mobility (Figs. 1 f, g, i) decreased steadily, while firmness and elastic recovery of cooked rice (the two trends were

TABLE 1. CHANGES IN PROPERTIES OF RAW AND PARBOILED RICE DURING AGEING[a]

| Property[b] | Maximum value[c] | Final value | |
|---|---|---|---|
| | Parboiled[d] | Raw | Parboiled[d] |
| EMC-S | 108 | 98 | 100 |
| $W_{96}$ | 106 | 93 | 96 |
| $W_{LT}$ | 110 | 81 | 92 |
| Slurry viscosity | 165 | - | 72 |
| $sA_{96}$ | | 93 | 83 |
| $sA_{LT}$ | | 80 | 66 |
| Firmness | | 138 | 127 |
| Elastic recovery | | 215 | 173 |
| Gel mobility | | 55 | 69 |

[a]Values are means of 5 non-waxy varieties and are expressed as percentages of the mean zero time values. For the first four properties, which initially rose and then fell with time, both the maximum point and final values after 40 months are shown. The last five properties rose or fell steadily with time; only their final values are shown. [b]Definitions of the properties are given under Materials and Methods. [c]Raw rice was tested only at the beginning and end of storage period. Hence, its maximum-point values are not available. [d]Values of parboiled rice are averages of mild and severe parboiled rice, both A and B treatments.

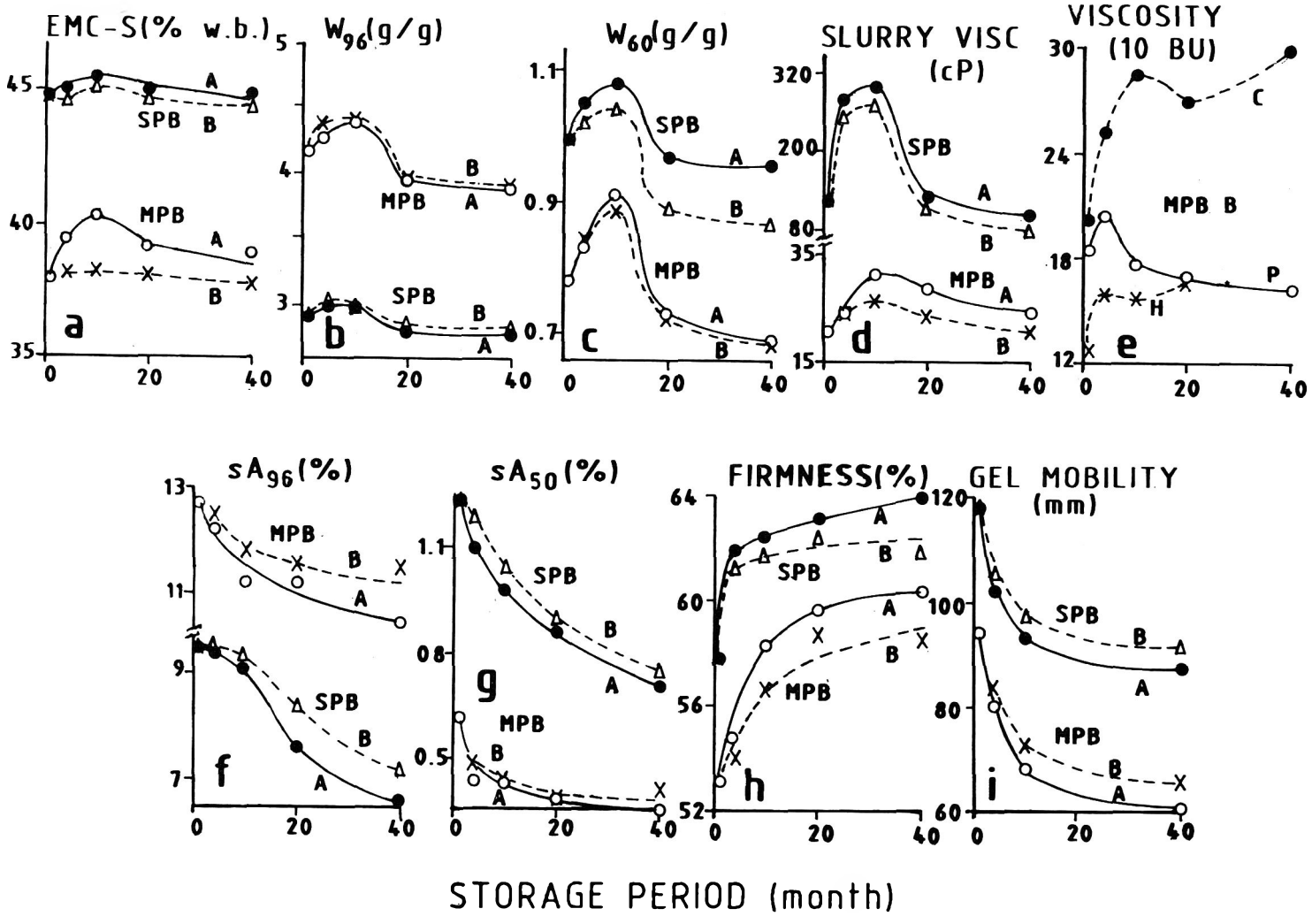


Fig. 1. Changes in various properties of mild (MPB) and severe (SPB) parboiled rice of 'IR-20' variety with time of storage at room temperature. Definitions of properties are given under Materials and Methods. **A.** raw paddy stored, then parboiled; **B.** parboiled rice stored. Brabender viscogram (12% slurry) values in Fig. e are P: peak viscosity, H: Viscosity after 95°C heating, **C:** viscosity after cooling to 50°C.

very similar, hence only firmness values are shown in Fig. 1 h) increased steadily. On the whole, the trends of A and B samples were also similar. This is clear evidence that even parboiled rice undergoes storage changes just like raw rice.

The pattern of Brabender viscograms of the mild parboiled B sample, depicted in Fig. 1e, is significant in this connection. The high breakdown (peak viscosity minus hot-paste viscosity) in the freshly harvested rice, its gradual decline and ultimate disappearance upon storage, as well as the progressively increasing setback (cold-paste minus peak viscosity) are typical of the ageing process. The mild parboiled A and the severe parboiled A and B samples gave essentially similar results and hence have not been shown in the above figure. Another instance is the progressive increase in the firmness of cooked rice with time of storage (Fig. 1 h) - one of the most perceptible sensory evidences of the ageing process. There was a concurrent progressive decrease in the stickiness of cooked rice also, as subjectively perceived, but this property was not quantitatively measured, as it has been repeatedly shown that firmness and stickiness of cooked rice were very highly and inversely correlated (Perez and Juliano 1979; Sowbhagya et al. 1987).

Average values of the five non-waxy varieties, both raw and parboiled, are shown in Table 1, expressed as mean % of the respective initial values. For all properties that initially increased and then decreased, both the maximum and the final values are shown (excluding raw rice, for which only the final values were tested). For properties that changed continuously, only the final values are presented (both raw and parboiled). The trends are similar to those shown in Fig. 1. Although there were some differences in the mean values between processing treatments (raw, mild and

severe parboiling), storage treatments (A, B) and varieties, statistical analysis by 't'-test showed that the differences were not significant in the majority of cases (detailed data are not shown).

Parboiled waxy rice gave a generally similar hydration (EMC-S, $W_{96}$) pattern, but its cooked-rice firmness did not significantly increase with storage time. Villareal et al (1976) too noted that the hardness of cooked raw waxy rice did not increase, nor did its stickiness decrease, over a period of 6 months.

*Mechanism of rice ageing :* It can be concluded that parboiled rice ages more or less just like raw rice. Apparently, whichever factors that are responsible for storage changes in the raw also operate in parboiled rice. Decrease in amylase activity during post-harvest storage (Sreenivasan 1939; Hogan 1963), action of free fatty acids (Yasumatsu et al. 1964), and cross-linking by carbonyl compounds produced by lipid auto-oxidation (Barber 1972) are some of the factors to which the ageing changes have been attributed. But the present results do not support any of these theories. One can rule out the possibility of amylases playing a role in the ageing process, as these enzymes should be destroyed during parboiling; yet, parboiled rice did age, not at once, but steadily over time. Similarly, since parboiled rice undergoes greater and faster lipid autooxidation than raw rice (Sowbhagya and Bhattacharya 1976), ageing changes should have been very much faster in the former, if the changes were caused by carbonyl cross-linking. Again, if free fatty acids were responsible for storage changes, then the changes should have been retarded in parboiled rice, for lipase is mostly destroyed during parboiling (Barber et al. 1983).

*New light on insoluble amylose :* Hot-water-insoluble amylose content of rice has been propounded as the key determinant of the varietal difference in the texture of cooked raw rice (Bhattacharya et al. 1982). The firmness of cooked rice increases and its stickiness decreases, as the insoluble amylose content of the variety increases, and *vice versa* (Sowbhagya et al. 1987; Kaur et al. 1991). It now appears that a similar situation may exist even in relation to changes in rice texture brought about by parboiling and ageing. The cooked grains of a variety become progressively harder and less sticky, as the rice is parboiled (Unnikrishnan and Bhattacharya 1987) or aged (Desikachar 1956). Significantly, this is accompanied

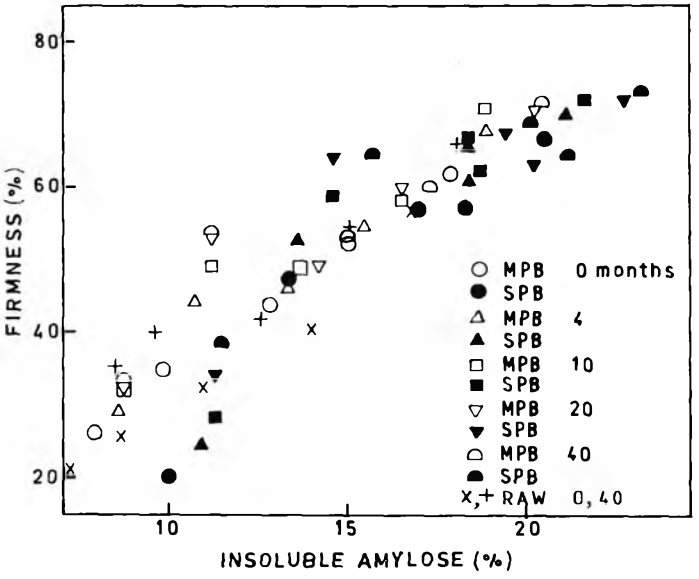


Fig. 2. Relationship between 96°-water-insoluble amylose content and viscoelastograph firmness of cooked grains of raw as well as mild (MPB) and severe (SPB) parboiled rice in five non-waxy varieties, after different periods of storage. Values of parboiled rice are averages of respective A and B treatments.

by a simultaneous progressive decrease in the solubility of the grain's amylose, both after parboiling (Ali and Bhattacharya 1972) and after ageing of raw (Indudhara swamy et al. 1978) as well as parboiled (Fig. 1f) rice. Unnikrishnan and Bhattacharya (1987) showed that insoluble amylose and cooked-rice firmness were correlated, not only in raw rice of 13 varieties but also after their parboiling under two levels, all the 39 samples giving almost a single regression curve ($r=0.977^{***}$). Now, it is seen in the present work with five varieties of raw and their two levels of parboiled rice that the above relationship holds for five periods of ageing as well (Fig. 2: $r=0.857^{***}$, for n =60). Thus, insoluble amylose may be an intrinsic basic quality attribute of rice, irrespective of variety, ageing and parboiling.

Recently, it has been shown that the insoluble amylose is actually an index of the long outer chains of its amylopectin molecule (Radhika Reddy et al. 1993). However, the estimated value of insoluble amylose increases, when rice is parboiled or aged, or both. The origin of this additional quantity of insoluble amylose is not known and is worthy of investigation.

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# Lipid Profile of Developing Oil Palm Fruit

S. GEORGE AND C. ARUMUGHAN*

Regional Research Laboratory,
Industrial Estate P.O., Thiruvananthapuram-695 019, India.

Patterns of accumulation of lipid classes and their fatty acid compositions at progressive stages of oil palm fruit development were determined. During the early stages of fruit development, the lipids were predominantly composed of polar lipids, partial glycerides and free fatty acids. By 24 weeks after anthesis, triacylglycerols accounted for 95% of the total lipids. The rapid phase of fat accumulation was between 16 and 20 weeks after anthesis. Higher contents of unsaturated fatty acids, in all the lipid classes were observed in early stages of fruit development. This trend was reversed towards the end of maturation. In the triacylglycerols, 16:0 and 18:1 contents showed significant increases during fat accumulation. Fatty acids 18:2 and 18:3 were associated with the phospholipids and glycolipids, respectively.

**Keywords** : Developing oil palm fruit, Phospholipid, Glycolipid, Diacylglycerol, Fatty acid, Monoacylglycerol, Fatty acid composition.

Although there have been a few reports on the characterization of fruit lipids of the oil palm during its development (Crombie and Hardman 1958; Bafor and Osagie 1986, 1988a, 1988b, 1989; Oo et al. 1986), a comprehensive study of the total characterization of the lipids of developing oil palm fruit has not been performed. The oil palm is an important future source of edible oil (Chada and Rethinam, 1991) and therefore, a basic knowledge of the lipids vis-a-vis other geographical regions is important. In our earlier studies, we reported on the histochemical changes in the mesocarp of oil palm (Mohan Kumar et al. 1994) and results of studies on extraction and evaluation of raw palm oil (Arumughan et al. 1989). The present study reports the lipid profile of developing oil palm fruit.

## Materials and Methods

*Sample details :* Oil palms of the 'tenera' variety (15 years old) were randomly selected from the germplasm garden of the Central Plantation Crops Research Institute, Palode, Thiruvananthapuram. Female inflorescences were identified. When more than two-third of the flowers on the inflorescence became receptive, the inflorescence was tagged. Samples were collected at two week intervals. Fruits from an entire bunch were separated and mixed well. A 500 g random, representative sample was taken for analysis. Samples for each development stage were collected from three different palms and analyzed in duplicate. Standard deviation was calculated. Bunch weight, fruit weight and percent mesocarp and kernel were determined. Moisture and oil percent were determined according to IUPAC methods (Paquot and Hautefenne 1987).

* Corresponding Author

*Isolation and separation of lipids :* Total lipids were extracted with chloroform-methanol (2:1, v/v), solvent mixture from fresh mesocarp as described by Goh et al (1982). About 20 mg of total lipid was spotted and separated into triacylglycerol, diacylglycerol, monoacylglycerol, free fatty acids and polar lipids by this layer chromatography (TLC) on 1 mm thick silica gel G (Merck, Bombay) using petroleum ether - diethyl ether-formic acid (60:40:1.6, v/v/v) (Ong et al. 1981). Lipid bands were detected by exposure to iodine vapour. The triacylglycerol, diacylglycerol, monoacylglycerol and free fatty acid bands were eluted from the gel with 5 ml x 4 portions of chloroform. The polar lipids were first eluted with acetone (10 ml x 4) to obtain the glycolipids followed by methanol (5 ml x 4) to extract the phospholipids.

*Estimation methods :* Phospholipids were estimated by elemental determination of phosphorus (Fiske and Subba Rao 1925). The phosphorus content was converted to phospholipid by multiplying with the factor appropriate for palm oil (Goh et al. 1982). Glycolipids were estimated from the hexose content, using anthrone-thiourea reagent (Southgate 1976). The quantity of glycolipid was calculated as digalactosyldiglyceride from the hexose content. For both phospholipid and glycolipid determinations, a correction for background absorbance due to silica gel was made using silica gel from the same chromatogram in the preparation of the blank.

The lipid classes separated by TLC were quantitated by conversion of the component fatty acids to methyl esters for analysis by gas-liquid chromatography using an internal standard (Kates 1986). An accurately weighed amount of internal standard, methyl pentadecanoate (Sigma Chemical Co., Missouri, USA) was added to the lipid sample,

prior to saponification and esterification. The methyl esters were then subjected to GLC. The amount of lipid sample was determined by relating the total area of the fatty acid peaks to the area of the peak for internal standard. A correction factor calculated from average molecular weight of the fatty acids, determined from the fatty acid composition, was used to convert the total amount of fatty acids to weight of the corresponding lipid class.

Methyl esters of the fatty acids were prepared by using IUPAC procedure (Paquot and Hautefenne 1987). A Hewlett Packard 5840 A Gas Chromatograph (Hewlett-Packard, Palo Alto, California) equipped with a flame ionisation detector was used. Methyl esters were analyzed on a 2m x 2mm (inner diam), 10% EGSS-X on Chromosorb W metal column. Injector and detector temperatures were maintained at 250 and 300°C, respectively. Column temperature was maintained isothermally at 180°C. Carrier gas was nitrogen at a flow rate of 20 ml/min. Methyl esters were identified with reference to standards and the peaks were quantitated by electronic integration.

## Results and Discussion

It is a unique feature that oil palm fruit yields two distinct commercially significant oils. Usually, the oil palm takes about 180 days for its full development (Hartley 1977). There was no appreciable formation of mesocarp constituents until 14 weeks after anthesis (Table 1). However, the development of endocarp was completed during this period. The subsequent changes beginning from 16 weeks after anthesis were largely in the mesocarp, as evidenced by the rapid increase in the dry matter and total lipids. The physical barrier of the fruit indicates two independent systems with sequential developmental stages and little scope for translocation of precursors between the endosperm and mesocarp. The duration of fruit development is known to be governed by geographic and agro-climatic variations and is between 150 and 180 days (Ng and Southworth 1973; Hartley 1977), as

TABLE 1. PHYSICAL PARAMETERS AND LIPID COMPOSITION OF DEVELOPING OIL PALM FRUIT MESOCARP

| Characteristic | Age of fruit after anthesis, weeks | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 |
| **Physical parameters** | | | | | | | | | | |
| Bunch weight, kg | 8.0 ± 3.0 | 13.3 ± 1.8 | 13.0 ± 2.0 | 14.3 ± 1.6 | 11.5 ± 2.0 | 19.0 ± 4.2 | 16.0 ± 4.0 | 14.0 ± 4.2 | 16.5 ± 1.7 | 17.5 ±1.7 |
| Fruit weight, g | 1.8 ± 0.4 | 4.4 ± 0.5 | 4.8 ± 1.1 | 6.8 ± 0.2 | 6.9 ± 2.1 | 7.3 ± 1.1 | 6.1 ± 1.0 | 8.6 ± 2.6 | 7.0 ± 0.2 | 6.6 ±0.6 |
| Mesocarp, % | 93.6 ± 1.2 | 73.5 ± 3.4 | 75.3 ±10.1 | 75.3 ± 2.4 | 78.5 ± 8.8 | 65.0 ±11.0 | 75.8 ± 7.8 | 73.5 ± 6.1 | 66.8 ± 7.9 | 75.2 ±0.1 |
| Kernel, % | - | - | 9.3 ± 5.3 | 9.9 ± 1.5 | 7.5 ± 5.0 | 10.4 ± 0.9 | 8.7 ± 4.3 | 7.4 ± 1.0 | 10.3 ± 4.1 | 8.2 ±0.3 |
| Shell, % | 6.4 ± 1.2 | 26.5 ± 3.4 | 15.5 ± 5.0 | 15.6 ± 0.6 | 14.0 ± 5.3 | 24.6 ±11.8 | 15.5 ± 4.6 | 19.2 ± 6.7 | 23.0 ± 3.8 | 16.6 ±0.4 |
| Moisture in mesocarp, % | 85.3 ± 0.7 | 85.2 ± 0.7 | 83.7 ± 0.8 | 83.8 ± 0.7 | 72.7 ± 0.6 | 74.4 ± 0.9 | 68.7 ± 0.6 | 42.5 ± 0.5 | 42.5 ± 0.5 | 41.4 ±0.7 |
| Moisture in kernel, % | - | - | 89.2 ± 0.9 | 67.1 ± 0.3 | 21.1 ± 0.7 | 26.2 ± 3.0 | 18.2 ± 2.0 | 15.3 ± 0.6 | 18.1 ± 0.7 | 14.9 ±1.6 |
| **Changes in lipid composition (g/100 g lipid)** | | | | | | | | | | |
| Phospholipid | 21.7 ± 1.4 | 37.8 ± 1.1 | 34.4 ± 0.7 | 45.4 ± 1.4 | 3.0 ± 0.5 | 1.9 ± 0.8 | 1.6 ± 0.3 | 0.9 ± 0.1 | 0.9 ± 0.5 | 0.6 ±0.2 |
| Glycolipid | 30.2 ± 1.2 | 15.5 ± 1.1 | 10.9 ± 0.2 | 21.4 ± 1.7 | 1.0 ± 0.0 | 0.8 ± 1.7 | 0.6 ± 0.0 | 0.6 ± 0.1 | 0.6 ± 0.2 | 0.2 ±0.1 |
| Triacyl-glycerol | 11.1 ± 0.4 | 11.4 ± 0.9 | ND | 11.6 ± 1.5 | 54.5 ± 1.6 | 87.8 ± 1.7 | 87.4 ± 0.9 | 88.9 ± 0.4 | 89.9 ± 0.9 | 95.3 ±2.3 |
| Diacyl-glycerol | 19.0 ± 0.2 | 18.5 ± 3.5 | ND | 8.8 ± 2.2 | 17.7 ± 0.9 | 4.2 ± 0.5 | 5.5 ± 0.6 | 4.3 ± 0.2 | 4.1 ± 0.3 | 1.9 ±1.4 |
| Monoacyl-glycerol | 6.9 ± 0.5 | 4.8 ± 1.1 | ND | 4.0 ± 0.8 | 6.2 ± 0.3 | 1.4 ± 0.9 | 1.5 ± 0.1 | 1.8 ± 0.4 | 1.7 ± 0.5 | 0.5 ±0.3 |
| Fatty acid | 9.0 ± 0.5 | 9.5 ± 1.9 | ND | 5.8 ± 1.6 | 15.7 ± 1.2 | 1.8 ± 0.7 | 1.5 ± 0.7 | 1.8 ± 0.3 | 1.3 ± 0.1 | 0.6 ±0.6 |
| **Oil content (% dry weight)** | | | | | | | | | | |
| Mesocarp | 1.5 ± 0.1 (0.2) | 1.7 ± 0.0 (0.3) | 2.4 ± 0.1 (0.4) | 2.2 ± 0.2 (0.4) | 27.9 ± 0.8 (7.6) | 35.7 ± 0.7 (9.1) | 49.1 ± 0.5 (15.4) | 66.2 ± 0.6 (38.1) | 73.2 ± 0.5 (42.3) | 77.2 ±0.9 (45.2) |
| Kernel | - | - | 43.5 ± 1.2 (4.7) | 42.9 ± 2.0 (14.1) | 37.6 ± 1.1 (29.7) | 40.0 ± 1.4 (29.5) | 44.2 ± 1.5 (36.2) | 44.9 ± 0.7 (38.0) | 46.1 ± 0.8 (37.8) | 46.3 ±1.0 (39.4) |

- : absent, ND : Not determined, Figures in parenthesis : g/100 g fresh tissue

TABLE 2. FATTY ACID COMPOSITION OF DEVELOPING OIL PALM FRUIT MESOCARP LIPIDS

| Fatty acid, wt % | Age of fruit after anthesis, weeks | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 4 | 8 | 12 | 14 | 16 | 18 | 20 | 22 | 24 |
| | | | | **Triacylglycerols** | | | | | |
| 12:0 | 1.7 | 3.6 | 2.6 | 0.2 | 0.6 | 0.2 | 0.2 | 0.2 | 0.6 |
| 14:0 | 1.6 | 4.6 | 1.4 | 0.5 | 0.9 | 0.7 | 1.2 | 1.5 | 1.8 |
| 16:0 | 19.6 | 34.4 | 32.7 | 34.8 | 46.7 | 41.0 | 40.0 | 43.0 | 42.6 |
| 18:0 | 3.6 | 6.4 | 9.0 | 5.6 | 6.1 | 4.6 | 4.9 | 4.1 | 4.1 |
| 18:1 | 17.4 | 30.5 | 22.6 | 47.8 | 36.3 | 44.2 | 45.5 | 41.0 | 41.7 |
| 18:2 | 47.7 | 15.9 | 25.1 | 10.5 | 8.9 | 8.9 | 7.8 | 9.6 | 9.0 |
| 18:3 | 8.4 | 4.6 | 6.6 | 0.6 | 0.5 | 0.3 | 0.5 | 0.6 | 0.4 |
| | | | | **Diacylglycerols** | | | | | |
| 12:0 | 1.2 | 8.2 | 4.9 | 4.3 | 1.9 | 1.8 | 1.7 | 1.4 | 1.3 |
| 14:0 | 1.0 | 4.8 | 5.9 | 2.5 | 1.0 | 8.2 | 1.3 | 1.6 | 1.6 |
| 16:0 | 29.8 | 41.5 | 41.0 | 26.6 | 33.3 | 24.9 | 29.3 | 27.6 | 32.2 |
| 18:0 | 5.6 | 6.0 | 7.1 | 8.2 | 11.5 | 8.5 | 13.2 | 9.8 | 9.0 |
| 18:1 | 33.1 | 11.9 | 15.4 | 47.5 | 36.8 | 48.0 | 42.8 | 47.4 | 39.9 |
| 18:2 | 26.6 | 23.6 | 18.9 | 10.1 | 15.3 | 8.7 | 11.0 | 11.6 | 15.2 |
| 18:3 | 2.9 | 4.0 | 6.9 | 0.7 | 0.3 | 0.1 | 0.8 | 0.7 | 0.8 |
| | | | | **Monoacylglycerols** | | | | | |
| 12:0 | 7.4 | 0.9 | 2.2 | 3.2 | 4.9 | 3.7 | 3.7 | 3.0 | 3.6 |
| 14:0 | 4.6 | 11.4 | 3.6 | 3.8 | 3.8 | 3.4 | 2.8 | 2.8 | 5.7 |
| 16:0 | 40.4 | 41.0 | 41.2 | 34.1 | 33.0 | 33.1 | 28.3 | 30.3 | 36.9 |
| 18:0 | 11.6 | 4.9 | 14.0 | 5.1 | 11.2 | 9.5 | 3.5 | 6.7 | 8.5 |
| 18:1 | 17.2 | 28.4 | 25.0 | 48.7 | 39.9 | 43.7 | 55.7 | 51.6 | 35.8 |
| 18:2 | 16.1 | 9.0 | 10.7 | 2.3 | 6.3 | 6.6 | 5.6 | 5.0 | 8.8 |
| 18:3 | 2.7 | 4.4 | 3.4 | 2.8 | 0.9 | - | 0.4 | 0.5 | 0.9 |
| | | | | **Fatty acids** | | | | | |
| 12:0 | 3.7 | 3.7 | 5.8 | 2.0 | 4.6 | 14.1 | 2.5 | 2.9 | 4.8 |
| 14:0 | 3.9 | 5.2 | 1.4 | 2.3 | 4.5 | 7.5 | 2.7 | 2.4 | 3.8 |
| 16:0 | 32.1 | 33.8 | 33.6 | 33.3 | 37.3 | 33.5 | 39.0 | 34.9 | 39.9 |
| 18:0 | 11.9 | 8.6 | 7.4 | 8.1 | 9.8 | 9.1 | 14.7 | 17.7 | 9.6 |
| 18:1 | 18.2 | 30.9 | 26.3 | 26.4 | 31.7 | 31.6 | 33.6 | 34.6 | 34.3 |
| 18:2 | 23.4 | 15.8 | 19.9 | 21.1 | 9.3 | 4.2 | 7.1 | 5.7 | 7.0 |
| 18:3 | 6.9 | 2.0 | 5.6 | 6.9 | 2.7 | - | 0.5 | 1.9 | 0.7 |
| | | | | **Phospholipids** | | | | | |
| 12:0 | 0.1 | 0.1 | 0.1 | 0.5 | 0.6 | 0.5 | 0.7 | 0.4 | 0.3 |
| 14:0 | 0.2 | 0.3 | 0.3 | 0.7 | 2.2 | 1.0 | 2.3 | 1.1 | 1.4 |
| 16:0 | 35.4 | 33.9 | 34.5 | 30.2 | 38.9 | 28.8 | 24.4 | 33.6 | 32.6 |
| 18:0 | 2.8 | 3.6 | 4.1 | 3.0 | 2.3 | 0.8 | 0.4 | 3.7 | 2.8 |
| 18:1 | 15.3 | 10.5 | 15.9 | 37.2 | 34.8 | 42.9 | 53.3 | 41.6 | 38.2 |
| 18:2 | 41.7 | 44.2 | 33.8 | 23.3 | 20.2 | 20.6 | 17.8 | 19.4 | 23.2 |
| 18:3 | 4.6 | 7.5 | 11.4 | 5.0 | 1.1 | 5.4 | 1.3 | 0.3 | 1.5 |
| | | | | **Glycolipids** | | | | | |
| 12:0 | 0.6 | 0.4 | 0.3 | 0.1 | 0.4 | 0.6 | 0.6 | 0.2 | 2.4 |
| 14:0 | 0.8 | 1.2 | 1.1 | 1.0 | 1.6 | 1.4 | 2.0 | 1.2 | 4.1 |
| 16:0 | 26.3 | 28.0 | 24.1 | 22.4 | 33.3 | 30.2 | 32.5 | 35.2 | 27.6 |
| 18:0 | 3.4 | 4.4 | 5.0 | 6.1 | 3.1 | 5.0 | 5.5 | 5.0 | 8.4 |
| 18:1 | 8.0 | 6.7 | 9.1 | 23.5 | 17.1 | 24.3 | 24.0 | 23.4 | 37.7 |
| 18:2 | 26.0 | 12.1 | 8.5 | 17.1 | 13.7 | 11.0 | 15.4 | 14.4 | 10.7 |
| 18:3 | 35.0 | 47.3 | 52.0 | 29.8 | 30.8 | 27.6 | 20.0 | 20.8 | 9.1 |

- : not detected

against 170 days in the present study. The fat formation in the mesocarp occurred towards the end of fruit development. Fixation of harvesting time is, therefore, very important for maximum oil recovery.

*Lipid profile of developing oil palm fruit* : During the early stages of fruit development, the lipids were predominantly composed of polar lipids (phospho- and glycolipids), partial glycerides (di-and monoacylglycerols) and free fatty acids; together comprising about 90% of the total lipids, the rest being triacylglycerol. The lipid profile began to change drastically between the fourteenth and sixteenth week, the shift of lipid biosynthesis being towards the formation of triacylglycerol (accounting for 95% by 24 weeks after anthesis). At this stage, polar lipids and partial glycerides became insignificant. During the early stages, the total lipids were largely accounted for by the structural lipids, with very little of the storage-form. The relative reduction in the non-triacylglycerol lipids is primarily due to the excessive synthesis of triacylglycerols and hence the dilution effect (Appelqvist 1975). The other explanation is that triacylglycerols are formed, either through the Kennedy pathway (α-glycerol phosphate) or via the phospholipid pathway (Gurr 1980). Both these routes involve phospholipids, and other partial glycerides including fatty acids as intermediate products, with a high turnover rate during the rapid phase of triacylglycerol formation, i.e., from 16 weeks onwards after anthesis. This would further explain the drastic reduction in the phospholipids and partial glycerides towards fruit maturation. The reduction in the proportion of glycolipids could be due to the degradation of photosynthetic tissue in the oil palm fruit, as it is known that glycolipid (particularly digalatosyldiacylglycerol) is a major constituent of chloroplast tissue (Harwood 1980).

There are several reports on the lipid profile of developing oilseeds, indicating similar trends (Hitchcock and Nichols 1971; Appelqvist 1975; Gurr 1980). Oo et al (1986) and Bafor and Osagie (1986, 1988a, 1988b) have reported the lipid profile for Malaysian and African oil palm fruit, respectively, and observed more or less similar results.

Table 2 shows the fatty acid profile of the lipid classes of developing oil palm fruit mesocarp. The general pattern of the fatty acid was that in the early stages of fruit development, a predominance of unsaturated fatty acids with a corresponding lower content of saturated fatty acids was observed. The trend was slightly reversed towards the end of fruit maturation. The major fatty acids were 16:0, 18:1 and 18:2 in all the neutral lipid classes. However, 18:3 was present in significant quantities during the early stages, among all the lipid classes.

With respect to the major acids, triacylglycerols exhibited a definite trend with 16:0 and 18:1 showing a gradual increase, and 18:2 and 18:3 registering the reverse. The transition was particularly noticeable at 12 and 16 weeks after anthesis. The other neutral lipid fractions did not exhibit such a remarkable change. The diacylglycerol and monoacylglycerol contained slightly lower proportion of 16:0, with higher levels of 18:1 in the respective stage of fruit maturation. The fatty acid pool also showed lower contents of 16:1 and 18:1 with corresponding higher levels of 12:0, 14:0 and 18:0, as compared to the fatty acids of the triacylglycerols. It may, therefore, be mentioned that 16:0 and 18:1 are better utilized for triacylglycerol synthesis in the oil palm fruit. Since lipase is specific to the primary positions (Galliard 1980), it is expected that the fatty acid released will mostly be 16:0, thereby leaving most of the 18:1 in the 2-position of the monoacylglycerols. From the fatty acid composition of the monoacylglycerol and free fatty acid, it may be stated that they were formed as intermediate products in triacylglycerol biosynthesis and not as lipolytic products.

Fatty acid profile of the phospholipids indicated that 16:0 was much lower and 18:2 was higher than those of the triacylglycerols, while 18:1 remained more or less the same. During fruit development, 16:0 remained almost unchanged, 18:1 registered a steep increase, 18:2 showed a rapid decrease, and 18:3 was present in substantial amount in glycolipid mostly at the cost of 18:2 and to some extent 18:1, as compared to phospholipids. In the case of glycolipids also, the level of 16:0 remained almost the same during the course of fruit development. However, 18:1 exhibited a steady increase, while 18:2 and 18:3 showed a downward trend. A general pattern of fatty acid association with a particular lipid class is perceptible, i.e., 16:0 and 18:1 with triacylglycerol, 18:2 with phospholipid and 18:3 with glycolipid. The biochemical mechanism to explain this phenomenon is yet to be investigated (Hitchcock and Nichols 1971).

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# Physical, Chemical and Cooking Characteristics of a 'Landrace' and Three Newly Developed Lentil Cultivars Grown in Jordan

K.I. EREIFEJ*[1] AND R.A. SHIBLI[2]

[1]Department of Nutrition and Animal Production, [2]Department of Plant Production, Jordan University of Science and Technology, P.O. Box 3030, Irbid, Jordan.

A landrace (Balady*) and three newly developed ('Jor-1', 'Jor-2' and 'Jor-3') lentil cultivars were studied for their physical as well as chemical properties, mineral content and cookability. Data showed significant differences ($P<0.05$) in their physical, chemical and mineral composition. The whole seeds of all cultivars required 35 min to cook at 100°C without previous soaking. Decortication and embryo removal did not greatly reduce the nutrient contents of these cultivars. Seed coats and embryos were found to be rich in minerals and nutrients.

**Keywords :** Lentil cultivars, 'Landrace', New cultivars, Jordan lentils, Proximate composition, Hectolitre weight, Thousand kernel weight, Cotyledons, Embryos, Seed coat, Mineral composition, Cooking characteristics.

Lentil (*Lens culinaris* Medik.) is a major food legume crop in Jordan, its annual production being 1571 tonnes in 1989 (Anon 1989). Lentil is relatively free of anti-nutritional factors, low in flatulence, and is an excellent source of proteins and amino acids (Bhatty 1988). Thus, it is a good complement to cereal proteins, particularly bread, which is most often consumed in Jordan. The chemical composition and quality of lentils have been investigated by many workers (Bhatty 1984; Bhatty et al. 1987; Abu-Shakra and Tannous 1981). Lentil yield improvement through breeding programs and/or introducing high yielding cultivars, has been the major effort of the Ministry of Agriculture in Jordan and three newly developed cultivars have emerged from these efforts (Haddad 1986).

In the present studies, a 'Landrace' ('Balady') and three newly developed lentil cultivars ('Jor-1', 'Jor-2' and 'Jor-3') were compared for their physico-chemical properties, mineral composition and cookability.

## Materials and Methods

*Lentil samples :* Representative samples of three lentil cultivars released in 1988 by Jordan University, and 'Balady' 'landrace' were planted in Maru Experimental Station (North Jordan, rainfall 380 mm). Cultivars and 'landrace' were planted in 4 $m^2$ plots in a randomized complete block design with four replications, plants were harvested at the dry stage and manually threshed. Sub-samples of 100 g for each replicate were milled on a Tecator 1093 cyclotec sample mill (Tecator, Sweden), the milled product was passed through a 0.40 mm screen and used for proximate and mineral analysis. Other sub-samples (500 g) were milled on Cemotic 1090 sample mill (Tecator, Sweden) at the highest setting (#7), and then the seed coats, embryos and dicotyledons were separated using an air blower. The mean weight of each fraction was recorded and the percentage was computed.

*Physical measurements :* Seed size (diam, mm) as mean of 100 measurements, colour (white seeds %), weight of one litre seeds (g/l), hydration capacity (% water uptake when 50 g sample soaked in 150 ml distilled water for 18 h at room temperature) were determined according to Hulse et al (1977). Weights of thousand grains were determined in quadruplicate and the average weight was recorded. The hard seed count (weight of the unswollen seeds/total weight of soaked seeds) was calculated as percentage.

*Chemical analysis :* Whole seeds, dicotyledons, seed coats and embryos were analyzed for moisture, proteins (N X 6.25), fat, crude fibre and ash according to AOAC (1984). Carbohydrate contents were calculated by difference. Gross energy value was calculated by multiplying proteins, fat and carbohydrate contents with factors of 4, 9 and 4, respectively (Khan et al. 1986).

*Mineral analysis :* Mineral contents (Ca, Na, K, Cu, Fe, Mn, Zn, and Mg) were determined by atomic absorption spectrophotometer (Pye Unicam, UK, Model SP9) using wet digestion with a mixture of nitric, sulphuric and perchloric acids (10:1:4). Phosphorus was determined according to Watanabe and Olsen (1965).

*Cookability :* Cooking time was determined according to Nikkuni et al (1988). Whole lentil seeds

* Corresponding Author

TABLE 1. PHYSICAL PROPERTIES OF LENTIL SEEDS OF FOUR CULTIVARS FROM JORDAN.

| | Cultivar | | | |
|---|---|---|---|---|
| | 'Balady' | 'Jor-1' | 'Jor-2' | 'Jor-3' |
| Size, mm | 4.1 b* | 4.7 a | 4.8[a] | 4.6[a] |
| White seed, % | 45.0 | 30.0 | 10.0 | 38.0 |
| One litre, g/l | 853.5[ab] | 831.7[c] | 855.8[a] | 843.2[b] |
| Hydration capacity, % | 97.9[ab] | 96.4[ab] | 98.3[a] | 95.7[b] |
| Hard seed, % | 10.1[a] | 5.6[b] | 5.6[b] | 6.0[b] |
| Seed coat, % | 9.2[a] | 8.1[a] | 8.1[a] | 8.1[a] |
| Dicotyledons, % | 85.3[b] | 82.9[c] | 85.8[b] | 87.0[a] |
| Embryo, % | 5.5[bc] | 9.1[a] | 6.2[b] | 4.9[c] |
| Thousand seed weight, g | 8.5[c] | 47.8[b] | 49.2[ab] | 53.9[a] |

* Means within rows having different letters are significantly different according to least significant differences (LSD) at 0.05.

(200 g) were cooked in 500 ml distilled water at 100°C. At regular intervals, 25 seeds were taken out and each seed was crushed with a finger by placing on a scale (4 kg, full scale). The weight necessary to smash it was recorded and the overall mean calculated.

*Statistical analysis* : The data were statistically analyzed and the least significant difference (LSD) among treatments was calculated according to Steel and Torrie (1980).

## Results and Discussion

*Physical properties* : 'Balady' lentil seeds were smaller in size (4.1 mm) than 'Jor-1', 'Jor-2' and 'Jor-3'. 'Balady' variety had equal portions of white and red seeds, while 'Jor-1' had 30% white seeds, and 'Jor-2' had only 10% white seeds. Red or yellow lentil seeds are liked better than the white seeds in Jordan. The hectolitre weights were also significantly different among the cultivars. 'Jor-2' absorbed the highest % of water and 'Jor-3' absorbed the least. 'Balady' had the highest (10.1%) hard to cook seeds, while other cultivars contained 5.0 - 6.0% hard to cook seeds (Table 1). The proportion of seed coat (9.2%) was slightly higher in the 'Balady' cultivar than in other varieties, while proportion of dicotyledons was comparable to other varieties. One thousand seed weights (TSW) (Table 1) were also significantly different among the four cultivars investigated.

*Chemical composition* : Data on proximate analysis and energy value for whole lentil seeds, cotyledons, embryos and seed coat are shown in Table 2. Protein content (whole seed) varied from 28.3 ('Jor-1') to 32.2% ('Jor-3'). These protein values are slightly higher than the ones reported by Khan et al (1986) on some lentil lines, but are comparable to the values reported by other workers (Bhatty et

TABLE 2. CHEMICAL COMPOSITION AND ENERGY VALUES OF LENTIL CULTIVARS AND THEIR FRACTIONS*.

| Cultivar | Protein, % | Fat, % | Carbo-hydrates, % | Fibre, % | Ash, % | Energy, Kcal/100 g |
|---|---|---|---|---|---|---|
| | | | **Whole seeds** | | | |
| 'Balady' | 29.7[ab]** | 1.5[a] | 60.3[a] | 5.0[a] | 3.5[a] | 373.5[b] |
| 'Jor-1' | 28.3[b] | 1.5[a] | 61.6[a] | 5.2[a] | 3.4[a] | 373.1[b] |
| 'Jor-2' | 31.4[a] | 1.5[a] | 60.1[a] | 3.7[ab] | 3.4[a] | 379.4[ab] |
| 'Jor-3' | 32.2[a] | 0.8[b] | 61.8[a] | 2.0[b] | 3.2[a] | 383.5[a] |
| | | | **Cotyledons** | | | |
| "Balady' | 32.3[a] | 1.2[a] | 60.9[a] | 1.9[a] | 3.6[a] | 384.0[a] |
| 'Jor-1' | 30.2[a] | 1.7[a] | 64.7[a] | 0.1[a] | 3.2[b] | 395.0[a] |
| 'Jor-2' | 31.1[a] | 1.1[a] | 64.3[a] | 0.2[a] | 3.3[b] | 391.2[a] |
| 'Jor-3' | 29.5[a] | 1.4[a] | 65.0[a] | 0.9[a] | 3.3[b] | 390.5[a] |
| | | | **Embryos** | | | |
| 'Balady' | 37.0[a] | 1.2[a] | 53.8[a] | 3.8[a] | 4.2[a] | 374.2[a] |
| 'Jor-1' | 36.3[ab] | 1.8[a] | 54.6[a] | 3.8[a] | 3.5[a] | 379.8[a] |
| 'Jor-2' | 34.5[c] | 1.9[a] | 56.6[a] | 3.0[a] | 3.9[a] | 382.1[a] |
| 'Jor-3' | 34.7[bc] | 1.9[a] | 54.3[a] | 5.1[a] | 4.0[a] | 373.1[a] |
| | | | **Seed coats** | | | |
| 'Balady' | 19.8[a] | 1.4[a] | 34.8b | 41.7[a] | 2.6[a] | 229.6[b] |
| 'Jor-1' | 12.3[b] | 0.9[a] | 44.9[ab] | 39.8[ab] | 2.1[a] | 236.9[ab] |
| 'Jor-2' | 12.8[b] | 1.0[a] | 53.7[a] | 29.7[bc] | 2.8[a] | 273.1[ab] |
| 'Jor-3' | 14.4[b] | 0.9[a] | 54.6[a] | 27.6[c] | 2.4[a] | 284.2[a] |

* Each value is an average of four determinations and values were calculated on dry basis.

** Means within columns having different letters are significantly different according to least significant difference (LSD) at 0.05.

al. 1987; Bhatty 1988). The fat contents of different fractions from various cultivars ranged from 0.8 to 1.9% and are comparable to the values reported by previous workers. The carbohydrate contents in various cultivars were practically the same (60.1 to 61.8%), while crude fibre contents varied from 2.0 to 5.2%. There were no significant differences in ash contents of the different cultivars, being in the range from 3.2 ('Jor-3') to 3.5% ('Balady'). The data are in consonance with values reported previously (Abu-Shakra and Tannous 1981; Bhatty et al. 1987; Bhatty 1988). Khan et al (1986) reported lower values (2.7%) for lentil lines grown in Pakistan.

All lentil cultivars had almost similar gross energy values, which were 373.5 ('Balady'), 373.1 ('Jor-1'), 379.4 ('Jor-2'); and 383.5 Kcal/100 g ('Jor-3'). These values are in agreement with values reported by Khan et al (1986), but are slightly lower than those reported by Batty et al (1987). The latter reported a value of 420 Kcal/100 g of lentil. The protein and fat contents of different cultivars did not change appreciably by removal of seed coat and embryo, but % fibre decreased considerably. As expected, seed coat contained considerably less amounts of proteins and carbohydrates than cotyledons and whole seeds. Protein content of embryos was highest among all fractions and whole seeds.

*Mineral contents :* The data on Ca, Na, K, P, Cu, Fe, Mn, Zn and Mg in whole seeds, cotyledons, embryos and seed coat are given in Table 1. All minerals varied significantly between cultivars except for K. The Ca content is in the range as reported by Bhatty (1984). K contents were lower than the lowest value (880 mg/100 g) reported by Bhatty (1988), probably due to genotype and environmental conditions. The values for P, Cu, Fe, Mn, Zn and

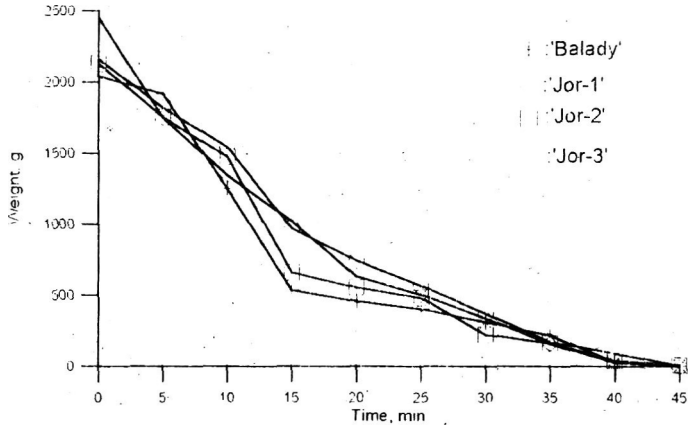


Fig. 1 Weight (g) required to crush cooked lentil seed at 100°C at different time intervals.

TABLE 3. MINERAL CONTENTS OF FOUR LENTIL CULTIVARS FROM JORDAN.

(Values, mg/100g*)

| Cultivar | Ca | Na | K | P | Cu | Fe | Mn | Zn | Mg |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | **Whole seeds** | | | | |
| 'Balady' | 42.3$^{b**}$ | 78.6$^{a}$ | 38.1$^{a}$ | 458.5$^{a}$ | 1.1$^{a}$ | 13.3$^{a}$ | 1.3$^{a}$ | 6.2$^{a}$ | 12.9$^{a}$ |
| 'Jor-1' | 32.0$^{c}$ | 58.7$^{c}$ | 34.4$^{a}$ | 421.6$^{b}$ | 1.0$^{ab}$ | 9.2$^{b}$ | 1.2$^{a}$ | 5.0$^{c}$ | 9.8$^{bc}$ |
| 'Jor-2' | 34.4$^{c}$ | 57.8$^{c}$ | 33.8$^{a}$ | 445.1$^{a}$ | 1.0$^{ab}$ | 9.5$^{b}$ | 1.0$^{b}$ | 5.6$^{b}$ | 9.3$^{c}$ |
| 'Jor-3' | 60.6$^{a}$ | 68.3$^{b}$ | 35.2$^{a}$ | 411.6$^{b}$ | 0.9$^{b}$ | 9.2$^{b}$ | 1.3$^{a}$ | 4.9$^{c}$ | 10.8$^{b}$ |
| | | | | | **Cotyledons** | | | | |
| Balady' | 18.4$^{b}$ | 60.4$^{a}$ | 35.6$^{a}$ | 463.4$^{b}$ | 1.1$^{b}$ | 10.2$^{a}$ | 1.1$^{c}$ | 5.9$^{a}$ | 10.3$^{a}$ |
| 'Jor-1' | 18.2$^{b}$ | 68.1$^{a}$ | 33.5$^{a}$ | 448.7$^{bc}$ | 1.0$^{c}$ | 8.8$^{ab}$ | 1.1$^{c}$ | 4.8$^{d}$ | 10.4$^{a}$ |
| 'Jor-2' | 17.2$^{c}$ | 56.8$^{a}$ | 32.0$^{a}$ | 490.4$^{a}$ | 1.2$^{a}$ | 10.2$^{a}$ | 1.2$^{b}$ | 5.7$^{b}$ | 10.5$^{a}$ |
| 'Jor-3' | 24.7$^{a}$ | 56.9$^{a}$ | 31.6$^{a}$ | 440.5$^{c}$ | 1.2$^{a}$ | 8.4$^{b}$ | 1.3$^{a}$ | 5.0$^{c}$ | 10.0$^{a}$ |
| | | | | | **Embryos** | | | | |
| Balady' | 81.4$^{b}$ | 122.2$^{a}$ | 128.3$^{a}$ | 510.3$^{a}$ | 1.3$^{a}$ | 14.4$^{a}$ | 2.5$^{b}$ | 9.3$^{a}$ | 36.1$^{a}$ |
| 'Jor-1' | 60.9$^{d}$ | 112.5$^{a}$ | 96.1$^{b}$ | 517.5$^{a}$ | 1.1$^{c}$ | 13.0$^{a}$ | 2.3$^{c}$ | 7.0$^{c}$ | 27.4$^{a}$ |
| 'Jor-2' | 77.1$^{c}$ | 93.3$^{b}$ | 79.2$^{a}$ | 447.5$^{a}$ | 1.3$^{a}$ | 16.3$^{a}$ | 2.5$^{b}$ | 9.5$^{a}$ | 50.3$^{a}$ |
| 'Jor-3' | 113.1$^{a}$ | 80.5$^{c}$ | 100.1$^{b}$ | 472.3$^{a}$ | 1.2$^{b}$ | 14.2$^{a}$ | 2.8$^{a}$ | 8.6$^{b}$ | 28.3$^{a}$ |
| | | | | | **Seed coats** | | | | |
| Balady' | 220.9$^{b}$ | 100.8$^{b}$ | 75.4$^{c}$ | 207.1$^{a}$ | 0.9$^{a}$ | 12.1$^{b}$ | 0.7$^{a}$ | 3.5$^{a}$ | 22.7$^{c}$ |
| 'Jor-1' | 281.7$^{a}$ | 121.8$^{a}$ | 121.4$^{a}$ | 166.2$^{c}$ | 0.6$^{a}$ | 12.2$^{b}$ | 0.5$^{b}$ | 2.9$^{c}$ | 34.1$^{a}$ |
| 'Jor-2' | 276.0$^{a}$ | 109.1$^{b}$ | 98.0$^{b}$ | 202.9$^{a}$ | 0.8$^{a}$ | 23.5$^{a}$ | 0.7$^{a}$ | 3.3$^{b}$ | 27.3$^{b}$ |
| 'Jor-3' | 285.7$^{a}$ | 20.2$^{c}$ | 14.7$^{d}$ | 184.2$^{b}$ | 0.6$^{a}$ | 22.4$^{a}$ | 0.5$^{b}$ | 3.4$^{ab}$ | 34.3$^{a}$ |

* Each value is an average of four determinations and values were calculated on dry basis. ** Means within columns having different letters are significantly different according to least significant difference (LSD) at 0.05.

Mg compare very well with those reported by Khan et al (1986) on lentil lines grown in Pakistan. There were no significant differences between P, Fe, and Mg contents of embryos. However, Ca, Na, K, Cu, Mn and Zn varied significantly. Mineral contents in seed coat also varied significantly except for Cu.

*Cookability* : Data on cookability of the four lentil cultivars are shown in Fig. 1. The weight (g) which was required to crush the cooked lentil seed at various lengths of time decreased from almost 2.5 kg at zero time to 250 g after 35 min. Cooking for further 10 min resulted in a thick slurry soup, and that type of soup is liked in the country. All cultivars needed 35 min at 100°C to cook without previous soaking.

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# Effect of Processing on the Functional Properties of Cowpea *(Vigna catjung)* in a Food System - *Wadian*

**SUSHMA THAKUR AND SHASHIKALA PUTTARAJ***

Department of Studies in Food Science and Nutrition,
University of Mysore, Manasagangothri, Mysore-570 006, India.

Effects of processing treatments viz., soaking fermentation and puffing on the functional properties of cowpea in *wadian*, a traditional dehydrated product (selected as food system in the present study) were determined and compared with those observed in a model system. Increases in the bulk density, viscosity and water absorption capacity, observed in the cowpea flour as a result of processing, showed further increase in cowpea *wadians*. The increase in viscosity was of the magnitude of 5 to 8-fold in all the *wadian* samples. Fat absorption capacity of raw *wadian* sample also increased, as compared to that of the raw cowpea flour, whereas it decreased in the processed *wadians*. All the *wadian* samples made from differently processed flours were of good quality and compared well with those of a commercial greengram *wadian* samples with respect to the physical and sensory characteristics.

**Keywords** : Cowpea, *Wadian*, Processing effect, Functional properties, Acceptability, Food *vs* model system.

Cowpea *(Vigna catjung)* is an important food legume of many tropical countries (IITA 1973). Its use in food has increased significantly in recent years (Gopalan 1992). Its potentiality in baby foods, such as *moin-moin* and *akara* has also been explored (McWatters 1980, 1983; McWatters and Chinnan 1985; McWatters et al. 1986). It is also felt that cowpea may replace other pulses in traditional Indian preparations. *Wadian*, traditionally prepared from either blackgram *(Phaseolus mungo)* or green gram *(Phaseolus aureus)*, is a popular dehydrated product in Northern India. *Wadian* is produced on a cottage scale and used in vegetable curries (Pruthi et al. 1981).

Nutritionally, cowpea is similar to other pulses in its protein content (Gopalan et al. 1989). However, its potentiality in food industry has not been exploited fully, probably because data available on its functional utility in suitable processed foods are limited (Okaka and Potter 1979; Padmashree et al. 1987). Previous studies, using a model system had shown an increase in bulk density, water and fat absorption capacities of cowpea flour due to various processing treatments, such as soaking, fermentation and puffing (Padmashree et al. 1987). Besides, effects of soaking, and cooking on the nutritional quality of cooked seeds have been reported (Bakr and Gawish 1991; 1992). Some reports have also appeared on the functional properties of normal and insect infested flours (Emefu et al. 1992). However, the observed proportions of cowpea flour have to be related to its performance in a food system, as these properties are desirable in bakery and dehydrated products (Kinsella 1976).

Hence, in the present study, an attempt has been made to determine the functional properties of processed cowpea flour in a selected food system-*wadian*, and to compare these properties with those observed in a model system. Acceptability of the product was also evaluated to assess the suitability of using cowpea flour in *wadian* preparation.

## Materials and Methods

*Cowpea samples* : Cowpea *(Vigna catjung)*, 'C-120' variety, was obtained from Karnataka State Seed Corporation, Bangalore, India. Seeds were cleaned manually to remove extraneous matter and stored in containers at room temperature (~28°c) until used.

*Processing treatments* : Cowpea seeds in 2 kg lots were subjected to three selected processing treatments. In case of soaking, cowpea was soaked for 3h in water (600 ml) at room temperature (~28°C), hand-dehulled and blended to form paste. For fermentation, the cowpea seeds were soaked in 1500 ml water (8h), hand-dehulled, blended with 400 ml water to form a paste, allowed to ferment naturally (16 h) at room temperature (~28°C). In case of puffing, cowpea was soaked in 1500 ml water at room temperature (~28°C) for 3 h, drained, puffed over sand at 250°C for 2 min in an iron girdle, hand-dehulled and air-classified to remove sand particles and husk. The puffed seeds were ground to flour in a grinder. The cowpea pastes, as processed above, were grouped into two batches. One batch was used for the preparation of *wadian*, while the other batch, except for the puffed seeds, was oven-dried at 50°C for 10-12 h to < 8% moisture content. Before analysis, all samples were

* Corresponding Author

passed through 60 mesh sieve (British standard).

Wadian *preparation* : Preliminary experiments were conducted to determine optimum conditions, based on the commercial method used for the preparation of greengram *wadian* (Pruthi et al. 1981). *Wadians* were prepared, using both raw and processed cowpea pastes. Raw cowpea flour (dehulled and milled in a grinder) and puffed cowpea flour were also made into paste with water (1100 ml and 1600 ml, respectively). Spices - salt (8%), chilli powder (2%) and asafoetida extract (0.2%) were added to the raw and processed cowpea pastes. The paste was made into balls weighing 40-45 g each. All the *wadian* samples (balls) were dried in an oven at 50°C for 8 h to < 9% moisture content.

*Analytical methods* : Moisture, proteins (Nx6.25), crude fat and total ash were determined by AOAC (1976) in three replicates. Carbohydrates were calculated by difference. Acidity and extent of non-enzymatic browning in *wadian* samples were estimated as per the procedure described by Ranganna (1986). Rehydration capacity of *wadian* was determined by frying a 5-g sample in oil at 200°C for 2 min, followed by boiling in 30 ml water for 5 min and draining. The samples were also hydrated in 10 times the weight of water at room temperature and were drained after 1, 2 and 3 h for recording the weights. Water absorption (%) was calculated at each stage (Ranganna 1986). Hardness of the *wadian* was measured using an Instron (Model 1140, Instron Limited, England).

*Functional properties* : Water and fat absorption capacities were determined by the methods of Sosulki (1962) and Sosulki et al (1976), respectively. Flour/*wadian* samples (5 g) were stirred manually in water (30 ml) or refined groundnut oil (25 ml) for 5 min in a graduated tube, allowed to stand for 30 min at room temperature (~28°C) and centrifuged at 4500 rpm for 25 min at room temperature. The volume of free liquid was measured and the retained volume was expressed as ml of water or oil absorbed/g of sample on dry weight basis. Bulk density was measured in triplicate by the method of Kinsella (1979). Sample (5 g) was weighed into a 15 ml graduated tube and packed tightly. The final volume was noted and expressed as g/ml. Viscosity was measured using a viscometer (Model HBT, Brookfield, Stoughton, Mass., USA). Spindle numbers 1 and 2 were used for all the samples with 1:10 flour to water ratio or more in case of *wadian* samples at 100 rpm speed.

*Sensory evaluation* : *Wadian* samples for sensory evaluation were deep-fried in refined groundnut oil at 200°C for 2 min, excess oil was removed using absorbent paper and then dipped in tomato curry for 1h. Tomato curry was prepared by seasoning tomatoes in two spoons of refined groundnut oil and cooked along with salt (6%) and pepper (2.4%), without water, till done. Coded samples consisting of whole *wadian* balls from raw and each of the three treatments and also a sample of commercial greengram *wadian* (control) were arranged in random and served warm to a semi-trained panel of 30 members (SEC-IFT 1964) for ranking of individual quality attributes, such as colour, appearance, texture, aroma, taste and after-taste (Table 1). The overall quality was scored on a 9 point scale (9= very good, 1=poor). In a few preliminary evaluation sessions, the panelists were trained to use the evaluation procedure and a quality description accommodating desirable and undesirable aspects in all the individual quality attributes was developed. The quality description was given to the panelists for reference during regular evaluation sessions for uniform understanding of the quality of individual attributes. Panelists were given a free hand to comment on the quality of *wadians*, particularly the defects, if any.

TABLE 1. DESIRABLE AND UNDESIRABLE QUALITIES OF *WADIAN* AS EMPLOYED IN SENSORY EVALUATION

| Quality attributes | Desirable qualities | Undesirable qualities |
|---|---|---|
| Colour | Brown, light brown cream, reddish brown | Dark brown, dull, not uniform colour |
| Appearance | Uniform in shape, flat, round | Non-uniform in shape, fragments |
| Texture or mouth feel | Soft, tender, chewy, firm | Fibrous, rubbery, hard |
| Aroma | Typical, fried pulse aroma, balanced, spicy | Beany, off-aroma |
| Taste | Balanced, spicy, fried pulse taste, after-taste pleasant | Salty, beany, raw, bitter after-taste, off-taste |

*Statistical analysis* : The data on proximate composition of raw/processed cowpea flours, compositional characteristics of dehydrated *wadians*, and the overall quality scores were subjected to analysis of variance and by Duncan's new multiple range test (Harter 1960). The ranked data for individual quality attributes were analyzed by Wilcoxon-Mann-Whitney's U-test (Sokal and Rohlf 1969).

TABLE 2. PROXIMATE COMPOSITION OF RAW AND PROCESSED COWPEA FLOURS AS WELL AS DEHYDRATED *WADIAN*

| Proximate composition* % | Cowpea flour | | | | | Dehydrated *wadian* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Raw | Soaked | Fermented | Puffed | SEm (12 df) | Raw | Soaked | Fermented | Puffed | Green-gram | SEm (15 df) |
| Moisture | 7.9$^{c}$ | 5.4$^{a}$ | 6.2$^{b}$ | 8.5$^{a}$ | ±0.05 | 8.20$^{a}$ | 4.5$^{a}$ | 9.2$^{c}$ | 6.6$^{c}$ | 5.6$^{b}$ | ±0.04 |
| Fat | 0.6$^{b}$ | 0.5$^{a}$ | 1.5$^{c}$ | 1.8$^{d}$ | ±0.01 | 1.8$^{c}$ | 2.0$^{d}$ | 2.0$^{d}$ | 1.3$^{b}$ | 1.0$^{a}$ | ±0.01 |
| Proteins | 24.1$^{b}$ | 23.5$^{a}$ | 25.1$^{c}$ | 25.6$^{a}$ | ±0.02 | 23.2$^{b}$ | 23.1$^{b}$ | 23.9$^{c}$ | 25.3$^{d}$ | 22.5$^{a}$ | ±0.01 |
| Total ash | 1.4$^{b}$ | 0.2$^{a}$ | 1.4$^{c}$ | 1.1$^{b}$ | ±0.01 | 4.2$^{c}$ | 4.4$^{d}$ | 3.2$^{b}$ | 5.6$^{e}$ | 3.1$^{a}$ | ±0.02 |
| Carbohydrates (by difference) | 66.0 | 70.4$^{c}$ | 65.8$^{b}$ | 63.0$^{a}$ | ±0.06 | 62.6$^{b}$ | 66.9$^{c}$ | 61.7$^{b}$ | 61.2$^{a}$ | 67.8$^{c}$ | ±0.03 |

*On dry basis. SEM (df) - Standard error of mean (degrees of freedom). Mean value bearing different superscripts a, b, c, d in rows differ significantly ($P \leq 0.05$).

## Results and Discussion

The proximate composition of the raw and processed cowpea flours as well as *wadian* is presented in Table 2. Processing did not have any profound effect on the proximate composition of cowpea flour. Compositional characteristics of *wadian* prepared from each treatment were within the range of raw and processed cowpea flours. The water uptake during the blending of cowpea-water mixture was highest in *wadian* made from puffed seeds and least in the *wadians* from fermented paste (Table 3). However, the quantity of *wadian* after drying was lowest for the product from puffed seeds and highest for that from fermented flours. Higher water imbibition capacity may be due to the enhanced swelling caused by heat treatment (Sosulki 1962). Titratable acidity differed depending on the treatment. Soaking of flour considerably increased acidity in the *wadian*, whereas it decreased in the product from puffed seeds. The extent of non-enzymatic browning was higher in the product from raw than that from the processed seeds. Apparently, sugar and amino acid interaction was greater in the product from raw flour, due to unaltered native protein.

TABLE 3. PHYSICAL CHARACTERS OF DEHYDRATED *WADIAN**

| Characteristics | Raw | Soaked | Fermented | Puffed |
|---|---|---|---|---|
| Water added during blending, ml | 1100 | 535 | 410 | 1600 |
| Weight of wet paste, g | 1380 | 2330 | 1920 | 1850 |
| Moisture of wet paste, % | 62 | 76 | 64 | 74 |
| Weight of *wadian*, g | 520 | 550 | 685 | 480 |
| Hardness of *wadian*, kg | 19.0 | 7.0 | 8.5 | 20 |
| Titratable acidity, % | 7.7 | 12.2 | 8.3 | 1.1 |
| Dehydration ratio | 2.66 | 4.24 | 2.80 | 3.85 |
| Non-enzymatic browning, OD | 0.14 | 0.05 | 0.03 | 0.09 |

* Values are mean of three replicates.

*Functional properties :* Processing treatments increased the bulk density, viscosity, water and fat absorption capacities of cowpea flour in the model system, as compared to flour from untreated raw cowpea (Table 4). Further increases in bulk density and viscosity were observed in all the *wadian* samples, whereas water absorption capacity showed differences depending on the treatment. The increase in viscosity was 5 to 8-fold in all the *wadian* samples. Water absorption capacity is considered a critical function in a dehydrated product (Kinsella 1976). While *wadian* from raw, soaked and fermented seeds showed further increase in water absorption capacity, it was found to decrease in *wadian* from puffed seeds, as compared to the puffed cowpea flour. However, the water absorption values of *wadian* from puffed seeds were still higher than those in *wadian* from fermented or raw seeds. Hydration properties, dispensability, water absorption, binding, swelling and viscosity are known to directly influence the characteristics of a food system (McWatters 1983). Water absorption capacity values are also shown to vary with the number and type of polar groups (Kuntz 1971) and polar amino acid content of cowpea has been reported to be 65.25 g/100 g proteins (Tamsama et al. 1969). The enhanced water absorption capacity in *wadian* from raw and fermented seeds may be due to denaturation of proteins during processing, which enhances the binding sites for water (Kinsella 1976). It has been suggested that the changes in starch also have a major effect on functionality (Hermansson et al. 1974). The decreased water absorption capacity in *wadian* from puffed seeds, comparing to that of the model (puffed cowpea flour) may, therefore, be attributed to changes in the starch molecule, and possibly to protein-carbohydrate interaction during the preparation of *wadian* (Hamid et al. 1984).

Fat absorption capacity is also important, as

TABLE 4. FUNCTIONAL PROPERTIES OF COWPEA FLOUR AND *WADIAN*•

| Treatment | Bulk density, g/ml | | Water absorption g/g flour | | Water absorption g/g protein | | Fat absorption g/g flour | | Fat absorption g/g protein | | Viscosity, cp | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | a | b | a | b | a | b | a | b | a | b |
| Raw | 0.79[a] | 1.11[a] | 0.62[a] | 0.78[a] | 2.58[a] | 3.39[a] | 0.20[a] | 0.98[c] | 0.83[a] | 4.26[c] | 12.8[a] | 64[a] |
| Soaked | 1.22[d] | 1.33[c] | 2.07[c] | 2.56[c] | 8.88[c] | 11.08[d] | 0.76[b] | 0.76[b] | 3.30[b] | 3.30[b] | 17.8[c] | 96[c] |
| Fermented | 0.84[b] | 1.35[c] | 1.06[b] | 1.27[b] | 4.24[b] | 5.30[b] | 0.95[c] | 0.63[a] | 3.80[c] | 2.62[a] | 16.4[b] | 80[b] |
| Puffed | 0.86[c] | 1.21[b] | 3.36[d] | 2.69[d] | 12.92[d] | 10.35[c] | 1.13[d] | 1.06[d] | 4.35[d] | 4.61[d] | 28.0[d] | 96[c] |
| Greengram | - | 4.88[d] | - | 3.12[c] | - | 13.55[c] | - | 1.04[d] | - | 4.70[c] | - | 80[b] |
| 5Em (12 df) | 0.0037 | 0.0060 | 0.0044 | 0.0062 | 0.0046 | 0.0038 | 0.0074 | 0.0076 | 0.0003 | 0.0048 | 0.0280 | 0.0001 |

a: cowpea flour; b : *wadian*; SEM(df) - Standard error of means (degrees of freedom) • Mean values bearing different superscripts a, b, c, d, e in column differ significantly (P≤0.05).

it improves the mouth feel and retains the flavour (Kinsella 1976). Fat absorption capacity of *wadian* from raw seeds improved further, over that from the raw cowpea flour. However, in the processed *wadian* samples, a decrease in the fat absorption capacity was observed, as compared to the corresponding processed flours in the model system. Fat absorption is primarily attributed to the proteins and is reported to be affected by temperature, size of particles and degree of denaturation (IITA 1973).

*Sensory properties* : The comparison of sensory quality for *wadian* samples and commercial greengram *wadian* (control) indicated no significant difference in taste and after-taste (Table 5). Colour, appearance, texture, aroma and quality of *wadian* from puffed seeds were significantly lower than those of *wadian* from other treatments, but these attributes were comparable in case of the other treatments. However, all the *wadian* samples were of good quality, with no perceivable beany flavour. The colour, according to the panelists, was attractive and comparable to that of greengram *wadian*.

The hardness values followed a similar trend, as those observed for overall quality in the sensory evaluation and indicated that the hardness values of the *wadian* are inversely related to overall quality scores. Rehydration capacity was also observed to be influenced by the hardness of the product (Fig. 1). It was obvious that harder structure resulted in lowered water inhibition by the product. Rehydration/hardness ratio as related to overall quality scores is shown in Fig. 2. It is evident that though *wadians* from fermented and soaked seeds have better texture, overall quality and hardness, the values were not significantly different from the products made from raw seeds and also compared well with those of the commercial greengram *wadian*.

TABLE 5. *WADIAN* QUALITY-RANK SUMS (30 PANELISTS)•

| Quality attributes | Treatments | | | | Green-gram *wadian* (control) |
|---|---|---|---|---|---|
| | Raw | Soaked | Fermented | Puffed | |
| Colour | 85[a] | 100[a] | 69[a] | 137[b] | 59[a] |
| Appearance | 79[a] | 92[a] | 77[a] | 135[b] | 67[a] |
| Texture | 86[a] | 70[a] | 78[a] | 116.5[b] | 99.5[a] |
| Aroma | 90[a] | 83[a] | 81[a] | 118[b] | 78[a] |
| Taste | 78.5[a] | 97.5[a] | 85[a] | 107[a] | 82[a] |
| After-taste | 84[a] | 90[a] | 79[a] | 110[b] | 87[a] |
| Overall quality mean (Max.10) | 6.6[a] | 6.2[a] | 7.2[a] | 4.7[b] | 6.6[a] |

• Rank sums/overall quality means in rows carrying different superscripts a, b, c differ significantly (P≤0.05).

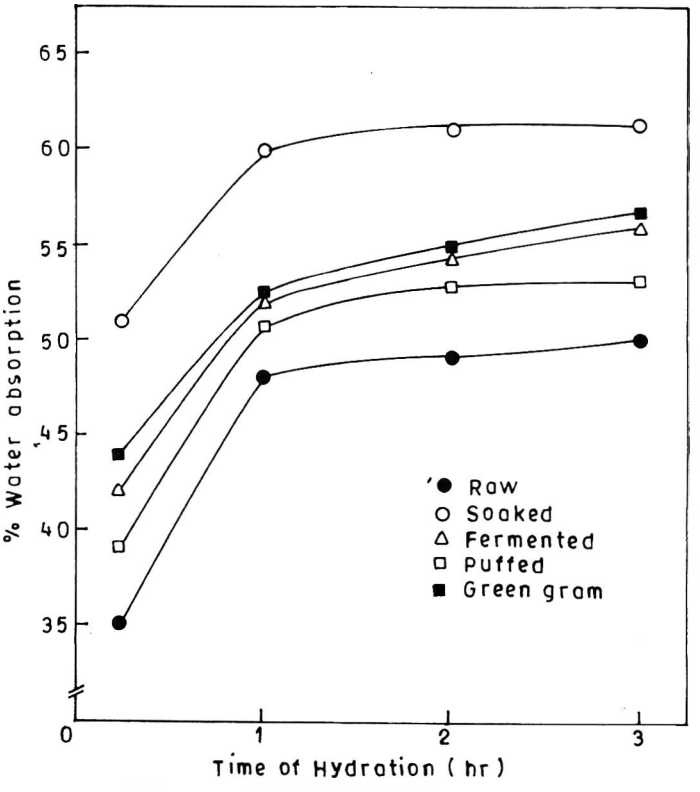


Fig. 1. Percent water absorption by *wadian*.

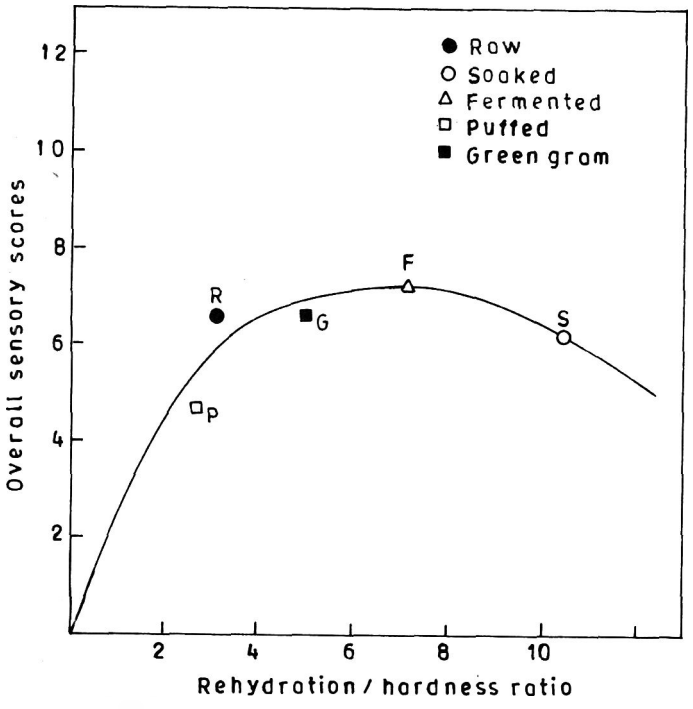


Fig. 2. Effect of rehydration/hardness ratio on the overall sensory score of *wadian*.

## Conclusion

Results of the study have shown that the functional utility of cowpea in foods may be predicted from that of a model system with regard to its hydration properties. The results also indicate that the processing conditions that would increase the hydration capacity of cowpea flour had a more favourable effect on physical and sensory characteristics of *wadians*. Sensory attributes of cowpea *wadians* were as good as those of traditional greengram *wadians*. Optimizing the condition for processing and using cowpea in traditional products would promote the utilization of this legume.

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# Factors Influencing the Response of Improvers to Commercial Indian Wheat Flours

R. RAVI AND P. HARIDAS RAO*

Department of Milling and Baking Technology,
Central Food Technological Research Institute, Mysore-570 013, India.

Effect of potassium bromate and ascorbic acid on rheological characteristics and bread making quality of Indian commercial wheat flours was studied. Ascorbic acid brought about greater change in the extensograph characteristics and improvement in the baking quality, as compared to potassium bromate. Soft wheat flour responded more than medium or hard wheat flours to improvers. The effect of potassium bromate on rheological characteristics was more pronounced, when pH of dough was lowered to less than 5.0. Potassium bromate and ascorbic acid brought about a greater improvement in milk bread as compared to other varieties such as plain, sugar, and fruit bread.

**Keywords** : Potassium bromate, Ascorbic acid, Indian commercial wheat flour, Rheology, Organoleptic evaluation, Formulation, Bread.

Improvers, like potassium bromate or ascorbic acid are used by some of the milling industries in India to improve the functional characteristics of flour (Menon 1990). It is known that the response of flour to these improvers depends on the quality of flour and the level used. For example, Finney et al (1973) reported that the response of Indian wheats to potassium bromate varied from negligible to extremely high among the 43 high yielding varieties. Singh et al (1978) reported beneficial effect of potassium bromate to varying degrees, but did not find the same, true with ascorbic acid or potassium iodate in five Punjab wheat varieties. On the other hand, Venkateswara Rao et al (1986) found improvement with ascorbic acid in some varieties of Punjab wheat. However, the information available is scanty on the effect of improvers on the quality of Indian wheat flours, which are normally milled from grits of wheat and are used by the baking industry in the country. Also, the effect of factors like pH, formulations, and processing conditions on the response of improvers to Indian commercial wheat flours is less known. Hence, studies were undertaken on the above aspects and the results are presented in this paper.

## Materials and Methods

*Wheat flour and bread :* Three different types of commercially milled wheat flours (*maida*) having different protein contents were procured from the local market and subjected to 'Remix' baking test (Irvine and McMullan 1960). Different varieties of bread like fruit, milk, plain and sweet were made, with or without improvers as per the formulations given in Table 1. The processing periods for mixing, fermentation, relaxation, proofing and baking at 220°C were 3, 120, 25, 55 and 25 min, respectively. All the above experiments were carried out in duplicate and in two different trials. Data were statistically evaluated by Duncan's new multiple range test (Duncan 1957).

TABLE 1. FORMULATIONS USED FOR DIFFERENT VARIETIES OF BREAD

| Ingredients | Bread (g/100 g wheat flour) | | | |
|---|---|---|---|---|
| | Plain | Fruit | Milk | Sweet |
| Compressed yeast | 2.0 | 3.0 | 3.0 | 3.0 |
| Sugar | 2.5 | 15.0 | 8.0 | 5.0 |
| Fat | 2.0 | 5.0 | 3.0 | 5.0 |
| Tutty fruity | - | 15.0 | - | - |
| Skim milk powder | - | - | 5.0 | - |

Salt, malt, GMS, and ammonium chloride were used in 1.0, 0.5, and 0.5 g quantities in each formulation. Water was added in preparation of doughs as required.

*Chemical and rheological characteristics :* Moisture, gluten, SDS - sedimentation value, falling

TABLE 2. QUALITY CHARACTERISTICS* OF COMMERCIAL WHEAT FLOURS

| | Flour samples | | |
|---|---|---|---|
| | A | B | C |
| Ash, % | 0.54 | 0.58 | 0.54 |
| Gluten (dry), % | 10.50 | 9.56 | 8.43 |
| SDS Sedimentation value, ml | 36.0 | 31.0 | 30.0 |
| Falling number, sec | 460 | 485 | 501 |
| Diastatic activity, mg/10 g flour | 302 | 210 | 235 |
| Colour grade value | 3.6 | 3.8 | 3.5 |
| Specific volume, ml/g | 4.24 | 4.20 | 4.18 |

* Expressed on 14% moisture basis

* Corresponding Author

TABLE 3. EFFECT OF IMPROVERS ON RHEOLOGICAL CHARACTERISTICS OF DOUGHS AND BREAD QUALITY FROM DIFFERENT FLOURS

| | | Flour A | | | Flour B | | | Flour C | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Extensograph | | Bread | Extensograph | | Bread | Extensograph | | Bread |
| Improvers | Level, ppm | Ratio figure | Sp. vol# ml/g | O.S* 100 | Ratio figure | Sp. vol# ml/g | O.S* 100 | Ratio figure | Sp. vol# ml/g | O.S* 100 |
| None | - | 3.12 | 4.24 | 75.0$^{h}$ | 2.54 | 4.20 | 68.2$^{g}$ | 2.93 | 4.18 | 65.9$^{h}$ |
| PB | 5 | 3.43 | 4.34 | 78.5$^{g}$ | 2.72 | 4.31 | 71.4$^{f}$ | 3.20 | 4.26 | 72.5$^{g}$ |
| " | 10 | 3.46 | 4.45 | 82.8$^{d}$ | 2.97 | 4.40 | 76.2$^{d}$ | 3.44 | 4.38 | 75.9$^{f}$ |
| " | 15 | 3.94 | 4.58 | 84.7$^{c}$ | 3.29 | 4.51 | 76.2$^{d}$ | 3.82 | 4.25 | 82.1$^{bc}$ |
| " | 20 | 4.24 | 4.43 | 81.2$^{f}$ | 3.75 | 4.41 | 75.1$^{d}$ | 4.29 | 4.20 | 82.0$^{bc}$ |
| AA | 50 | 7.33 | 4.50 | 81.3$^{ef}$ | 6.71 | 4.45 | 81.2$^{c}$ | 6.25 | 4.40 | 78.5$^{d}$ |
| " | 100 | 8.27 | 4.57 | 86.2$^{ab}$ | > 7.40 | 4.51 | 83.1 | 6.78 | 4.48 | 81.2$^{c}$ |
| " | 150 | 8.80 | 4.46 | 85.1$^{cb}$ | > 8.00 | 4.50 | 84.2$^{a}$ | 7.72 | 4.51 | 83.5$^{a}$ |
| " | 200 | 9.90 | 4.31 | 84.8$^{c}$ | > 8.33 | 4.42 | 84.5$^{a}$ | 8.18 | 4.45 | 82.9$^{ba}$ |
| PB+AA | 5+50 | 7.45 | 4.56 | 82.5$^{de}$ | > 7.87 | 4.41 | 80.5$^{c}$ | 8.00 | 4.30 | 76.8$^{ef}$ |
| " | 5+100 | 8.52 | 4.73 | 85.1$^{bc}$ | > 9.09 | 4.30 | 83.5$^{ab}$ | 8.28 | 4.28 | 78.1$^{d}$ |
| " | 10+50 | 9.10 | 4.48 | 86.5$^{a}$ | > 9.52 | 4.30 | 83.1$^{b}$ | 9.17 | 4.25 | 81.5$^{c}$ |
| " | 10+100 | 11.11 | 4.13 | 81.8$^{def}$ | > 10.52 | 4.25 | 78.2$^{c}$ | 10.55 | 4.18 | 77.5$^{de}$ |

* : Mean of four observations. Means in the same column bearing different superscripts differ significantly ($p \leq 0.05$). SEM ± 0.40 (39df) for all sets. # Specific volume, O.S: Overall score, PB : Potassium bromate, AA: Ascorbic acid.

number, diastatic activity, and colour grade value as well as farinograph and extensograph characteristics were determined according to standard methods of AACC (1986). The rheological parameters were determined by varying the pH of dough from near neutral to acid pH (5.8 to 4.5) with lactic acid, as the response of these improvers, particularly potassium bromate is reported to depend on pH (Bushuk and Hlynka 1960). Studies were also carried out at different levels of potassium bromate (0-20 ppm) and ascorbic acid (0-200 ppm), individually or in combination, added to both the flours. Breads were evaluated after cooling for 12 h for physical and sensory characteristics. Loaf volume was measured using a loaf volume meter. (Mallock and Cook 1930) Sensory parameters such as crust

TABLE 4. EFFECT OF pH ON THE RHEOLOGICAL PROPERTIES AND BAKING QUALITY OF FLOUR A CONTAINING IMPROVERS

| pH | Improvers | Level, ppm | Farinograph | Extensograph | Baking quality | |
|---|---|---|---|---|---|---|
| | | | Stability, min | Ratio figure | Specific volume, ml/g | Overall score*, 100 |
| 5.8 | None | - | 6.30 | 3.12 | 4.24 | 75.0$^{b}$ |
| 5.8 | PB | 15 | 6.50 | 3.93 | 4.58 | 84.7$^{h}$ |
| 5.8 | AA | 100 | 6.30 | 8.27 | 4.57 | 86.2$^{a}$ |
| 5.5 | None | 0 | 6.35 | 3.50 | 4.71 | 86.1$^{a}$ |
| 5.5 | PB | 15 | 5.45 | 4.12 | 4.82 | 87.5$^{i}$ |
| 5.5 | AA | 100 | 5.30 | 9.50 | 4.72 | 78.8$^{e}$ |
| 5.0 | None | 0 | 6.15 | 4.45 | 4.47 | 81.4$^{f}$ |
| 5.0 | PB | 15 | 5.45 | 5.37 | 4.55 | 75.1$^{b}$ |
| 5.0 | AA | 100 | 5.00 | 10.98 | 4.50 | 71.9$^{e}$ |
| 4.5 | None | 0 | 4.15 | 8.19 | 4.38 | 68.0$^{d}$ |
| 4.5 | PB | 15 | 4.30 | 10.55 | 4.41 | 70.8$^{e}$ |
| 4.5 | AA | 100 | 4.00 | 16.66 | 4.11 | 68.2$^{d}$ |
| | | | | | | SEM ± 0.40 (36df) |

PB : Potassium bromate, AA: Ascorbic acid, * Mean of four observations.
Means bearing different superscripts differ significantly ($p \leq 0.05$)

colour, symmetry of the loaf, crumb colour, texture, uniformity of the grain and taste were assessed by a panel of six judges.

**Results and Discussion**

*Quality characteristics of flour :* Commercial flours showed variations in some of the important quality characteristics, particularly with respect to gluten content and SDS-sedimentation value (Table 2). Flour A was more towards medium hard type, while flours B and C were found to be soft type. The softer nature of flours B and C was confirmed by lower specific loaf volume and overall quality of breads made from these flours, as compared to flour A.

*Effect of improvers on rheological characteristics :* The farinograph studies indicated negligible effect on various characteristics, except marginal increase in stability, with increase in the levels of improvers as reported earlier (Ikezoe and Tipples 1968; Maninder Kaur and Bains 1979). Incorporation of improvers, in general, stiffened the dough, as indicated by increase in the ratio figure for different flours (Table 3). Similar observation was earlier made by Merritt and Bailey (1945). Increases in levels of improvers increased the resistance to extension and decreased the extensibility, as reflected in the ratio figures of flours A, B and C. Further, the effect of ascorbic acid on the ratio figures was greater than that of potassium bromate. In other words, the stiffening action of ascorbic acid on the doughs, was greater than that of potassium bromate. The effect of a mixture of potassium bromate and ascorbic acid in different proportions on the

TABLE 5. RESPONSE OF IMPROVERS ON THE QUALITY OF BREAD AS AFFECTED BY FAT AND SUGAR

| Additive level, % | Improver, ppm | Flour A | | Flour B | | Flour C | |
|---|---|---|---|---|---|---|---|
| | | Specific volume, ml/g | Overall# score, 100 | Specific volume, ml/g | Overall# score, 100 | Specific volume, ml/g | Overall# score, 100 |
| | | | | **Fat** | | | |
| 0 | None | 3.64 | $61.5^{h}$ | 3.50 | $58.8^{b}$ | 3.43 | $56.0^{f}$ |
| 0 | PB 15 | 3.79 | $63.0^{g}$ | 3.67 | $60.1^{a}$ | 3.39 | $58.7^{e}$ |
| 0 | AA 100 | 3.66 | $60.3^{i}$ | 3.53 | $60.9^{a}$ | 3.52 | $60.8^{d}$ |
| 0.5 | None | 4.02 | $72.1^{f}$ | 4.04 | $62.3^{c}$ | 3.95 | $60.2^{d}$ |
| 0.5 | PB 15 | 4.18 | $73.8^{c}$ | 4.05 | $64.5^{d}$ | 3.87 | $65.8^{c}$ |
| 0.5 | AA 100 | 3.82 | $73.9^{c}$ | 4.09 | $66.3^{c}$ | 4.05 | $65.0^{c}$ |
| 2.0 | None | 4.24 | $75.0^{c}$ | 4.12 | $68.2^{f}$ | 4.08 | $65.9^{c}$ |
| 2.0 | PB 15 | 4.58 | $84.7^{e}$ | 4.51 | $76.2^{g}$ | 4.58 | $82.1^{a}$ |
| 2.0 | AA 100 | 4.57 | $86.2^{b}$ | 4.51 | $83.1^{i}$ | 4.48 | $81.2^{a}$ |
| 5.0 | None | 4.35 | $82.4^{d}$ | 4.38 | $80.5^{h}$ | 4.50 | $80.7^{b}$ |
| 5.0 | PB 15 | 4.60 | $86.8^{ab}$ | 4.43 | $85.5^{j}$ | 4.45 | $85.5^{g}$ |
| 5.0 | AA 100 | 4.89 | $87.7^{a}$ | 4.67 | $87.0^{k}$ | 4.59 | $88.8^{h}$ |
| | | | SEM ± 0.39 (36df) | | ± 0.40 (36df) | | ± 0.40 (36df) |
| | | | | **Sugar** | | | |
| 0 | None | 3.87 | $71.8^{a}$ | 3.38 | $60.0^{a}$ | 3.30 | $60.0^{b}$ |
| 0 | PB 15 | 4.28 | $78.2^{b}$ | 3.71 | $77.3^{b}$ | 3.86 | $76.8^{c}$ |
| 0 | AA 100 | 3.97 | $73.2^{c}$ | 3.56 | $70.5^{c}$ | 3.65 | $70.3^{d}$ |
| 2.5 | None | 4.24 | $75.0^{d}$ | 4.12 | $68.2^{d}$ | 4.08 | $65.9^{e}$ |
| 2.5 | PB 15 | 4.58 | $84.7^{e}$ | 4.51 | $76.2^{e}$ | 4.58 | $82.1^{a}$ |
| 2.5 | AA 100 | 4.57 | $86.2^{f}$ | 4.51 | $83.2^{f}$ | 4.48 | $81.2^{a}$ |
| 8.0 | None | 4.37 | $89.3^{g}$ | 5.01 | $85.5^{g}$ | 4.73 | $81.8^{a}$ |
| 8.0 | PB 15 | 4.44 | $91.5^{h}$ | 5.35 | $88.8^{h}$ | 4.88 | $83.5^{f}$ |
| 8.0 | AA 100 | 4.88 | $93.6^{i}$ | 5.89 | $91.3^{i}$ | 4.97 | $87.6^{g}$ |
| | | | SEM ± 0.40 (27df) | | ± 0.39 (27df) | | ± 0.40 (27df) |

PB : Potassium bromate, AA : Ascorbic acid, # : Mean of four observations. Means in the same column bearing different superscripts differ significantly ($p \leq 0.05$).

extensograph characteristics of soft type flours B and C was apparently synergistic in nature.

During resting of the moulded dough for 45, 90 and 135 min, resistance to extension increased gradually by 20 to 30 BU, in case of dough without improvers and by 40 to 75 BU in dough containing improvers. Among the improvers ascorbic acid effected greater increase on resting, as compared to potassium bromate.

*Effect of improvers on quality of bread :* Improvers when added at certain levels, individually or in combination, showed improvement in the loaf volume as well as overall quality of bread made from different flours. Improvement was more pronounced, when 10-15 ppm potassium bromate or 100-150 ppm ascorbic acid was used in the formulation. Greater improvement in the overall quality was observed in flour C, as compared to other flours (Table 3). Ascorbic acid brought about greater improvement in bread quality. Potassium bromate, when added along with ascorbic acid, showed negligible additional advantage, and had an adverse effect on the quality of bread at higher levels.

*Effect of dough pH on response of improvers and bread quality :* Reduction in pH from 5.8 to 4.5 decreased the farinograph stability of flour A from 6.30 to 4.15 min (Table 4), which corroborated with the report of Harinder and Bains (1990). However, the effect was only marginal with the reduction in pH upto 5.0. Resistance to extension and stiffness (ratio figure) increased with reduction in pH to 4.5. Tsen (1966) attributed it to reduction in -SH groups, like the action of improvers. Potassium bromate at 15 ppm and ascorbic acid at 100 ppm levels effected changes in the extensograph characteristics of dough, which were greater with ascorbic acid than with potassium bromate (Table 4). Reduction in dough pH from 5.8 to 5.5 increased the loaf volume and improved the quality of bread. Further reduction in dough pH, with or without improvers, adversely affected the crumb grain and bread quality.

*Effect of ingredients on response of improvers :* Breads made with 0-5.0% levels of added fat indicated that the improvers responded better at higher levels of fat in all the three flours. The response of ascorbic acid was greater than that of potassium bromate at highest level of 5% of fat tested in formulations (Table 5). There was an increase in volume and overall quality of bread with increase in the level of sugar in the formulation.

TABLE 6. EFFECT OF PROCESSING CONDITIONS ON THE QUALITY OF BREAD FROM FLOUR A

| Process time | Improver, ppm | Specific volume, ml/g | Overall# score, 100 |
|---|---|---|---|
| **Fermentation time, h** | | | |
| 1.0 | None | 4.00 | 73.0$^{e}$ |
| 2.0 | None | 4.24 | 75.0$^{d}$ |
| 3.0 | None | 4.11 | 70.0$^{a}$ |
| 1.0 | PB 15 | 4.30 | 81.0$^{b}$ |
| 2.0 | PB 15 | 4.58 | 84.7$^{f}$ |
| 3.0 | PB 15 | 4.20 | 82.0$^{ab}$ |
| 1.0 | AA 100 | 4.35 | 81.0$^{b}$ |
| 2.0 | AA 100 | 4.57 | 86.2$^{g}$ |
| 3.0 | AA 100 | 4.15 | 83.0$^{d}$ |
| **Proofing time, min** | | | |
| 55 | None | 4.24 | 75.0$^{e}$ |
| 65 | None | 4.20 | 70.0$^{f}$ |
| 75 | None | 3.84 | 68.0$^{g}$ |
| 55 | PB 15 | 4.58 | 84.7$^{b}$ |
| 65 | PB 15 | 4.78 | 91.0$^{a}$ |
| 75 | PB 15 | 4.48 | 82.0$^{d}$ |
| 55 | AA 100 | 4.57 | 86.2$^{c}$ |
| 65 | AA 100 | 4.80 | 90.8$^{a}$ |
| 75 | AA 100 | 4.40 | 84.0$^{b}$ |

PB : Potassium bromate, AA : Ascorbic acid, # Mean of four observations. Means in the same column bearing different superscripts differ significantly ($P \leq 0.05$). SEM ± 0.40 (27df) for both sets.

(Harold and Johnson 1951). Potassium bromate and ascorbic acid showed greater improvement in specific volume and overall quality of bread prepared without added sugar and with added sugar at 8% level, respectively, in all the three flours (Table 5).

*Effect of processing conditions on response of improvers :* Changes in specific volume as well as overall quality of bread, made from flour A with and without improvers, as influenced by fermentation and proofing times are indicated in Table 6. The results indicated that optimum fermentation time of 2 h was not affected, as a result of addition of improvers. When the fermentation period was increased to 3 h, the quality was adversely affected in bread made both with and without improvers. This indicated that the fermentation tolerance was not influenced by the improvers and this was found to be true for all the experimental flours. Specific loaf volume increased in all breads, made either with or without improvers, upto 55 min of proofing period. However, increasing the proofing time to 65 min, further increased the loaf volume only in

TABLE 7. EFFECT OF POTASSIUM BROMATE AND ASCORBIC ACID ON QUALITY OF VARIETY BREADS FROM DIFFERENT FLOURS

| Additives | Flour A | | Flour B | | Flour C | |
|---|---|---|---|---|---|---|
| | Specific volume, ml/g | Overall# score, 100 | Specific volume, ml/g | Overall# score, 100 | Specific volume, ml/g | Overall# score, 100 |
| | | | **Plain bread** | | | |
| None | 4.15 | 85.7$^{b}$ | 4.25 | 80.3$^{b}$ | 3.89 | 76.5$^{a}$ |
| PB 15 | 4.20 | 88.6$^{a}$ | 4.26 | 85.0$^{a}$ | 3.93 | 78.8$^{b}$ |
| AA 100 | 4.18 | 89.3$^{a}$ | 4.36 | 86.0$^{a}$ | 3.95 | 80.3$^{c}$ |
| | | | **Milk bread** | | | |
| None | 4.18 | 75.0$^{a}$ | 3.88 | 70.7$^{b}$ | 3.80 | 70.0$^{a}$ |
| PB 15 | 4.47 | 85.6$^{b}$ | 4.16 | 85.8$^{a}$ | 4.21 | 85.7$^{b}$ |
| AA 100 | 4.63 | 87.0$^{c}$ | 4.24 | 86.3$^{a}$ | 4.29 | 87.1$^{b}$ |
| | | | **Sweet bread** | | | |
| None | 4.62 | 87.0$^{b}$ | 3.33 | 85.2$^{a}$ | 3.65 | 83.0$^{a}$ |
| PB 15 | 4.67 | 85.0$^{a}$ | 3.50 | 84.1$^{a}$ | 3.61 | 84.0$^{a}$ |
| AA 100 | 4.75 | 88.3$^{a}$ | 3.54 | 87.8$^{b}$ | 3.76 | 84.0$^{a}$ |
| | | | **Fruit bread** | | | |
| None | 3.64 | 78.0$^{a}$ | 3.40 | 82.1$^{a}$ | 3.41 | 82.7$^{a}$ |
| PB 15 | 3.60 | 81.0$^{b}$ | 3.66 | 84.3$^{b}$ | 3.51 | 85.0$^{b}$ |
| AA 100 | 3.83 | 83.0$^{c}$ | 3.85 | 87.5$^{c}$ | 3.86 | 88.0$^{c}$ |

PB : Potassium bromate, AA : Ascorbic acid, # : Mean of four observations. Means in the same column bearing different superscripts differ significantly ($p \leq 0.05$). SEM ± 0.41 (9df) for all sets.

breads containing potassium bromate or ascorbic acid. This was found to be true in all the three types of flours tested. This indicated that loaves made with improvers, if proofed for a slightly longer period, would further improve the quality of bread, probably because of the crumb strengthening action of the oxidants. (Geddes and Larmour 1933).

*Response of improvers in different varieties of bread :* Specific volume and overall quality of plain, milk, fruit and sweet breads, as affected by the improvers, are given in Table 7. Use of either potassium bromate or ascorbic acid brought about maximum improvement in milk bread, as compared to sweet or fruit bread. This was probably because of the requirement of improvers to counteract the adverse effect of chemical factors of milk powder (Ashworth and Herbert 1942). Ascorbic acid brought about greater improvement than did potassium bromate in all varieties of bread.

This study indicated that ascorbic acid brought about greater change in the rheological characteristics and improvement in the quality of bread based on Indian commercial wheat flours. Incorporation of mixture of potassium bromate and ascorbic acid, however, was not found to have any additional benefit. Among different varieties of bread, the effect of improvers was greater in milk bread, than fruit, sweet and plain breads in that order.

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# A Spectrophotometric Method for Phosphine Residue Determination in Legumes

J.R. RANGASWAMY* AND N. GUNASEKARAN

Infestation Control and Protectants Department,
Central Food Technological Research Institute, Mysore - 570 013, India.

A simple spectrophotometric method for the determination of phosphine residue in legumes has been developed. It is based on the reaction of phosphine with silver nitrate in iso-propanol to form a pale yellow chromophore, with an absorption maximum at 436 nm. It gave a linear relationship between the absorbance at 436 nm and the amount of phosphine in the range of 0.02 to 0.48 µg/3 ml reagent. The crop extractives from the legumes showed a low absorbance of 0.008 at 436 nm, and did not interfere in the chromophore reaction. The method is sensitive with determination limit of 0.015 µg phosphine and can be applied for determination of 0.02 µg residue in 10g legume samples. Phosphine residue on legumes, when extracted with a known volume of 2% aqueous isopropanolic silver nitrate, formed egg yellow chromophore with absorption maximum at the same range. Recovery of fortified phosphine from a closed system ranged from 97 to 98%.

**Keywords** : Phosphine residues, Legumes, Phosphine holding capacity, Spectrophotometric method.

A number of methods are available for determination of phosphine residues in foods. Dumas (1978, 1980) flushed the phosphine residue, desorbed from wheat, with nitrogen into a trap cooled with dry ice for quantification by gas chromatography. Nowicki (1978) has reported a gas chromatographic screening method with flame photometric detection for determining phosphine residues in wheat. Kashi and Muthu (1975) employed a mixed indicator strip method for determination of phosphine in the head space of commodities. Rangaswamy (1984, 1988, 1990; Rangaswamy and Sasikala 1990; Rangaswamy and Muthu 1985) reported sensitive spectrophotometric methods for the determination of phosphine residues in wheat, rice types, cashew kernels and coffee seeds. These methods reported for other commodities could not be used for phosphine residue determination in legumes, as the crop extractives interfered in the formation of chromophore.

The present study describes a new, sensitive and simple spectrophotometric method for the determination of phosphine residues in legumes. It is based on the reaction between phosphine and silver nitrate in aqueous isopropanol.

## Materials and Methods

Stock solution of silver nitrate was prepared by dissolving 150 mg crystals in 50 ml glass distilled water. Two ml of this solution was diluted to 100 ml with distilled isopropanol (BDH grade), to obtain the solution containing 60 µg silver nitrate/ml. Standard phosphine was liberated from phosfume pellet and stored over water in gas burette. All chemicals used were of analytical quality.

*Preparation of a standard graph :* Three ml aliquots of 2% aqueous isopropanolic silver nitrate solution was pipetted individually into 6 ml tubes (5.5 cm x 11 mm internal diam), and these were closed with gas-tight rubber septum. Air (50-200 µl), containing phosphine in the range of 0.015-0.50 µg, was injected into the reagent, using long needled gas-tight micro-syringe. Equal volume of air, without any phosphine, was injected into 3 ml reagent to serve as blank. The tubes were allowed to stand for 20 min for development of chromophore and the absorbance was measured against blank at 436 nm in Spectronic 21 (Baush and Lomb, New York). The absorbance plotted against amounts of phosphine, yielded straight line over a range of 0.02 to 0.48/g phosphine/3 ml reagent.

*Phosphine recovery from legumes :* Three different procedures were employed :

*Procedure A.* Legumes (10 g) were fumigated with phosphine at 3.0 to 300 µg doses in 50 ml conical flasks, with side spout fitted with rubber septum to permit injection of phosphine with 500 and 100 µl pressure Lok gas-tight syringe. The content was left undisturbed for 48 h before removing glass stopper for 30 sec to drive out free phosphine in head space. A known volume (18 to 21 ml) of 2% aqueous isopropanolic silver nitrate solution was added quickly to the flask and the stopper replaced tightly. The extraction of phosphine and development of chromophore were allowed to proceed for 20 min with intermittent shaking. The

* Corresponding Author

extract was filtered through a Whatman No. 1 filter paper (9 cm diam). The absorbance was read as above against a crop control filtrate prepared similarly. Blank experiments without legume in pre-calibrated 50 ml conical flasks at every level of phosphine were run, to assess the extent of absorption of added phosphine by glass, grease and rubber septum. After 48 h, known aliquots of phosphine-borne air were withdrawn with micro gas-tight syringe and analyzed as described as above.

*Procedure B.* Fumigation of legumes (10g) with the same doses of phosphine as in case of procedure A, crop control and blank experiments were carried out. After 48 h, fumigation, stopper of the flask was removed and the content was transferred quickly to 50 ml B24 stoppered tube, containing known volumes (12 to 21 ml) of aqueous isopropanolic silver nitrate solution and the tubes were tightly stoppered immediately. The other details are as per procedure A.

*Procedure C.* Legumes (200 g) were exposed to 0.5 to 1.5 ml phosphine for one week in 250 ml stoppered conical flasks as in case of procedure A. An equal amount of phosphine was injected into an another similar pre-calibrated empty flask to serve as control. At the end of one week, the concentration of phosphine in headspace over legumes and in the control flask were determined by withdrawing known aliquots of phosphine, borne air. After airing the contents by opening the stopper, the void space of each commodity in the flask was determined by evacuating the flasks and injecting air into them with 100 ml gas-tight syringe. The total volume of air injected is taken as the void volume of the commodity. The amount of phosphine taken up by 200 g legumes was computed by subtracting headspace concentration from that in the control flask.

*Residue analysis of legumes :* Legumes (2 kg), in duplicate, were fumigated in 4 l flask at a dose of 6 tablets/tonne by placing phosfume (36 mg) in a paper pack underneath the legumes. The flasks were closed with gas-tight silicon greased glass stopper. Nearly 12 mg of phosphine should have been produced by 36 mg phosfume pellet. At the end of one week exposure at room temperature (28-30°C), phosphine residue in pre-aired samples was determined by the present method. The remaining legumes were aired by spreading in a thin layer for 24 h in the open and the phosphine residue was again determined, as in the procedure described. Phosphine residue was determined by extracting 10 g legumes with known volume (15 ml) of

TABLE 1. RECOVERY OF FORTIFIED PHOSPHINE (μg)* FROM LEGUMES

| Legumes | Phosphine residue in the legume, μg | | | | | | |
|---|---|---|---|---|---|---|---|
| | Level of fortification of phosphine, μg | | | | | | |
| | 3 | 6 | 24 | 60 | 120 | 150 | 300 |
| | | | **Procedure A** | | | | |
| Empty flask | 2.91 | 5.82 | 23.30 | 58.23 | 116.46 | 145.58 | 291.15 |
| Greengram | 0.31 | 0.38 | 6.38 | 18.31 | 28.89 | 45.78 | 63.00 |
| Greengram *dhal* | 0.77 | 1.13 | 1.59 | 2.93 | 16.90 | 25.90 | 34.30 |
| Blackgram | 0.47 | 3.99 | 8.02 | 14.27 | 15.89 | 24.68 | 32.83 |
| Blackgram *dhal* | 0.05 | 0.06 | 0.25 | 3.20 | 5.86 | 9.75 | 11.11 |
| *Bengal*gram | 0.66 | 1.76 | 7.59 | 15.56 | 21.02 | 40.20 | 114.66 |
| *Bengal*gram *dhal* | 0.21 | 0.30 | 5.37 | 12.28 | 12.44 | 12.50 | 27.36 |
| Fieldbean | 0.16 | 0.44 | 2.80 | 5.57 | 8.22 | 9.30 | 9.61 |
| Fieldbean *dhal* | 0.06 | 0.11 | 0.39 | 0.63 | 2.40 | 4.76 | 6.19 |
| | | | **Procedure B** | | | | |
| Greengram | 0.21 | 0.22 | 0.31 | 1.29 | 2.90 | 4.78 | 10.80 |
| Greengram *dhal* | 0.11 | 0.14 | 0.74 | 1.03 | 1.12 | 1.15 | 2.95 |
| Blackgram | 0.27 | 0.40 | 0.42 | 1.10 | 2.46 | 3.62 | 10.40 |
| Blackgram *dhal* | 0.13 | 0.15 | 0.19 | 0.38 | 0.63 | 0.69 | 1.60 |
| *Bengal*gram | 0.09 | 0.18 | 0.23 | 0.83 | 1.37 | 2.04 | 0.72 |
| *Bengal*gram *dhal* | 0.01 | 0.56 | 0.59 | 0.71 | 1.83 | 2.68 | 7.56 |
| Fieldbean | 0.07 | 0.13 | 0.13 | 0.24 | 0.39 | 0.62 | 2.08 |
| Fieldbean *dhal* | 0.004 | 0.006 | 0.007 | 0.002 | 0.030 | 0.090 | 0.550 |

* Mean of 6 trials $P<0.001$

2% aqueous isopropanolic silver nitrate (60 µg/ml) solution for 20 min, filtered and the absorbance was read at 436 nm against the corresponding crop control filtrate as blank. Ten g unfumigated legumes were similarly extracted and used as blank.

**Results and Discussion**

Although the phosphine-silver nitrate chromophore formed in aqueous medium (Rangaswamy 1984) has an absorption maximum at 400 nm, the egg-yellow chromophore formed due to interaction of phosphine and silver nitrate in 2% aqueous isopropanol has the absorption maximum at 436 nm. The chromophore showed the same absorption maximum, when these legumes are soaked in 2% aqueous isopropanolic silver nitrate reagent. Hence, the absorbance was read at 436 nm against the corresponding crop control as blank. The linear relationship between the absorbance at 436 nm and concentration of phosphine is obeyed between 0.02 and 0.48 µg phosphine/3ml. Mean values of 12 replicate determinations were calculated and mean of the standard deviations at various points is ±0.0025. The minimum weight of phosphine needed to produce a measurable chromophore is 0.015 µg.

Generally, grams afford higher recoveries of fortified phosphine, compared to corresponding *dhal*, by procedures A and B (Table 1), which confirms earlier observations that the rough surfaces of the husks of coffee seeds and paddy, retain more of phosphine compared to smooth surface of the seed and grains. *Bengal*gram affords the highest recoveries and field bean *dhal* the lowest by procedure A (Table 1), while greengram and blackgram afford highest recoveries and field bean *dhal* the lowest by procedure B (Table 1). Although the field bean *dhal*, compared to other *dhals*, has larger surface, it has given the lowest recoveries of phosphine by both the procedures. This again confirms earlier observations that it is the rough nature of the surface and perhaps its constituents, which play an important role in phosphine retention, compared to surface area. By procedure B (commodity transfer method), *Bengal*gram shows lower recoveries than *Bengal*gram *dhals*. This may be due to faster dissipation of phosphine from the former during transfer of *Bengal*gram from fumigation flask to the tube containing the reagent. By both the procedures, the amount of phosphine recovered increases, as the dosed phosphine increases from 3 to 300 µg. If this is taken as an index of the amount of phosphine fortified with legumes, then these values

TABLE 2. RECOVERY OF FORTIFIED PHOSPHINE (µG)* BY PROCEDURE C

| Description | Fortified phosphine, µg | | |
|---|---|---|---|
| | 600 | 1200 | 1800 |
| Control flask | 582.28 (97%) | 1164.50 (97%) | 1764.58 (98%) |
| **Greengram** | | | |
| Headspace, µg | 105.78 | 191.13 | 309.37 |
| Absorbed, µg | 476.50 (81.5%) | 973.42 (83.6%) | 1459.21 (82.5%) |
| **Greengram *dhal*** | | | |
| Headspace, µg | 107.77 | 208.31 | 356.11 |
| Absorbed, µg | 474.51 (81.5%) | 956.25 (82.1%) | 1048.47 (79.8%) |
| **Blackgram** | | | |
| Headspace, µg | 280.13 | 548.99 | 806.42 |
| Absorbed, µg | 302.15 (51.9%) | 615.57 (52.8%) | 958.16 (54.3%) |
| **Blackgram *dhal*** | | | |
| Headspace, µg | 34.96 | 122.07 | 189.12 |
| Absorbed, µg | 547.32 (93.9%) | 1042.49 (89.5%) | 1275.46 (89.3%) |
| ***Bengal*gram** | | | |
| Headspace, µg | 380.56 | 754.50 | 1196.88 |
| Absorbed, µg | 201.72 (34.6%) | 410.06 (35.2%) | 567.70 (32.2%) |
| ***Bengal*gram *dhal*** | | | |
| Headspace, µg | 140.72 | 314.11 | 465.52 |
| Absorbed, µg | 441.56 (76.8%) | 850.39 (73.0%) | 1295.76 (73.8%) |
| **Field bean** | | | |
| Headspace, µg | 192.18 | 435.11 | 601.31 |
| Absorbed, µg | 390.10 (66.9%) | 729.45 (62.6%) | 1163.27 (65.9%) |
| **Field bean *dhal*** | | | |
| Headspace, µg | 78.35 | 175.83 | 216.68 |
| Absorbed, µg | 503.93 (86.5%) | 988.73 (84.9%) | 1547.90 (87.7%) |

* Mean of 18 trials ($P < 0.001$)

indicate complete recovery of fortified phosphine, thereby suggesting the efficiency of the method. This is because, unlike with solid or liquid pesticides, complete recovery of fortified fumigant is very difficult to establish due to fugacity of the gas. Some amount of fumigant escapes at every stage of sampling. It is likely that some portion of phosphine that has settled on these legumes might have also escaped, when the stopper is removed for 30 sec (procedure A), and while transferring the material from flask to the tube containing reagent (in procedure B).

In general, the recoveries by procedure B are lower, at any given dose, than those by procedure A. This is because of the loss of phosphine, loosely

TABLE 3. PHOSPHINE RESIDUE (ppm) IN PRE-AIRED AND ONE DAY AIRED LEGUME (DOSE 6 TABLETS/TON)

| Commodity | Pre-aired | One-day aired |
|---|---|---|
| Greengram | 0.0710 | 0.015 |
| Greengram *dhal* | 0.0970 | 0.013 |
| *Bengal*gram | 0.0140 | 0.008 |
| *Bengal*gram *dhal* | 0.0162 | 0.008 |
| Field bean | 0.0160 | 0.004 |
| Field bean *dhal* | 0.0040 | Nil |

• Mean of 4 trials ($P<0.001$)

held by legumes, as these are disturbed during transfer of legumes to reagent in stoppered tube. Within the limits of phosphine-holding capacity of the legume, 10 g of a particular legume would hold only a definite amount of phosphine under given set of conditions, including the dosage, as shown in Table 1, and that amount has been completely recovered. At all the doses, there is 97 to 98% recovery of added phosphine from empty flasks.

The aim of the procedure C (Table 2) is to determine phosphine residue on the legume by computing the headspace concentration and the amount of added phosphine remaining after absorption by glass and grease. Values in Table 2 show 97 to 98% recovery of added phosphine from empty flasks. By this procedure also, the residue on legumes decreases, as the dosed phosphine decreases. The phosphine retention on greengram is 476.50 µg at 600 µg dose, while it is 1459.40 µg at 1800 µg dose. Blackgram, *Bengal*gram and field beans showed lower phosphine holding capacity, than their corresponding *dhals*. Blackgram *dhal* shows the highest mean phosphine-holding capacity of 91.2%, while *Bengal*gram shows the lowest mean capacity of 34.8%. There is no difference between greengram and its *dhal* in their phosphine-holding capacity. The values shown in Table 2 indicate the amount of phosphine held during fumigation, when the legumes are not disturbed. Phosphine residues on these legumes, when fumigated at 6 tablets/ton (Table 3) indicate that the method can be applied for determination of phosphine residues, as the crop extractives from legumes do not interfere with the development of the chromophore. Even the pre-aerated residues of phosphine on these legumes are less than the permissible limit of 0.1 ppm, thus making them suitable for phosphine fumigation. One-day aired samples show that phosphine residue is negligible and is nil on field bean *dhal*. The field bean *dhal* shows the highest loss (100%) of phosphine residue, while greengram shows the least loss (11.8%) during one-day aeration.

Legumes during storage are highly prone to infestation with *Callosobruchus chinensis* and *C. maculatus*. These beetles bore holes to lay eggs and the larvae feed on seeds of legumes. They attack a wide variety of grams and pulses (split grams) during storage. Although some traditional methods such as mixing them with clay dust or applying mud paste to prevent insect infestation have been practised by some people, there are no reports about the fumigation being practised for legumes. Phosphine fumigation of legumes is a promising proposition to prevent the loss due to infestation of pulse beetles (Rangaswamy and Gunasekaran, under communication). The sensitive method reported here, thus, is of vital importance.

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# Standardization of Parameters for Enhancing the Shelf-life of Boiled Egg

T.S. SATYANARAYANA RAO*[1], HEMAPRAKASH REDDY[2] AND K.S. JAYARAMAN

Defence Food Research Laboratory, Mysore - 570 011, India.

Data on different heat treatment methods for hard-cooking of eggs in as such or packed forms (polypropylene or paper-aluminium-foil polyethylene laminating pouch) showed similar ease of peeling. Coating of hard-cooked eggs with different wax emulsions showed no benefit. Hard-cooked eggs had a shelf-life of 7 and 25 days at ambient and refrigerated temperatures without any packing. These were acceptable organoleptically and free of pathogens.

**Keywords** : Hard-cooked eggs, Enhanced shelf-life, Coating, Packing, Acceptability, Pathogens.

At present, troops are issued mostly fresh eggs, in cardboard or plastic egg filler flats. Consequently, the breakage and damage account for about 4-5% of eggs during transit. The eggs are often transported and stored at the temperature prevailing at high altitudes and in ships, submarines and difficult forward areas, thereby getting subjected to severe high or low temperatures. The spoiled eggs pose health hazards because of known presence of *salmonella* (Satyanarayana Rao 1979). Since hard-cooked eggs are not fragile, these can be transported in plastic filler flats without much further packing and these containers can be used repeatedly after washing. Data on reliable shelf-life under such conditions are of vital importance to our defence personnel. Hence, an attempt has been made in the present studies to standardize the methods of boiling of eggs for ease of peeling, coating, packing and extending their shelf-life.

Fresh eggs weighing 50-60 g (grade A) of "White Leg Horn" birds were obtained from a local poultry farm. The eggs were held at ambient temperature (19-26°C) for three days to increase the pH of the egg contents to 8.9, as this helps in easy peeling of eggs after boiling (Meehan et al. 1961). All the eggs were washed in running cold water, drained, dried under the fan, weighed individually and placed in a clean plastic filler flats. All the chemicals used were of AR quality. Waxes were procured from local market, while polyolefin wax PED 521 (Hochest Limited, Germany) was supplied *gratis* by M/s Polyolefins Industries Limited, Bombay. Packaging materials like polypropylene (75μ) and paper-aluminium foil polyethylene laminate (0.012 mm) were procured from M/s India Foil Ltd., Calcutta.

*Composition and preparation of wax emulsions:* Wax emulsions contained carnauba wax, paraffin wax, sugar cane wax, oleic acid and triethanolamine at 20:20:40:8:25 parts. The waxes were heated in a stainless steel vessel to 95-100°C, before adding the emulsifiers with constant stirring. Boiling water (about 500 ml) was added in small doses with continuous stirring, until the emulsion was completely formed. The heating was discontinued and the content was cooled to room temperature. Sodium benzoate (1% w/w) was added, wherever required and the final volume was made upto 1 l with cold water.

*Processing of eggs:* The cleaned eggs were subjected to four different types of heat treatments as follows : (a) eggs were immersed in water and then heated to bring the water to boiling temperature, held for 15 to 30 min and cooled immediately in chilling water, (b) eggs were immersed in boiling water and held in for 15 to 30 min, (c) eggs were cooked by steam in an open autoclave for 10-30 min and, (d) eggs were cooked in hot air oven at 100°C for 40 to 70 min and cooled immediately in chilling water. The hard-cooked eggs were dipped in different types of oil coating (heavy or light grade liquid paraffins) and wax emulsions (with or without preservative) for various periods ranging from 5 to 10 min and then dried under the fan.

*Analytical aspects :* The fresh and hard-cooked eggs were weighed. pH of the fresh egg melange was determined (Elico pH meter Model 1012) after thorough mixing of albumen and yolk. In case of hard-cooked eggs, the albumen and yolk were mixed in distilled water (50 ml) before measuring the pH. The moisture contents of albumen, yolk and whole eggs were determined as per AOAC (1960) method. Beta-carotene was determined as described earlier (Satyanarayana Rao 1992). Microbiological examination for viable counts, spores, *E. coli*, *Staphylococcus*, psychrophilic organisms and

* *Corresponding Author.* [1] *Present address 59/D5 'ARKA' 2nd Main, Yadavagiri, Mysore-570 020;* [2] *Department of Nutrition and Food Science, College of Arts and Science, Drexel University, Philadelphia, USA.*

TABLE 1. LOSS IN WEIGHT OF BOILED EGG, WITH OR WITHOUT COATING (PARAFFIN) AND WITH PRESERVATIVE DURING STORAGE AT AMBIENT TEMPERATURE (19-26°C)

| Sl. No. | Eggs coated with paraffin oil (without benzoic acid) | | | Eggs coated with paraffin oil + benzoic acid | | | Control | | |
|---|---|---|---|---|---|---|---|---|---|
| | Days | | | Days | | | Days | | |
| | 4 | 7 | 15 | 4 | 7 | 15 | 4 | 7 | 15 |
| 1 | 0.09 | 0.12[c] | - | 0.39 | 0.70[c] | - | 1.20 | 1.98 | 3.30 |
| 2 | 0.32 | 0.52[c] | - | 0.62[a] | 0.16[a] | 4.92[a] | 1.33 | 2.73[c] | - |
| 3 | 1.11[a] | 5.10[b] | - | 0.16 | 0.48[c] | - | 1.07 | 1.96[c] | - |
| 4 | 0.47 | 2.76[a] | 3.08[a] | 0.32 | 2.03[a] | 3.38[a] | 0.97 | 1.63 | 2.70 |
| 5 | 0.37 | 0.57 | 1.12[a] | 0.36 | 3.01[b] | - | 0.98 | 1.72 | 2.80 |
| 6 | 4.08[b] | - | - | 1.00[a] | 3.25[a] | 7.58[a] | 1.22 | 2.02 | 3.37 |
| 7 | 0.38 | 0.58 | 0.95 | 0.53 | 0.81 | 4.82[a] | 1.39 | 2.19 | 3.38 |
| 8 | 0.45 | 4.09[a] | 8.87[a] | 0.28 | 0.56 | 0.64 | 1.43 | 2.15 | 3.39 |
| 9 | 0.38 | 0.51 | 0.87 | 2.17[a] | 6.99[a] | 8.30 | 1.20 | 2.06 | 3.46 |
| Average loss, % | 0.35 | 0.46 | 0.91 | 0.34 | 0.64 | 0.64 | 1.20 | 1.98 | 3.2 |

a = Exudate coming out of the eggs, b= Eggs with distinct off-odour and rejected, c = Eggs taken for organoleptic evaluation.

yeast plus moulds were determined (APHA 1960). Organoleptic evaluation of eggs was conducted by 10 semi-trained panel of judges on a 9-point Hedonic scale (Wilson and Solesberg 1942). Stored hard-cooked and treated eggs were compared with control (freshly hard-cooked) at 2, 4, 7 and 15 days and 7, 25, 35 and 45 days at ambient and refrigerated temperatures, respectively.

Preliminary evaluation of the hard-cooked eggs obtained by four different types of heat treatments, either in free or in packed form, showed easy peeling characteristics and good overall acceptability, but with same shelf-life. However, a greenish yellow exudate was seen oozing out from the packed eggs at ambient temperature and the eggs had a distinct off-odour after 2 days. In contrast, the eggs kept in egg filler flats (control) did not show any exudate or off-odour and were acceptable up to a period of 7 days at ambient temperature.

The average weight losses were of the order of 0.34 to 0.35% at the end of 7 days for eggs coated with and without preservatives, respectively (Table 1). The losses increased further at the end of 15 days storage. The overall acceptability scores of coated eggs with and without preservative were 6.16 and 6.33, respectively at the end of 7 days, as against 7.16 obtained for control. Moisture content is a critical factor for acceptability of hard-cooked eggs and its loss was negligible in both albumen and yolk during storage (Table 2). The pH of albumen decreased, while that of the yolk and whole egg increased. The changes in beta-carotene

TABLE 2. CHANGES IN MOISTURE CONTENT, WEIGHT LOSS, pH, BETA-CAROTENE AND ORGANOLEPTIC QUALITIES OF BOILED EGGS WITH OR WITHOUT WAX COATING DURING STORAGE AT REFRIGERATED TEMPERATURE (4°C)

| | | | Control | | | | 15% wax | | | | 20% wax | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Days | | | | Days | | | | Days | | | |
| | | Initial | 7 | 25 | 35 | 45 | 7 | 25 | 35 | 45 | 7 | 25 | 35 | 45 |
| Moisture, % | Alb | 86.7 | 85.6 | 84.2 | 8.30 | 8.20 | 86.0 | 84.8 | 83.6 | 81.8 | 86.0 | 84.5 | 83.4 | 82.1 |
| | Yolk | 52.8 | 52.6 | 52.1 | 53.0 | 51.8 | 52.0 | 52.4 | 52.0 | 52.1 | 51.9 | 52.3 | 52.2 | 52.6 |
| Weight loss,% | | - | 1.3 | 2.8 | 4.1 | 5.3 | 0.9 | 2.3 | 3.4 | 5.1 | 0.8 | 2.4 | 3.3 | 4.9 |
| pH | Alb | 8.81 | 8.54 | 8.50 | 8.36 | 8.25 | 8.45 | 8.40 | 8.22 | 8.14 | 8.19 | 8.13 | 8.20 | 8.09 |
| | Yolk | 6.65 | 7.42 | 8.22 | 8.28 | 8.65 | 7.40 | 8.23 | 8.20 | 8.50 | 7.38 | 8.01 | 8.15 | 8.40 |
| | Whole egg | 7.33 | 7.82 | 8.30 | 8.31 | 8.50 | 7.85 | 8.31 | 8.19 | 8.40 | 7.55 | 8.10 | 8.18 | 8.21 |
| β-carotene, µg/100g | | 38 | 36 | 40 | 38 | 36 | 34 | 36 | 36 | 34 | 38 | 36 | 34 | 36 |
| Organoleptic score | | 7.3 | 6.9 | 6.9 | 5.2 | - | 6.2 | 5.0 | 5.0 | - | 6.2 | 6.1 | 5.0+ | - |

+ slight off-taste noticed. pH values of the fresh albumen, fresh yolk and fresh whole egg were 9.20, 6.15 and 7.36, respectively.

TABLE 3. MICROBIOLOGICAL COUNTS OF HARD-COOKED EGGS COATED WITH WAX AND STORED AT REFRIGERATED TEMPERATURE (4°C)

| | Control | | 15% wax | | 20% wax | |
|---|---|---|---|---|---|---|
| | 35 days | 45 days | 35 days | 45 days | 35 days | 45 days |
| Total plate counts | 210 | 6300 | 2000 | 44000 | 2000 | 27000 |
| *E. Coli* | 30 | Nil | 2000 | 25000 | 2000 | 3900 |
| *Streptococcus* | 40 | Nil | 150 | Nil | 190 | Nil |
| Psychrophillic | 20 | Nil | 20 | Nil | 20 | Nil |
| Yeast and moulds | 10 | 50 | 30 | 50 | 10 | 90 |

Spores were nil in all the cases

were negligible (Table 2). The organoleptic score of whole egg was 6.9 at the end of 25 days in control sample, whereas treated eggs were unacceptable after the same period of storage, as score was less than 6, a level which is considered as indicative of development of off-flavour (Fryd and Hanson 1944).

The total plate counts of hard-cooked eggs with or without coating and after refrigerated storage for 45 days showed increases both in control and treated samples, (Table 3), while spores were completely absent. *E. coli* counts were higher in coated samples, when compared to control. A few *streptococcus* and psychrophillic bacteria were observed at 35 days, but these were absent at the end of 45 days. Similar observations have been made by earlier workers (Acton and Johnson 1973; McCready 1973). Yeast and moulds increased in both control and coated eggs. In case of hard-cooked eggs, with or without coating, packed either in polypropylene or in PFP pouches and stored for 50 days at refrigerated temperature, the total counts were higher in PFP-packed than in polypropylene packed eggs in control samples. *Streptococci* were present in all the samples and these were more in PFP-packed wax-coated eggs than in PP packed samples. A few but a negligible counts of moulds and yeasts were found throughout the storage period in case of eggs packed in both the packaging materials. Spores and psychrophillic bacteria were completely absent. (Table 4).

The eggs coated with polyolefin wax (data not presented) and stored at ambient temperature in filler flats, showed a greenish yellow excudate during storage. This excudate has been shown to be due to the formation of ferrous sulphide (Fromm 1960; Imai 1980; Nath et al. 1971). This may be responsible for the unacceptability of hard-cooked eggs.

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TABLE 4. CHANGES IN MOISTURE CONTENT, WEIGHT LOSS, pH, BETA-CAROTENE AND ORGANOLEPTIC QUALITIES OF BOILED EGGS WITH OR WITHOUT WAX COATING DURING STORAGE AT REFRIGERATED TEMPERATURE (4°C)

| Days/Pkg. material | Control | | | | 15% wax | | | | 20% wax | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TPC | *E.coli* | *Strept* | Y&M | TPC | *E.coli* | *Strept* | Y&M | TPC | *E.coli* | *Strept* | Y&M |
| 14 | | | | | | | | | | | | |
| PP | 22000 | - | 140 | 80 | 10 | - | 490 | 90 | 3200 | - | 1750 | 70 |
| PFP | 116000 | - | 480 | 30 | 200 | - | 50 | 90 | 7900 | 2400 | 20 | 100 |
| 20 | | | | | | | | | | | | |
| PP | 89000 | - | 2000 | 20 | 7900 | - | 2000 | - | 28000 | - | 2000 | - |
| PFP | 210000 | - | 39000 | 60 | 180000 | - | 2000 | - | 160000 | - | 316000 | 10 |
| 40 | | | | | | | | | | | | |
| PP | 1000 | - | 200 | 10 | 1900 | - | 40 | - | - | - | 2000 | 10 |
| PFP | 700 | 13000 | 78000 | - | 260 | 80 | 6200 | - | 80000 | 30 | 2000 | - |
| 50 | | | | | | | | | | | | |
| PP | 360000 | 156000 | 4600 | - | 52000 | 27000 | 75000 | - | 310000 | - | 410 | 40 |
| PFP | 24000 | 31000 | 550000 | 30 | 260000 | 90000 | 84000 | - | 340000 | 750 | 20000 | - |

Spores and psychrophillic were absent. TPC = Total plate counts, *Strept* = *Streptococcus*, Y&M = Yeast and moulds, PP = Polypropylene (75μ); PFP = Paper-aluminium foil (0.12 mm) polyethylene laminate pouch

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# Synergetic Effect of Pressure and Enzyme Treatment for Tenderization of Spent Hen Meat

S.K. MENDIRATTA* AND P.C. PANDA[1]

Division of Livestock Products Technology,
Indian Veterinary Research Institute, Izatnagar-243 122, India.

To explore the potential of combined pressure and enzyme treatment for tenderization of spent hen meat, papain solution (48 mg/kg live wt) was injected intravenously, 30 min prior to slaughter, and pressure-treated (14 kg/cm² for 1 h) at 20° and 60°C at pre-and post-rigor stages, respectively. Significantly lower pH, higher water holding capacity and lower cooking losses were recorded in pressure or pressure plus enzyme-treated samples both at pre- and post-rigor stage treatments. Tenderness and acceptability scores indicated that pressure treatment at post-rigor stage was equally effective as enzyme treatment, but more effective than pre-rigor pressure treatment. Best desirable results could be achieved by synergetic treatment of enzyme and pressure at post-rigor stage.

**Keywords** : Spent hen meat, Tenderization, Enzyme treatment, Pressure treatment, Pre-and post-rigor treatments, Water holding capacity, Cooking loss, Acceptability.

There is a growing problem of disposal of spent hen meat, which comprises about 46% of total poultry meat (Kondaiah and Panda 1987), although some amount was being used for chicken patties (Kondaiah et al. 1992). This is due to inherent qualitative differences, specially tenderness, between broiler and spent hen meat (Sachdev and Verma 1990). The well recognized methods of tenderization, like ageing and electrical stimulation, have their own limitations for exploitation in the industrial sector (Sinha and Panda 1985). It has been reported that enzymes bring about tenderization of spent hen meat by breaking the cross-links of collagen molecules (Sachdev and Verma 1990; Haleem et al. 1970). Beneficial effects of pressure treatment on myofibrillar component of toughness of muscle tissue are well known (Macfarlane 1985). Therefore, an investigation was undertaken to explore the possibility of use of combination of pressure and enzyme treatment to improve the tenderness.

Forty 'White Leg horn' spent hens of about 18 months old were used for each of the three trials. Half of the hens were treated with the enzyme, 30 min prior to slaughter. Crude papain (Activity-1 Anson unit/g) solution in distilled water (8 mg/ml) was injected intravenously into wing vein (6 ml/kg live weight). Pressure treatment at pre-rigor or post-rigor stage was given to untreated and enzyme-treated carcasses in a pressure vessel (mild steel, 20.5 cm diam and 21.5 cm height) fitted with inlet valve and pressure gauge. Samples packed in polyethylene bags were placed in pressure vessel and bolted to air tightness. Air pressure upto 14 kg/cm² was applied through the inlet valve using an air compressor. For pressure treatment at pre-rigor stage, pressure vessel packed with samples was immersed in 20±1°C water bath. The pressure treatment for post-rigor stage was similarly carried out at 60±1°C. Samples were then kept for 1 h in pressure vessel and taken out after releasing the pressure. In order to avoid variation in anatomical cuts, only leg cuts were taken for further analysis.

*Chemical and organoleptic analysis* : Moisture and total protein contents were determined by the methods of AOAC (1984). Procedure described by Bouton et al (1971) was used to measure pH. Water holding capacity (WHC) was estimated by following the press technique of Whiting and Jenkins (1981). Meat samples from different treatments were cooked in an autoclave at 1 kg/cm² for 15 min, after addition of 1% salt, and evaluated organoleptically by 15 semi-trained taste panelists. A 9-point Hedonic scale (ranged from like extremely to dislike extremely) was used for the purpose. Cooking losses were also recorded (Sinha and Panda 1985). Results were statistically analyzed and interpreted by using two factor factorial CRD (Steel and Torrie 1960).

No significant differences were recorded in moisture and total protein contents, after different treatments at pre- or post-rigor stage (Table 1). The pH values of pressure-treated samples were significantly ($P<0.05$) lower, than those of control or enzyme-treated samples at pre- or post-rigor stage. The pH values of control and enzyme-treated samples did not differ significantly at pre- or post-

* *Corresponding Author.* [1] *Present address : Department of Animal Products Technology, C.C.S. Haryana Agricultural University, Hisar - 125 004, India.*

TABLE 1. CHEMICAL COMPOSITION AND ORGANOLEPTIC EVALUATION AFTER DIFFERENT TREATMENTS AT PRE- AND POST-RIGOR STAGE

| Attribute | Pre-rigor stage | | | | Post-rigor stage | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Pressure | Enzyme | Enzyme + Pressure | Control | Pressure | Enzyme | Enzyme + Pressure |
| **Chemical composition** | | | | | | | | |
| Moisture, % | 69.4 ±1.4 | 69.2 ±1.3 | 69.6 ±1.7 | 69.6 ±1.2 | 68.6 ±1.4 | 68.4 ±1.4 | 69.3 ±1.4 | 68.6 ±1.6 |
| Proteins, % | 18.4 ±1.0 | 18.7 ±1.3 | 17.9 ±1.1 | 18.6 ±0.8 | 18.5 ±1.0 | 19.1 ±1.0 | 18.4 ±1.1 | 18.6 ±1.0 |
| pH | $6.1^{b}$ ±0.1 | $5.8^{a}$ ±0.1 | $6.1^{b}$ ±0.1 | $5.8^{a}$ ±0.1 | $5.8^{b}$ ±0.1 | $5.7^{a}$ ±0.1 | $5.7^{b}$ ±0.1 | $5.7^{a}$ ±0.1 |
| WHC* | $24.1^{a}$ ±1.4 | $26.1^{c}$ ±1.4 | $25.3^{b}$ ±0.7 | $26.5^{c}$ ±1.1 | $26.2^{a}$ ±1.0 | $28.1^{bc}$ ±1.6 | $27.5^{b}$ ±1.3 | $28.9^{c}$ ±1.3 |
| Cooking loss, % | $19.8^{c}$ ±1.4 | $17.1^{ab}$ ±1.4 | $17.5^{b}$ ±1.4 | $16.1^{a}$ ±1.6 | $18.6^{c}$ ±1.5 | $15.7^{ab}$ ±1.1 | $16.3^{b}$ ±1.5 | $15.0^{a}$ ±1.3 |
| **Organoleptic evaluation** | | | | | | | | |
| Tenderness | 5.8 ±0.7 | 7.4 ±0.4 | 7.9 ±0.5 | 8.2 ±0.5 | 5.9 ±0.4 | 7.6 ±0.5 | 7.9 ±0.5 | 8.0 ±0.5 |
| Juiciness | 7.3 ±1.0 | 7.3 ±0.5 | 7.7 ±0.8 | 8.0 ±0.5 | 7.3 ±0.6 | 7.6 ±0.5 | 7.8 ±0.6 | 7.9 ±0.5 |
| Colour | 7.5 ±0.4 | 7.4 ±0.3 | 7.6 ±0.3 | 7.4 ±0.4 | 7.6 ±0.7 | 7.5 ±0.7 | 7.9 ±0.3 | 7.6 ±0.5 |
| Flavour | 7.2 ±0.7 | 6.9 ±0.6 | 6.9 ±0.6 | 7.1 ±0.8 | 7.4 ±0.8 | 7.1 ±0.6 | 7.2 ±0.4 | 7.2 ±0.5 |
| Acceptability | 6.0 ±0.4 | 6.9 ±0.4 | 7.8 ±0.5 | 8.0 ±0.5 | 6.1 ±0.2 | 7.6 ±0.5 | 8.0 ±0.4 | 8.3 ±0.5 |

Figures with same superscripts linewise (for pre- and post-rigor stage treatments separately) do not differ significantly at $P<0.05$.
* Water holding capacity.

rigor stage. Lower pH in pressure-treated samples might be due to increased rate of glycolysis during pressure application (Macfarlane 1973; Macfarlane et al. 1982). Water holding capacity (WHC) of pressure and/or enzyme-treated samples was significantly ($P<0.05$) higher than control. Riffero and Holmes (1983) and Macfarlane (1974) also reported an increase in WHC due to pressure treatment. Improvement in WHC of enzyme-treated samples in the present study is consistent with the findings of Sinha and Panda (1985). Cooking losses in all the treated samples were significantly ($P<0.05$) less than control. Cooking losses were lowest in enzyme + pressure, followed by pressure, enzyme and control, all at pre- or post-rigor stage. Lower cooking losses reported in pressure (Macfarlane et al. 1976; Riffero and Holmes 1983) and enzyme-treated sample (Sinha and Panda 1985) can be related to higher WHC (Mendiratta and Panda 1992).

Organoleptic evaluation scores after different treatments at pre- or post-rigor (Table 1) indicated higher tenderness and acceptability scores for enzyme + pressure-treated samples, followed by enzyme, pressure and control. There were not much differences in juiciness, colour and flavour scores. The overall acceptability scoring in decreasing order was, enzyme + pressure (post-rigor) > enzyme (post-rigor) > enzyme + pressure (pre-rigor) > enzyme (pre-rigor) > control (post-rigor) > control (pre-rigor), indicating that pressure-treatment at post-rigor stage (at 60°C) was more effective, than at pre-rigor stage (at 20°C). Best acceptable meat could thus be produced by combining enzyme and pressure-treatments at post-rigor stage.

Bouton et al (1977) and Ratcliff et al (1977) indicated that disruption of myofibrillar protein was mainly responsible for the tenderizing effect of pressure-treatment. Horgan (1981) was of the view that cathepsin B was responsible for the breakdown of sarcoplasmic reticulum in muscle held under pressure. Gonzalez (1983) and Elgasim et al (1983) have claimed that pre-rigor pressure-treatment resulted in rupture of lysosomal membranes and release of catheptic enzymes. Kurth (1986) accounted an increase in activity of cathepsin $B_1$ under pressure

and reported greater activity of enzyme at higher temperature, used along with pressure. Macfarlane (1985) reported that the tenderizing effect of pressure-treatment was due to histological changes, specially disintegration of 'Z' lines. Higher tenderizing effect of synergetic treatment of enzyme and pressure can be explained by the fact that proteolytic enzyme has more effect on background toughness i.e. toughness due to collagen, whereas pressure acts mainly on myofibrillar component of toughness (Macfarlane 1985). Brooks et al (1985) reported that pressure alone or with heat caused collagen triple helix to unwind and thus rendered the collagen more susceptible to enzyme. Pressure-treatment has also been reported to increase the permeability of enzyme to mycfilaments (Macfarlane 1985).

The study indicated that application of pressure to muscle had a positive response on tenderness and its potential usefulness in industrial application. Since treatment favourably accelerated post-mortem glycolysis in pre-rigor meat with no myofibrillar toughness, it can be exploited for processing hot deboned pre-rigor meat, immediately after dressing. The pressure tenderization at post-rigor stage is also useful in reducing the cooking loss and increasing the tenderness of muscle, the only disadvantage being the additional application of heat to raise its temperature to 60°C during pressure-treatment. Further study is required to find out the influence of time and temperature of application of pressure + enzyme treatment in improving functional properties, nutritional value, shelf-life and tenderization of meat, before recommending the same for commercial application.

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# Photoacoustic Study of the Moisture Content in Instant Skim Milk Powder at $(\upsilon_3+2\upsilon_2)$ Coupled Vibrational-Rotational Transition of Free Water; Construction and Evaluation of Photoacoustic Spectrometer

ANDRAS MIKLOS[1] AND DANE BICANIC[2]*

[1]Institute of Isotopes of the Hungarian Academy of Sciences,
P.O. Box 77, H-1525, Budapest, Hungary.
[2]Laser Photoacoustic Laboratory, Department of Agricultural Engineering and Physics,
Wageningen Agricultural University, Bomenweg 4, 6703 HD Wageningen, The Netherlands.

Photoacoustic spectroscopy with a high pressure xenon lamp as a radiation source was used to determine the moisture content in specimens of skim milk powder at wavelengths of 1500 and 1550 nm. Linear relationship between the photoacoustic signal and the concentration (within 3.7 and 10%) was found. Facts that eventually should lead to the minimization of detector size and to substantially improved performance of the present photoacoustic cell (stainless steel) design are discussed.

**Keywords** : Near infrared, Photoacoustic spectroscopy, Skim milk powder, Moisture analysis.

In the recent years, there has been considerable interest in the photoacoustic and photothermal effects as well as their applications in methods for optical and thermal characterization of solid, liquid and gaseous specimens (Bicanic 1992). Basically, the modulated incident radiation heats the sample at the same periodicity rate (Bell 1881). This phenomenon is then detected by acoustical or optical means. If the exciting radiation is of sufficient power to produce the measurable signal, the photoacoustic and photothermal effects can be employed in any spectral region (Mandelis 1992; Bicanic et al. 1994). The most important advantage of photoacoustic, photothermal and other related methods, over the conventional techniques such as transmission and reflectance, is their suitability to a wide variety of samples, including optically opaque and powdered products (Adams et al. 1978; Rosencwaig 1980; Jin et al. 1982; Belton 1984).

Milk and various milk products belong to the categories that were studied using a photoacoustic, photothermal or another scheme in near and midinfrared regions. Photoacoustic kinetic studies of Maillard browning reaction in tablets made out of lypophilized raw milk was reported (N'soukpoe-Kossi et al. 1988). Likewise, the investigation of various powdered milks and can-sterilized infant formulas subjected to different heat treatments prior to spray-drying, has been carried out (Martel et al. 1989; Martel and Paquin 1989).

When manufactured, the moisture content of dried milk products is about 3%, but this value increases with time due to water absorption during storage. The possibility to measure and control the amount of moisture is, therefore, of importance for the quality assessment of these products. In this work, the photoacoustic method has been applied to investigate its potential in detecting moisture content in the instant skim milk powder. The most commonly used method for moisture analysis (Egan et al. 1981; Wetzel 1983) is a diffuse reflectance, which provides good results in the near infrared for a wide range of samples (Osborne and Fearn 1986). The broad absorption bands in the infrared are mainly attributable to overtones of fundamental vibration modes of particular bonds (-CH, -OH, -NH) or the combination bands due to interaction between various functional group vibrations (McQueen 1990). Fundamental oxygen-hydrogen vibrations of the non-bonded, free hydroxyl group are centered at 2.7 to 2.9 microns and first overtone at about 1.4 micron, while a strong combination brand $(\upsilon_3+2\upsilon_2)$ is observed at 1.9 micron (Matissek et al. 1992). For bonded hydroxyl group, the first overtone and the combination band occur at 1.4 and 2.2 microns, respectively (Bonner and Curry 1970). It is thus possible to determine free water, bound water or both by measurement of absorption at 1.9, 2.2 and 1.4 microns.

The experimental arrangement with a 1000 Watt high pressure lamp L (Oriel Corporation, Stamford, Ct., USA) employing 976C1 Xe source (Canrad Hanovia Inc., Newark NJ, USA), that was used in this study is shown in Fig.1. Rare gas

* Corresponding Author

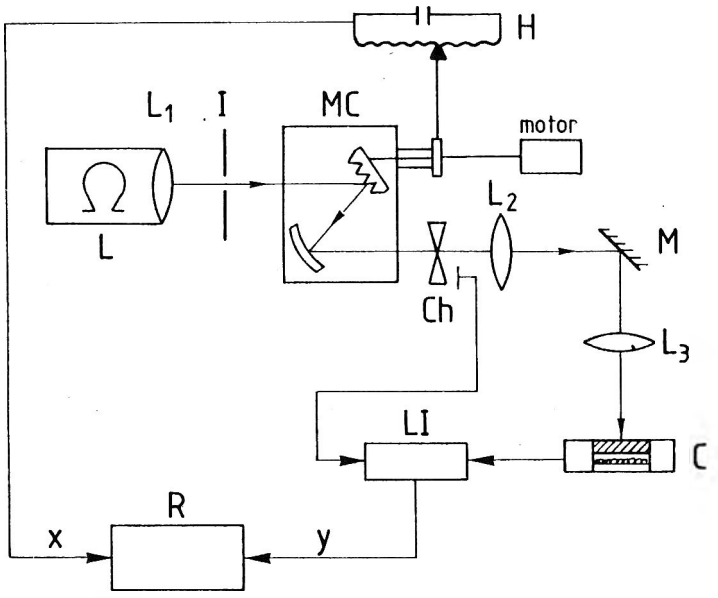


Fig. 1. Schematic diagram of the experimental set-up

continuous sources exhibit intense narrow line emission that is being superimposed on continuous background in the near infrared region (Kimmit 1970), thereby making it very difficult to correct the spectra for variations in source intensity with the wavelength. The lamp output is focussed into the entrance slit of Zeiss M4QIII 185-2500 nm monochromator (Carl Zeiss Oberkochen, Germany) MC (slit width 0.5 mm) by means of a lens $L_1$ (f=250 mm). The radiation leaving the monochromator was mechanically modulated (19 Hz) using 197 EG&G precision light chopper Ch (EG&G Instruments Ltd., Wokingham, England) prior to being collected (lens $L_2$ with f=100 mm focal length), reflected at the plane mirror M and then focussed again with the lens $L_3$ (f=50mm) through the quartz window into the photoacoustic cell C as shown in

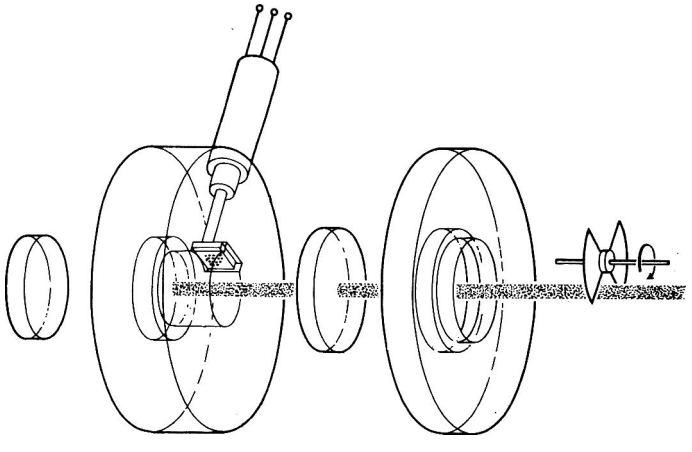

Fig. 2. The exploded view of the photoacoustic cell for powdered samples.

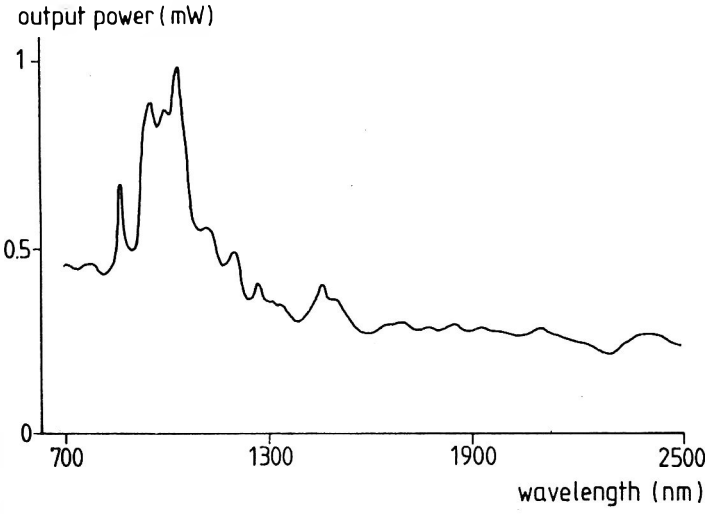


Fig. 3. The power spectrum of high pressure xenon lamp.

Fig.1. For optimum signal/noise ratio, present design requires the sample that is sealed inside the cell. The exploded view of the PA cell manufactured from a stainless steel and insulated from the table vibrations is shown in Fig. 2. It consists of a solid lower section with the bottom plate (diam 60 mm) carrying the sample cup (diam 9 mm, height 2 mm) and the upper cover with the entrance window. The lower and upper sections are put together by means of an "O" ring and by tightening two locking nuts. The miniature M37 electret microphone (Microte BV, Amsterdam, The Netherlands) is mounted flush with the cell's inner wall. The signal detected by the microphone is fed into Ithaco 3691B two phase lock-in amplifier LI (Ithaco, Ithaca, NY, USA) the output (Y) which is displayed versus the monochromator wavelength (X) on a HP 7090 A (Hewlett Packard Company, Palo Alto, Ca., USA) measurement X-Y plotting system. The output of

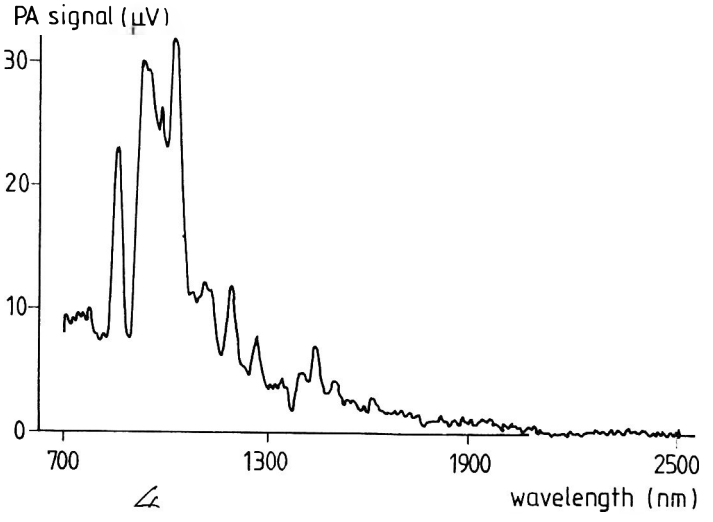


Fig. 4. The photoacoustic spectrum of the photoacoustic cell loaded with the carbon black.

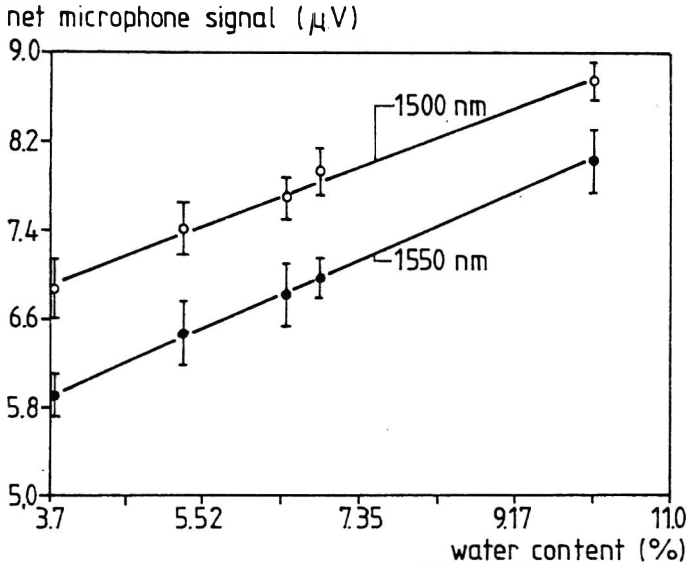


Fig. 5. The photoacoustic spectrum of empty cell.

monochromator was calibrated using the continuously varying (0-22 kohms) Helipot resistor H connected to a home made 6 V d.c. power supply. The magnitude of input signal (X) is then proportional to the position of the sliding contact between the two terminals. Elmeckanic RSM 50/8 (Elkmeckanic BV, Amsterdam, The Netherlands) motor drives simultaneously the prism and Helipot that is coupled to the motor by means of a belt. The ratiometer R constructed in own electronic workshop enables normalization of the PA signal to the incident power.

In order to find out the amount of power reaching the PA cell, the calibrated power meter Photon Control 25S (Photon Control, Cambridge, United Kingdom) was placed immediately behind the chopper. The maximum power (1 mW at 0.15 mm slit width) was observed in the spectral region between 900 and 1000 nm (Fig.3). The output

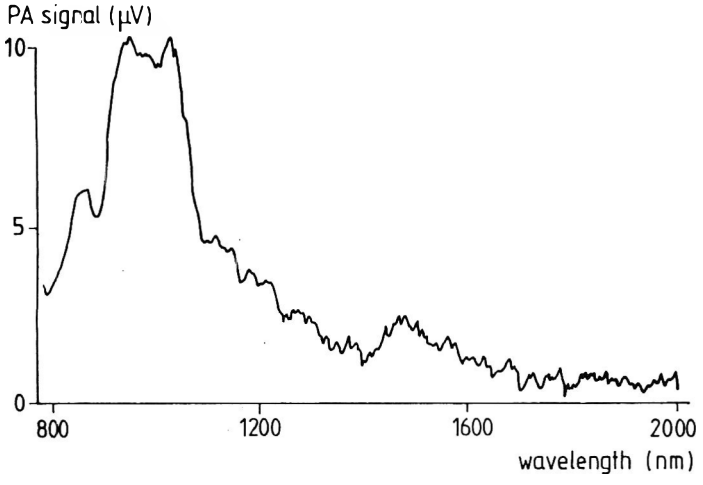


Fig. 6. The photoacoustic specrum of skim milk powder.

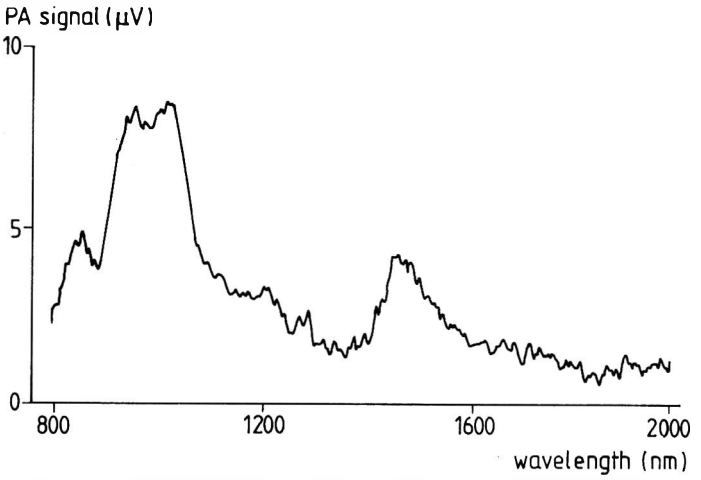


Fig. 7. The photoacoustic signal versus the moisture content in samples of instant skim milk powders at two different excitation wavelengths (1500 and 1550 nm).

power near 1500 nm was approximately 400 microwatts under the same conditions. The stability of lamp between 700 and 1000 nm was measured with a fast photovoltaic silicon detector Centronic OSD-5 (Centronic, Croydon, England) and the lock-in amplifier, yielding a standard deviation smaller than 0.2%. At 1 mW input power level, the signal magnitude was typically 10 mV with 1 microvolt noise (when the source is blocked). The photoacoustic spectrum of xenon lamp (Fig. 4) was determined by recording the microphone signal obtained from the cell loaded with the carbon black. The spectrum resembles that of lamp output power shown in Fig. 3. The PA spectrum from the empty cell is displayed in Fig. 5. The similarity between the two spectra indicates that the cell acts as a grey body radiator with the emissivity factor between 0.2 and 0.3. Comparison of the spectrum recorded with the cell loaded with milk powder (Fig. 6) to that obtained with the empty cell (Fig. 5) reveals pronounced differences only in the near infrared region centered at 1550 nm. Since $(\upsilon_3+2\upsilon_2)$ coupled vibrational-rotational transition of free water is found at 1457 nm (McQueen 1990), this region was selected for further measurements.

Reproducible quantity of representative sample is brought into the cup with the upper section of the cell being removed. Due to relatively large (8mm x 3mm) slit image in the focal plane, sufficient care had to be exercised, when repositioning the cell with each measurement (the resetability < 2%, the repeatability < 5%). The transient peaks from electrical mains power line influenced considerably the microphone signal. This disturbing effect was observed to be practically independent of the sensitivity knob setting of lock-in amplifier and

thus imposes an ultimate limit on detection sensitivity, when measuring small signal differences caused by a varying water content. It is for this reason that relatively broad slit width (0.15 mm) had to be used in order to allow large lock-in signals (of the order of 10 microvolts with the average fluctuations of 3%).

The photoacoustic signals of five carefully prepared samples (each having a different % moisture content, i.e., 3.75,5.3, 6.5, 6.9 and 10.1) were measured (slit width 0.15 mm) at two different wavelengths, i.e., 1500 and 1550 nm, respectively. The measuring procedure proceeded as follows : the cell was loaded with the samples (starting with 3.7%) and the measurement performed at 1500 and 1550 nm, respectively; this was repeated three times. The cell was emptied and then loaded with the new samples (5.3%). Again, the consecutive observations were made at the two wavelengths, as stated above. The series of measurements were completed by applying the above procedure to remaining samples. At that stage, the entire sequence was traversed again, but this time using fresh samples of same concentrations. Readings were taken over a time span of several days. The effective integration time of the lock-in amplifier was 3 min with the lock-in integration time of 30 sec and linear averaging (3 sec sampling time at 64 repetitive scans).

The results of these experiments are shown in Fig. 7. The magnitude of the photoacoustic signal depends obviously on the moisture content in the milk powder. The relationship appears linear, but the slopes of two lines are slightly different. Standard deviation was first calculated for both, the total signal and the background signal (empty cell) and the net signal magnitude obtained by substracting vectorially the background signal from the brutto signal. The error bars in Fig. 7 represent the sum of standard deviations of both, the total signal and background signal. Neither of the plots shown in Fig.7 does extrapolate to zero signal for the zero water content, which is probably due to residual absorption caused by constituents other than water (butter fat, for example) present in the instant skimmed milk powder (Walstra 1965). For the sake of completeness, it is stressed that only one type of skim milk powder (of varying moisture contents) was investigated in this study. Additional studies are needed to ascertain the effect of powder composition (lactose, proteins, lipids) on the photoacoustic signal.

In conclusion, the feasibility of photoacoustic method for measuring the moisture content (3.75-10%) in instant skim milk powder was demonstrated. The performance of present set-up is limited by the low output of the source and the high background signal, due to the choice of a cell material. The combined use of presently available diode laser (that emits as high as 20 mW at 1500 nm) and photoacoustic cell made from material with negligible absorption (glass) in the wavelength range of interest, would therefore improve the sensitivity. In addition, the use of a diode laser eliminates the need for a monochromator and the chopper, since the laser can be electronically modulated. Furthermore, the reduction of the present cell volume (by at least a factor of 10) is easily achievable.

Future cell design will also feature the microphone separated from the cell's volume, in order to avoid scattered radiation from reaching the microphone's membrane. Ensuring the proper degree of acoustic matching between microphone and the sampling volume offers one order of magnitude improvement in the signal to noise ratio. The new cell offers the possibility for rapid inter-changing of samples without a need for frequent demounting of the windows. Such photoacoustic device might eventually develop into a low-cost and compact instrument suitable for a non-destructive routine analysis in a dairy science and other agro-food industries with performance superior to that of standard methods of moisture determination.

The fact that the sample is sealed inside the cell, inhibits considerably the applicability of photoacoustic method for one-line detection. An attempt to construct an open-end photoacoustic cell by bringing the sample into contact with a pyroelectric detector met only with moderate success (Kocsany, personal communication, 1988). The response of such a contact detector was shown to be dependent on a grain size of the powder. Moreover, the signal magnitude decreases faster for contact detector, than it does for the photoacoustic cell.

Finally, since data collected by the photoacoustic measurements are intrinsically only relative, some absolute standard is required, when the quantitative measurements are being considered. Initial steps in this direction are already realized in a differential system (Favier et al. 1993) used for photoacoustic characterization of various flours and spices (Favier at al. 1994).

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# Effects of Processing and *In vitro* Proteolytic Enzyme Action on Bambarra Groundnut Hemagglutinin

PHILIPPA C. OJIMELUKWE[1], CHRISTIAN C. ONUOHA[2] AND ZAK A. OBANU[2]

[1]School of Food Science and Technology, Abia State University
P M B 200 Uturu, Abia State, Nigeria,

[2]Department of Food Science and Technology, University of Nigeria, Nsukka, Nigeria.

Hemagglutinating protein extracts were prepared from bambarra groundnut seeds, before and after processing and assayed for activity using red blood cells of the cow. Effects of soaking, boiling of whole seeds, cooking with trona, (sodium sesquicarbonate) autoclaving, fermentation and roasting of flour samples on hemagglutinating activity were determined. Heat treatment of flour samples for up to 2 h was found to be the best method for elimination of hemagglutinating activity. Effects of proteolytic enzyme action on the hemagglutination of human red blood cells were evaluated using pepsin, trypsin and protease. Treatment with protease was the most effective in reducing hemagglutinating activity. Blood group O was not measurably agglutinated in the presence of proteolytic enzymes. Characterization based on solubility showed that only the albumin and globulin protein fractions possessed hemagglutinating properties.

**Keywords** : Hemagglutinin, Bambarra groundnut, Processing methods, Proteolytic enzymes, Solubility properties.

A major deterrent to effective utilization of legumes is the preponderance of anti-nutritional factors found in these foods (Wilson et al. 1972; Pusztai and Palmer 1977; Cristofaro et al. 1974). Hemagglutinins are one of the very important anti-nutritional factors, because of their deleterious physiological effects (Liener et al. 1986). These are sugar specific, cell agglutinating mitogenic proteins, that interact with specific blood group substances, promote cell adhesion and have insulin-like effect on fat cells (Liener et al. 1986). If unaffected by proteolysis, after ingestion, hemagglutinins can impair the absorption of other nutrients by binding to the walls of the intestinal villi and changing their conformation, thereby reducing nutrient utilization (Liener et al. 1986). Quite a number of hemagglutinins have been purified and characterized (Dahlgreen et al. 1970; Marshall and Lauda 1975; Pusztai and Palmer 1977), but such studies have not been carried out for lesser known legumes and the effects of heat treatment of enzymes have not been studied in all the legumes except a few (Bansal et al. 1988; Batra et al. 1986; Ghorpade et al. 1986; Ramamani 1986). Bambarra groundnut is one of the legumes of the Papillionaceae family. Its agronomy, physiology and composition have been studied (Okigbo 1978; Lawani and Rachie 1975; Johnson 1968). Anti-nutritional components such as trypsin inhibitors, flatulent oligosaccharides and tannins have been studied in some bambarra groundnut cultivars (Poulter 1981; Ojimelukwe and Ayernor 1992), but its hemagglutinating protein has not received enough research attention. The present study was, therefore, undertaken to investigate the effect of processing treatments and proteolytic enzyme action on bambarra groundnut hemagglutinin.

Whole bambarra groundnut seeds (cream-coloured cultivar), purchased from Nsukka market, were cleaned with water and air-dried to 10% moisture for 12 h. Seeds were used in whole form, and ground to pass through 1 mm mesh sieve in a sample mill (Cyclotex 1053, Tecator Instruments). Pepsin, trypsin and protease were from Sigma, USA. Other reagents were of analytical grade.

Hemagglutinating protein extracts were prepared by a modification of the method of Dahlgreen et al (1970). Ground seeds were equilibrated in a mechanical shaker for 2 h (whole flour : solvent ratio = 1:20), using phosphate buffered saline (1% NaCl in phosphate buffer, pH 7.4) and centrifuged at 2,000 **g** for 20 min at 4°C. Crude extracts were assayed for hemagglutinating activity in microtitre plates by double dilution, using red blood cells of the cow, pig and goat. Extracts were further purified by precipitation with ammonium sulphate, after acidification to pH 5.0 and centrifuged again at 20,000 **g** for 20 min at 40°C. The supernatant was dialyzed against phosphate buffer (pH 6.8) and used as the buffer extract of hemagglutinin.

Animal red blood cells (cow) were obtained from the abbatoir at Nsukka market (Enugu State, Nigeria) during exsanguination. Human blood was obtained from normal healthy individuals. Blood grouping tests were carried out before

* *Corresponding Author*

hemagglutination studies. A modification of the method of Liener (1955) was used for the preparation of standard red blood cells. The scheme is outlined below:

Collect blood in Alsevier's solution/ anticoagulant (1:1 ratio)

↓

Centrifuge at 5,000 **g** for 5 min to obtain crude red blood cells

↓

Wash crude red blood cells with 0.1% NaCl in phosphate buffered saline pH 7.4

↓

Suspend in fresh phosphate buffered saline to give an absorbance of 2.0 at 620 nm of the untrypsinized red blood cell suspension

↓

Include with 1% trypsin and wash again to obtain trypsinized red blood cells.

The following processing treatments were given to bambarra groundnut samples of 10 g size. Finally, ground bambarra groundnut seeds were heated in an enamel ware for 1, 2, 3 and 4 h in an air oven. Whole seeds were boiled in 10 times their own volume of water for 30 min, 1 h and 2 h, with or without addition of trona (sodium sesquicarbonate). Whole seeds were soaked for 12 h and dried at 60°C for 5 h in an air oven. Seed samples were autoclaved at 121°C for 1 h. Seeds were also soaked for 6 h, fermented for 48 h and dried at 60°C for 5 h. Fermentation of soaked whole seeds was carried out at room temperature in wrapped banana leaves. Buffer extracts were prepared from processed seeds and flour for use in hemagglutination assays, using red blood cells of the cow.

The enzymes pepsin, trypsin and protease (0.01%) were incubated *in vitro* with 2% suspension of human red blood cells at 25°C for 1 h. Using bambarra groundnut hemagglutinin extracts, assays were carried out according to the spectrophotometric method of Liener (1955). For solubility studies, distilled water, 5% NaCl, 70% ethanol and 0.02 N NaOH were used to extract bambarra groundnut flour (1:1 ratio). The extracts were centrifuged at 20,000 **g** in a refrigerated centrifuge, precipitated with ammonium sulphate (50% saturation) and assayed for activity using red blood cells of the cow.

TABLE 1. AGGLUTINATION OF COW, GOAT AND PIG ERYTHROCYTES BY CRUDE BAMBARRA GROUNDNUT HEMAGGLUTININ

| Erythrocyte source | Volume of agglutination (ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1.0 | 0.9 | 0.8 | 0.7 | 0.6 | 0.5 | 0.4 | 0.3 | 0.2 | 0.1 |
| Cow | ++ | ++ | ++ | ++ | + | + | + | + | + | + |
| Goat | ++ | ++ | ++ | + | + | + | + | + | + | + |
| Pig | + | + | + | – | – | – | – | – | – | – |

++ : Pronounced agglutination, + : Positive agglutination, – : No observable agglutination.

Table 1 shows the extent of hemagglutination of animal (cow, goat and pig) red blood cells, by crude bambarra groundnut hemagglutinin extracts. Pig red blood cells were agglutinated by higher concentrations of agglutinins, indicating that these have lower affinity for hemagglutinins, than the red blood cells of cow and goat. Total red cell counts for cow, goat and pig red blood cells used for hemagglutination experiments were $25.44 \times 10^6$, $45.90 \times 10^6$ and $22.02 \times 10^6$, respectively.

Table 2 shows the effect of processing treatments on hemagglutination of red blood cells of the cow by bambarra groundnut hemagglutinin. Dry heat treatment of flour samples for up to 2 h achieved total elimination of hemagglutinating activity. Cooking of seeds with trona was least effective in reducing hemagglutinating activity of bambarra groundnut hemagglutinin. Statistically, the differences were significant between the total hemagglutinating activity values of samples treated with trona and those given ordinary moist heat treatment ($p \leq 0.05$). Cooking for 2 h reduced the protein content as determined by the method of Lowry et al (1951), possibly due to leaching of soluble proteins. In addition, the hemagglutinating activity was also reduced. Cooking is known to soften the seed, improve cell wall plasticity, facilitate cell expansion and reduce cell adhesion, thereby allowing the cell components to undergo changes during moist heat treatment (Quast and Da Silva 1977). Protracted cooking is known to reduce the nutritional quality (Ekpenyong and Borchers 1981). Processing treatments used in the present study are known to reduce hemagglutinating activity and toxicity of legumes, while the degree of elimination is dependent on the biological properties of the hemagglutinin (Grant 1986). Bambarra groundnut is popularly consumed in some parts of Nigeria as a steamed flour. Since size reduction, in combination with heat treatment, enhances elimination of hemagglutinating activity, this practice should be

TABLE 2. EFFECT OF PROCESSING ON HEMAGGLUTINATION OF COW RED BLOOD CELLS BY BAMBARRA GROUNDNUT HEMAGGLUTININ

| Sample | Processing treatment | Protein content, mg/ml | Number of hemagglutinating units, x | Specific activity, mg/proteins | Total activity, HU |
|---|---|---|---|---|---|
| Bambarra groundnut seeds | Cooking, 30 min | 6.29 | 2.74 | 4.35 | 2051.17$^{c}$ |
| | Cooking, 1 h | 5.57 | 2.64 | 4.74 | 1981.39$^{c}$ |
| | Cooking with trona, 1 h | 7.23 | 4.69 | 6.48 | 3515.95$^{a}$ |
| | Cooking, 2 h | 3.25 | 2.06 | 6.33 | 1541.96$^{cd}$ |
| | Autoclaving at 121°C for 1 h | 5.53 | 3.75 | 6.78 | 2812.42$^{b}$ |
| | Soaking,12 h | 5.03 | 2.55 | 5.46 | 1916.43$^{c}$ |
| | Fermentation,48 h | 5.42 | 3.01 | 5.56 | 2260.14$^{c}$ |
| Bambarra groundnut flour | Dry heat treatment | | | | |
| | 30 min at 100°C | 6.29 | 2.14 | 3.44 | 1622.82$^{d}$ |
| | 1 h at 100°C | 6.29 | 1.91 | 3.04 | 1431.12$^{d}$ |
| | 2 h at 100°C | 6.29 | - | - | - |
| | 3 h at 100°C | 6.29 | - | - | - |
| | 4 h at 100° | 6.29 | - | - | - |

Values carrying the same letters are not significantly different from one another ($p \leq 0.05$) - : No measurable agglutination

encouraged to avoid the hemagglutinin problem.

Table 3 shows the specific and total hemagglutinating activities of bambarra groundnut hemagglutinin, in the presence of proteolytic enzymes. Specific activities in the presence of pepsin and trypsin were higher in the agglutinating medium than in the presence of protease. For human blood group O erythrocytes, the sample extracts did not show measurable agglutination in the presence of proteolytic enzymes. This suggests that protease may have better hydrolytic effect on the hemagglutinin molecule. Earlier literature also indicates that modifications of the hemagglutinin molecule by proteolytic enzymes may lead to conformational changes, that reduce their capacity for cell agglutination (Burger 1973). Also, not all proteolytic digestions may lead to inactivation. On the other hand, incubation of red blood cells with proteolytic enzymes, followed by washing with saline, to remove digestion products is known to increase the agglutinability of such red blood cells and reduce the quantity of hemagglutinins required for agglutination (Burger 1973). Incubation of red blood cells with proteolytic enzymes is known to cause surface modifications and changes in the surface zeta potential (Burger 1973).

Only the albumin and globulin fractions of bambarra groundnut protein fractions had hemagglutinating properties (22.37 and 25.15 HU/ml, respectively).

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TABLE 3. HEMAGGLUTINATION OF HUMAN RED BLOOD CELLS BY BAMBARRA GROUNDNUT HEMAGGLUTININ IN THE PRESENCE OF PROTEOLYTIC ENZYMES

| Sample extract | Human blood groups | | | |
|---|---|---|---|---|
| | A | | B | |
| | Specific activity, mg/protein | Total activity, HU | Specific activity mg/protein | Total activity, HU |
| Bambarra groundnut + pepsin | 18.81 | 1191.98 | 13.59 | 861.30 |
| Bambarra groundnut + protease | - | - | 13.15 | 833.62 |
| Bambarra groundnut + trypsin | 25.43 | 1161.60 | 15.24 | 965.65 |
| No enzyme | 18.29 | 1158.98 | 13.38 | 848.10 |

- : No measurable agglutination was observed for group 'O' erythrocytes.

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# Hypocholesterolemic/Hypolipidemic Effect of Dietary Fibres from Outer Dry Skin of Garlic and Onion

S. VADHERA*[1], AMANDEEP K. PUNIA[1] AND G.L. SONI[2]

[1] Department of Biochemistry, [2]Department of Microbiology,
Punjab Agricultural University, Ludhiana - 141004, India.

Fibres from the outer skin of onion and garlic lowered total lipids, total cholesterol and glyceride levels of plasma and tissues in the rats. The effect was prominent in case of garlic skin fibres. The fibres from onion skin are potently anti-atherogenic, as evidenced by increased high density lipoprotein cholesterol/low density lipoprotein cholesterol ratio. The hypocholesterolemic action of these fibres could not be attributed solely to the bile acid excretion or cholesterol catabolism. No direct relationship between hypocholesterolemic/hypolipidemic effect of these fibres to their *in vitro* bile acid absorption or water holding capacity could be established.

**Keywords** : Hypolipidemia, Hypocholesterolemia, Atherosclerosis, Fibre, Onion, Garlic, Outer dry skin.

Hypercholesterolemia and hyperlipidemia are major risk factors in the development of atherosclerosis (Goldstein and Brown 1987). There has always been a search for agents, which can lower the blood cholesterol levels, particularly of low density lipoprotein fraction. The lowering of blood cholesterol levels can be achieved with the help of drugs, either by inhibiting endogenous synthesis and/or by lowering cholesterol absorption from the intestine (Sedaghat et al. 1975), but such an approach is never free from side effects (Sabine 1977) The changes in the dietary regimens would thus, be the most suitable approach for lowering cholesterol level. Increased use of refined diets leads to low intake of dietary fibre. A good part of vegetables (skin, stalk and peels) with its unavailable carbohydrates is discarded, though these portions may have potential biomedical benefits. Therefore, in the present study, the fibres prepared from outer dry skin of onion and garlic have been used as dietary supplements to ascertain their hypocholesterolemic/hypolipidemic action.

Fibre was prepared from the outer dry skin of onion (*Allium cepa*) and garlic (*Allium sativum*) according to the method of Eastwood and Mitchell (1976). The fibre was analyzed for cellulose (Crampton and Maynard 1938), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin (Goering and Van Soest 1970) and ash (AOAC 1980). The physico-chemical analysis included determination of water holding capacity and bile acid adsorption (Singh et al. 1983).

Male albino rats, about 6 weeks old and each weighing 60-70 g, were divided into 3 groups of six animals each and were given experimental hypercholesterolemic diets, which consisted of (%) starch 64, oil 10, salt mixture 4, yeast powder 1, cellulose/dietary fibre 5, casein 15 and cholesterol 1. At the end of four weeks of feeding, the animals were sacrificed and their blood as well as different organs were collected for biochemical analysis. Total lipids (Folch et al. 1957), total cholesterol (Zak et al. 1953), phospholipids (Ames 1966), free fatty acids (Lowry and Tinsley 1976) and high density lipoprotein cholesterol (Lopes Virilla et al. 1977) were determined. Bile acid excretion was determined from the faecal mass collected during the last week of feeding trial (Boyd et al. 1966).

Fibre from outer skins of onion and garlic had comparable ADF, cellulose and lignin. In contrast, hemicellulose content and water holding capacity of garlic skin fibre were higher than those of onion skin fibre (Table 1). Inclusion of fibre from either of the source in the hypercholesterolemic diets of albino rats did not interfere with food intake or its utilization for growth (Table 2). Moreover, these fibres did not react to show any adverse effect on organ weight indices or haematological parameters,

TABLE 1. PROXIMATE COMPOSITION (%) AND WATER HOLDING CAPACITY (g/g) OF FIBRES FROM SKIN OF ONION AND GARLIC

| | Onion skin | Garlic skin |
|---|---|---|
| Neutral detergent fibre | 41.5 ± 2.3 | 47.1 ± 2.1[a] |
| Acid detergent fibre | 39.0 ± 1.4 | 40.7 ± 1.8 |
| Cellulose | 35.3 ± 1.2 | 36.5 ± 1.5 |
| Lignin | 3.6 ± 0.1 | 4.1 ± 0.2[a] |
| Hemicellulose | 2.5 ± 0.1 | 6.4 ± 0.4[a] |
| Mineral | 7.0 ± 0.3 | 6.3 ± 0.4 |
| Water holding capacity | 10.5 ± 0.8 | 12.7 ± 0.9[b] |

Significantly different from that of onion skin fibre
[a]$p<0.01$ [b]$p<0.05$

* Corresponding Author

TABLE 2. FOOD INTAKE (g), GAIN IN BODY WEIGHT (g), PLASMA (Mg/Dl), HEPATIC AND CARDIAC LIPID PROFILE AND BILE ACID EXCRETION OF RATS GIVEN HYPERCHOLESTEROLEMIC DIETS CONTAINING DIFFERENT FIBRES (VALUES ARE MEAN ± SD)

| Paramters | Fibre source | | |
|---|---|---|---|
| | Cellulose (control) | Onion | Garlic |
| Food intake, g | 248.0 ± 16.6 | 263.0 ± 22.5 | 236.6 ± 17.8 |
| Gain in body weight, g | 42.0 ± 4.2 | 42.4 ± 4.3 | 38.6 ± 4.4 |
| **Plasma lipids (mg/dl)** | | | |
| Total lipids | 1033.3 ± 51.6 | 713.2 ± 68.1[a] | 600.0 ± 1.1[a] |
| Cholesterol | 209.0 ± 35.0 | 117.8 ± 14.1[a] | 124.1 ± 32.2[a] |
| Phospholipids (PL) | 260.0 ± 33.5 | 290.0 ± 26.9[b] | 275.0 ± 20.8 |
| Free fatty acids | 26.9 ± 2.8 | 19.6 ± 2.3[a] | 22.3 ± 2.9[b] |
| Glycerides | 535.8 ± 39.8 | 285.8 ± 25.9[a] | 178.1 ± 41.3[a] |
| Cholesterol/PL ratio | 0.8 ± 0.1 | 0.4 ± 0.1[a] | 0.5 ± 0.1[a] |
| High density lipoprotein cholesterol (HDLc) | 32.3 ± 5.5 | 43.8 ± 10.1[b] | 46.4 ± 5.0[a] |
| Low density lipoprotein cholesterol (LDLc) | 69.6 ± 10.2 | 17.0 ± 3.8[a] | 42.1 ± 6.0[a] |
| Very low density lipoprotein cholesterol (VLDLc) | 107.2 ± 16.3 | 57.0 ± 12.5[a] | 35.6 ± 8.1[a] |
| HDLc : LDLc ratio | 0.46 ± 0.1 | 2.67 ± 0.2[a] | 1.10 ± 0.1[a] |
| **Liver lipids (mg/g)** | | | |
| Total lipids | 191.2 ± 27.3 | 163.3 ± 26.1a | 173.3 ± 17.9 |
| Cholesterol | 30.3 ± 5.0 | 22.5 ± 7.0[a] | 27.4 ± 4.3 |
| Phospholipids | 21.6 ± 1.3 | 13.7 ± 5.64[a] | 25.7 ± 2.5 |
| Free fatty acids | 21.2 ± 5.0 | 15.3 ± 5.13[b] | 22.2 ± 2.6 |
| Glycerides | 117.4 ± 11.6 | 111.7 ± 11.2 | 97.9 ± 9.7[b] |
| **Heart lipids (mg/g)** | | | |
| Total lipids | 51.9 ± 6.26 | 45.4 ± 5.42 | 28.8 ± 4.63[a] |
| Cholesterol | 4.9 ± 0.49 | 4.3 ± 0.38[b] | 2.7 ± 0.31[a] |
| Phospholipids | 20.3 ± 2.81 | 19.2 ± 2.15 | 10.8 ± 1.61[a] |
| Free fatty acids | 7.4 ± 1.10 | 9.0 ± 1.26[b] | 4.2 ± 0.74[a] |
| Glycerides | 19.3 ± 2.14 | 12.8 ± 1.57[a] | 11.0 ± 1.38[a] |
| **Bile acid excretion** | | | |
| mg/g faecal matter | 1.44 ± 0.32 | 1.80 ± 0.31[b] | 2.04 ± 0.29[b] |
| mg/day | 2.18 ± 0.32 | 2.23 ± 0.35 | 1.91 ± 0.30 |

[a]$p < 0.01$, [b]$p < 0.05$

such as haemoglobin level, RBC count and packed cell volume.

Both garlic and onion skin fibres lowered the total lipids, cholesterol, glycerides, cholesterol to phospholipid ratio and cholesterol of LDL and VLDL fractions. In case of garlic, the effect was more prominent for total lipids, glycerides and cholesterol of VLDL fraction. In contrast, the effect of onion fibres was more prominent in case of total plasma cholesterol and cholesterol of LDL fraction (Table 2). Since the ratio of HDL cholesterol to LDL cholesterol is inversely related to the development of coronary heart disease and serves as an important predictor of development of atherosclerosis (Goldstein and Brown 1987), the increased ratio observed in fibre-fed groups indicates that both these fibres have anti-atherogenic properties and can thus protect against development of atherosclerosis. Of these two fibres, onion skin fibre reduced the risk more than garlic skin fibre. The lipid and cholesterol lowering effects of these fibres are also reflected in the hepatic and cardiac tissues (Table 2). Lowering of plasma and hepatic lipids by different fibre sources have also been reported (Singh et al. 1983). The beneficial effects of these fibres are further reflected by the substantial reduction of hepatic degenerative changes, particularly with onion skin fibre. The decreased fatty degenerative changes observed in hepatic cells are more or less in agreement with the biochemical changes in lipid levels of plasma and liver.

Fibre-fed groups had higher faecal bile acid

TABLE 3. BILE ACID ADSORPTION (% ADSORPTION) BY ONION AND GARLIC SKIN FIBRES

| Time, min | Cholic acid | | Deoxycholic acid | |
|---|---|---|---|---|
| | Onion | Garlic | Onion | Garlic |
| 30 | 12.5 ± 0.6 | 9.6 ± 0.7[a] | 15.3 ± 0.8 | 21.4 ± 1.1[a] |
| 60 | 15.1 ± 0.8 | 14.2 ± 0.8[a] | 22.5 ± 1.3 | 27.5 ± 1.3[a] |
| 90 | 17.2 ± 1.1 | 16.8 ± 1.1 | 26.9 ± 1.7 | 34.6 ± 1.2[a] |
| 120 | 17.8 ± 0.8 | 18.0 ± 1.0 | 30.7 ± 1.9 | 38.3 ± 2.1[a] |
| 150 | 19.1 ± 1.0 | 18.4 ± 1.2 | 31.5 ± 2.4 | 39.1 ± 1.8[a] |

[a] Significantly different from that of onion. ($p < 0.01$)

excretion, which could be due to their adsorption fibres (Table 3). But the degree of bile acids adsorption could not be related to any single component of these fibres. Adsorption of bile acids has been directly related to the lignin content of fibre from cereals and pulses in earlier studies (Uberoi et al. 1992; Agte and Joshi 1991). But such a relationship does not appear to hold good with these fibres. Certain other minor dietary components such as polyphenols and saponins (Oakenfull and Fenwick 1978) are known to be associated with these fibres and these may contribute to their hypocholesterolemic action (Oakenfull 1981). The hypocholesterolemic action of these fibres could be due to the combination of mechanisms enhancing cholesterol catabolism and/or suppressing its absorption and endogenous synthesis. Moreover, both these fibres are good water binders (Table 1) and can be used as dietary supplements as well as humectants in certain food products. The appreciable hypolipidemic/hypocholesterolemic action proves their usefulness as a dietary supplement in various food blends.

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# Development of a Continuous Motorized Peeler for Raw Mangoes

**S.C. MANDHAR* AND G. SENTHIL KUMARAN**

Section of Agricultural Engineering,
Indian Institute of Horticultural Research, Bangalore - 560 089, India.

A raw mango peeler for pickle, *chutney* and mango powder production has been developed. It consists of power transmission, concave wheel, guide drum, casing and frame. The inlet for unpeeled mangoes to concave and the outlet for peeled mangoes are provided to the casing. The concave wheel has sharp projections and revolves at 250 rpm, with 2 hp motor. The guide drum revolves at 2 rpm and maintains the continuous feeding and discharge of the mangoes. The rotation of mangoes in the elliptical chamber, formed between concave wheel (with sharp projections), guide and casing, peels the mangoes. The mango peeler had a capacity of 200 kg/h with single concave wheel of 900 mm diam, in comparison to 7.5 kg/person in manual peeling. The cost of peeling is Rs. 45/tonne, in comparison to Rs. 667/tonne by manual peeling.

**Keywords :** Motorized peeler, Raw mangoes, Power transmission, Concave wheel, Guide drum, Casing, Peeling capacity.

Mango is the most important fruit crop of India, accounting for 42% of area under fruit cultivation (Bose 1985). Around 9.45 million tonnes of mangoes are produced in India and about 1% is processed to different products (Pruthi 1992). Mango pickle and *chutney* are the delicacies of Indian sub-continent and about 9.5, 3.6, 3.2 and 0.09% of the processed mangoes are used for pickle, mango slices in brine, mango *chutney* and mango powder production, respectively (Pruthi 1992). *Chutney* and mango powder (*amchur*) production require peeled raw mangoes. The peeling is done manually, due to non-availability of mechanized peelers. Manual peeling is not only tedious and time consuming, but is also difficult to manage, because of labour scarcity during the mango processing season. Hence, a motorized peeler was developed earlier for raw fruits (Mandhar and Senthil Kumaran 1993), but it required about 1 min batch time to peel 4-8 fruits. The capacities for mango and papaya were 120 and 200 kg/h, respectively. Therefore, a continuous motorized peeler of the peeling capacity of 200 kg mangoes/h has been developed and its features and performance are reported in the present communication.

The principle of peeling is to rotate the fruit in an enclosed chamber, against the sharp projections on the inner surface. The detailed drawing of the peeler is shown in Fig. 1. The main parts of the peeler are : frame, power transmission, concave wheels, guide drum and casing.

*Frame :* It supports all the parts of the peeler and is fabricated out of 50 x 50 x 6 mm mild steel (m.s.) angles in case of the prototype. The power transmission and casing are rigidly fitted on this. The concave wheel and the guide drum are fitted with ball bearings and supported on bearing block on the frame and the shaft of concave wheel for free rotation, respectively.

*Power transmission :* The prototype is operated by 2 hp, AC motor, fitted on frame. Necessary V-pulleys, gears and counter-shafts are provided to rotate the concave wheel at 250 rpm and guide drum at 2 rpm.

*Concave wheels :* The prototype has only one concave wheel of 900 mm diam and 125 mm width. This rotates around horizontal axis at 250 rpm. An elliptical cavity is provided all along the circumference of the concave wheel. The concave wheel is fabricated from stainless steel (S.S.) flat and the cavity from S.S. sheet. The width of the cavity (minor axis of ellipse) is 70 mm and depth of cavity (semi-major axis) is 90 mm. All along the internal surface, 6 mm diam holes, with the density of 550 holes/sq mt are drilled. Half the circumferences of the holes are projected upward

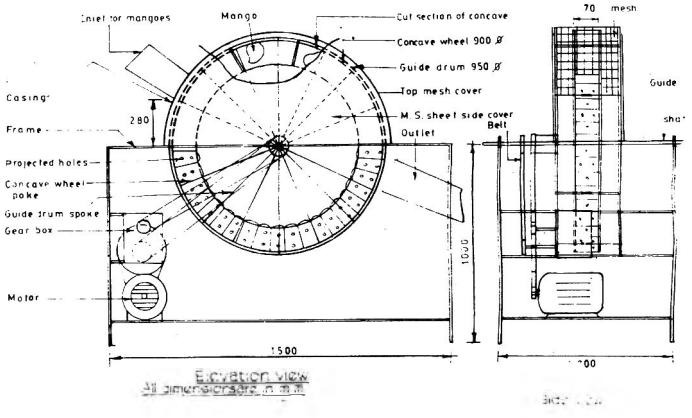


Fig. 1. Continuous motorized peeler

* *Corresponding Author*

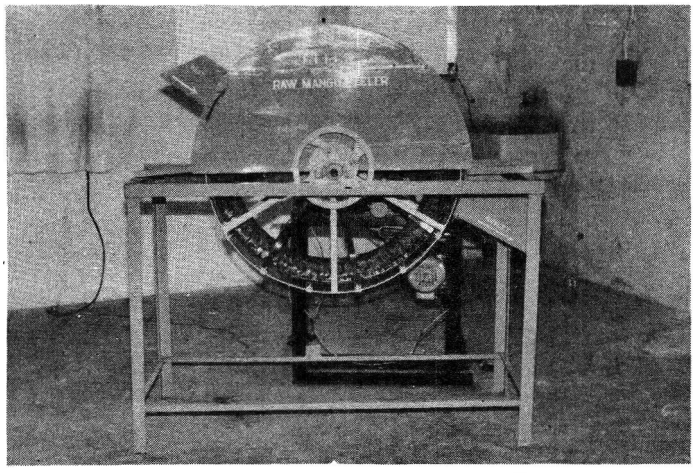

Fig.2. Continuous motorized peeler for raw mangoes

to a height of 2 mm and sharpened. These sharp projections peel the fruits and guide the peel to pass through holes to avoid clogging of peel at the peeling edge.

*Guide drum :* The diam of guide drum is 950 mm and width 255 mm. The guide wheels are fabricated from m.s. flats. These are connected with guide holders to form guide drum. The material used for fabrication of guide holder is S.S. flat and 16 guide holders are provided along the periphery of the drum. One guide of S.S. is welded to each guide holder. The width of guide is 45 mm and length 100 mm. The guides are accommodated in the cavity of the concave wheel. The inside edge of the guide is semi-circle of 45 mm diam to avoid contact with the concave wheel and projections inside the cavity. The guide drum rotates at 2 rpm, along the same axis and direction, as of the concave wheel.

*Casing :* It is provided for holding the mangoes inside peeling chambers and to guide the peel for collection and disposal. The inlet chute for raw mangoes and outlet chute for peeled raw mangoes are provided in the casing of the peeler. The upper half of the concave wheel and guide drum is covered by the semi-circular casing. Both sides of casing are of m.s. sheet for peel collection and have a diam of 1050 mm. The top of the casing is fabricated out of S.S. welded mesh and has a width of 300 mm. The size of openings in S.S. mesh is 25 x 25 mm. This mesh retains the fruits in the peeling chambers and helps the operator to see the condition of peeling. The size of inlet opening for unpeeled mangoes is 100 x 100 mm and provided with necessary chute. The outlet chute for peeled mangoes is provided at the end of the casing.

*Operation :* The unpeeled raw mangoes are fed manually through inlet chute (Fig. 2). These are enclosed in the peeling chamber by the movement of the guide. The chamber is formed by 2 guides, concave cavity and top casing. The movement of projections, at a speed of 12 min/sec rotation, peels the mangoes evenly. The rotation of guide takes the mangoes to the end of the casing, where these drop in the outlet chute and get collected in the desired container.

The performance of peeler of 'Neelam' variety mangoes (average dimensions : Length 77 mm, diam 58 mm and weight 142 g) is given in Table 1. The peeling capacity was found to be 200 kg/h for a single operator against 7.5 kg/h/person in manual peeling. The cost of peeling for one tonne mangoes was worked out to be Rs. 45, in comparison to Rs. 667 by manual peeling. The cost of pulp production was calculated to be Rs. 6,320/tonne, in comparison to Rs. 6,482/tonne for manual peeling.

The peeler was found to be suitable for mangoes of dimensions upto length 80 mm and diam 60 mm. The maximum dimension of the mango to be peeled is limited by the cavity size provided on concave wheel. The diam and number of concave wheels can be increased to enhance the capacity of the peeler. Three concave wheels with different sizes of cavities can be provided in the peeler to peel three different grade sizes (big, medium and small) of fruits from ungraded mangoes. A mango size grader can be used to grade and feed the respective concave wheel of the peeler. The estimated capacity for a peeler with 3 concave wheels of 1800 mm diam will be about one tonne/h. The estimated power requirement for this peeler will be around 10 hp.

The machine may be used for peeling papaya

TABLE 1. PERFORMANCE OF MANGO PEELER FOR 'NEELAM' VARIETY

| Performance | Manual peeling | Mango peeler |
|---|---|---|
| Capacity, kg/h/person | 7.5 | 200 |
| Unpeeled mango weight, g | 144 | 142 |
| Peeled mango weight, g | 130 | 118 |
| Recoverable pulp, % of total weight of mango | 72 | 64 |
| Peel removed, % of total weight of mango | 10 | 18 |
| Cost of peeling, Rs./tonne of mango (labour cost Rs. 40/day) | 667 | 45 |
| Cost pulp production, Rs./tonne (mango cost Rs. 4.0/kg) | 6,482 | 6,320 |

and other hard raw fruits by changing the size of cavity of the concave wheel and guide drum.

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# Use of Troponin for Species Identification of Cattle and Buffalo Meats

Y. SAISEKHAR AND P.M. REDDY*

Department of Meat Science and Technology,
College of Veterinary Science, Tirupati - 517 502, India.

Troponin was used as antigen to raise antibody in rabbits for the purpose of identification of the species of fresh, cooked as well as decomposed cattle and buffalo meats by agar gel double immunodiffusion (DID) test. The rabbit anti-buffalo troponin serum (RAB) showed cross reactions, whereas the rabbit anti-cattle troponin serum (RAC) showed no cross reactions and was species specific. However, the (RAB) serum could be made monospecific by adsorbing on glutaraldehyde for detecting adulteration of fresh buffalo meat upto 1% and also decomposed buffalo meat. But, it did not detect cooked buffalo meat. The RAC serum, which was species specific, could detect fresh cattle meat up to 10% adulteration with other meats, in addition to detection of decomposed cattle meat. It could not detect cooked meats of cattle origin.

**Keywords** : Troponin, Agar gel double immunodiffusion, Rabbit anti-cattle troponin sera, Rabbit anti-buffalo troponin sera, Adulteration.

Species identification of meat is of importance in food inspection, quality control programmes and veterinary forensic medicine. The methods extensively used for identification of species origin of meats were mostly serological (Reddy et al. 1990; Sherikar et al. 1987). Other methods such as immuno assay, gel electrophoresis of mitochondria have also been used (Kang'ethe and Lindqvist 1987; Bandyopadhyay et al. 1985). In quest for developing species-specific antibodies, Milgrom et al (1965) described the presence of heat-stable antigens in adrenal glands. Babiker et al (1981) used polyacrylamide gel electrophoresis (PAGE) technique and found that horse meat cooked at 120°C for 20 min retained only high molecular weight myofibrillar muscle protein fractions. Thermostable muscle proteins and adrenal proteins from sheep, goat, ox and buffalo resolve into fairly distinct bands in sodium dodecyl sulphate gel electrophoresis, ranging in molecular weights from 35,000-37,000 daltons corresponding to tropomyosin and troponin fractions, which are myofibrillar proteins (Radhakrishna et al. 1989).

The present study was undertaken to develop species-specific antibodies against fresh muscle troponin of buffalo and cattle to detect adulteration of meats with fresh, cooked or decomposed buffalo and cattle meats.

*Antigen preparation :* Troponin antigen was prepared according to the method of Greaser and Gergely (1971), which is schematically presented in Fig. 1.

*Raising antisera :* The modified procedure of Milgrom et al (1963) was followed. Six healthy rabbits, divided into two groups and weighing 1.5 kg each, were immunized with particular species of troponin antigen. One ml of (9 mg/ml) troponin antigen was emulsified with one ml of Freunds complete adjuvent and injected at the rate of 0.6 ml per rabbit. Half of the 0.6 ml dose (0.3 ml) was given intradermally at several sites on shaved back and the remaining half dose was administered intramuscularly in the hind leg. Three injections were given on 1st, 2nd and 3rd weeks. Further three injections were given in a similar fashion with incomplete Freunds adjuvent on 4, 6 and 9th weeks of immunization. Serum was harvested one week after the last day of injection and was preserved with thiomersal 1 in 10,000 concentration. The harvested sera were concentrated by using 10% polyethylene glycol/ammonium sulphate precipitation method. Cross-reacting antibodies were adsorbed using the method described by Talwar (1983).

*Preparation of test meat extract :* Fresh, heated and decomposed meat extracts of 20% concentration were prepared by homogenizing 20 g fresh/cooked/decomposed/muscle in 100 ml NaCl (0.14 M) solution. Adulteration of meats (1 to 10%) was achieved by mixing 20% fresh and cooked muscle saline extracts of different species origin. Agar gel immuno diffusion test was carried out as described by Ouchterlony (1949).

The double immuno diffusion test with rabbit anti-buffalo fresh muscle troponin [RAB (F)] serum showed presence of one sharp, thick and fine line of precipitation with homologous antigen and as a

* Corresponding Author.

| | |
|---|---|
| 1 kg ground muscle + 2 litres solution containing 20 mM KCl, 1 mM $KHCO_3$, 0.1 mM $CaCl_2$ and 0.1 mM Dithiothreitol (DTT) | Stirred for 5 min, filtered, residue washed four times similarly. |
| ↓ | |
| Residue + 2 litres of 95% ehanol | Filtered after 10 min, repeated twice |
| ↓ | |
| Residue + 2 litres of diethyl ether | Washed thrice for 10 min, residue allowed to dry at room temperature for 2-3 h |
| ↓ | |
| Dried powder + 2 litres solution containing 1 M KCl, 25 mM tris(pH 8), 0.1 mM $CaCl_2$ and 1 mM DTT | Overnight extraction at room temperature and later filtered |
| ↓ | |
| Residue + 1 litre 1 M KCl | Extraction, two extracts combined and cooled to 4°C |
| ↓ | |
| Extract + ammonium sulphate | 40% saturation, centrifuged after 30 min. |
| ↓ | |
| Supernatant + ammonium sulphate | 60% saturation, centrifuged |
| ↓ | |
| Precipitate + 500 ml solution containing 5 mM tris (pH 7.5), 0.1 mM $CaCl_2$ and 0.1 mM DTT) | Dialyzed against 15 litres of same solution for 6 h, repeated with fresh solution overnight. |
| ↓ | |
| Contents of dialysis sac + solid KCl added to a concentration of 1 M + 1 M KCl solution added to make 1 litre, adjusted pH to 4.6 with HCl. | Tropomyosin precipitate was removed by centrifugation. |
| ↓ | |
| Supernatant pH adjusted to 7 with KOH + ammonium sulphate. Precipitate + solution with 5 mM tris (pH 7.5), 0.1 mM $CaCl_2$, and 0.1 mM DTT | 70% saturation centrifuged.<br>Dialyzed overnight against the same solution. |
| ↓ | |
| Contents of dialysis sac + solid KCl added to a concentration of 1 M, pH adjusted to 4.6 | Precipitate removed by centrifugation, neutralized the supernatant. |
| ↓ | |
| Supernatant + 2 mM Tris (pH 7.5) solution | Dialyzed against 2 litres of 2 mM tris solution. Dialysis repeated until Nessler's reaction was negative. |
| ↓ | |
| Troponin | |

Fig. 1. Flow-chart for preparation of antigen

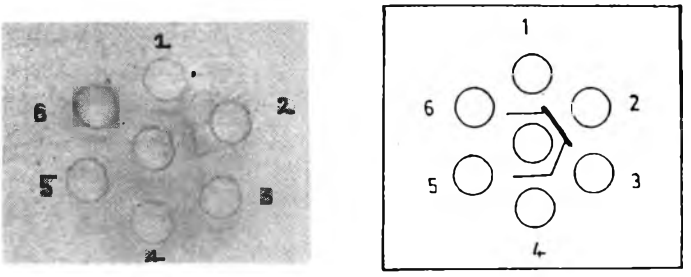


Fig. 2. DID : Central well : RAB (F) troponin serum. Peripheral wells 1: Cattle meat extract fresh, 2: Buffalo meat extract fresh, 3: Sheep meat extract fresh, 4: Goat meat extract fresh, 5: Pig meat extract fresh, 6: Chicken meat extract fresh.

spur with cattle or sheep muscle extract antigen (Fig. 2). The line of precipitation was fused completely with the other line of precipitation observed with goat fresh muscle extract antigen, thereby confirming the cross-reactivity amongst the four species (buffalo, cattle, sheep and goat) studied. No precipitation reaction against all the heated muscle extract antigens (buffalo, cattle, sheep and goat) was observed, thereby indicating that the antibody raised with fresh muscle troponin antigen failed to react with heated antigen. Presence of cross-reacting antibodies against cattle, sheep or goat, but not against pork or chicken in rabbit anti-buffalo fresh muscle troponin serum, indicated that the cattle, sheep or goat were more closely related to buffalo as compared to pig and chicken. Also, the cattle are more closely related to buffalo, as compared to sheep or goat. A similar view was expressed by Sherikar et al (1979).

The cross reacting antibodies in the RAB (F) troponin serum, could be completely eliminated upon absorption with glutaraldehyde and the serum made monospecific, giving one line of precipitation with homologous fresh muscle antigen only. The adsorbed serum could detect buffalo meat to a minimum level of 1% adulteration in cattle/sheep/goat meats. The results were repeatable as the adsorbed serum reacted specifically with 20 different samples of homologous fresh muscle extracts and no reaction with heterologous samples was observed. However, the serum could not detect cooked meat of homologous/heterologous species.

The rabbit anti-cattle fresh muscle troponin [RAC(F)] serum was found to be monospecific. Even without adsorption, it reacted with fresh cattle meat to a minimum level of 10%, when adulterated with buffalo/mutton/chevon, giving a single line of precipitation. The serum did not react with heated homologous (cattle) muscle extract antigens.

Radhakrishna et al (1989) also reported that the anti-serum raised against troponin in rabbits revealed species-specificity in agar gel double immuno diffusion experiments. The RAB (F) or RAC (F) troponin anti-sera also reacted with respective homologous (buffalo or cattle) decomposed meat extracts.

Thus, it is possible to detect the presence of cattle and buffalo meat (fresh and decomposed, but not cooked) with the anti-troponin sera, viz., RAB (F) and RAC (F) as experienced also with anti-troponin sera of chicken and turkey (Hayden 1977).

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# Physical Characteristics of Potato Flour Made from Six Potato Varieties

SUNEETA PANT AND K. KULSHRESTHA*

Department of Food and Nutrition,
G.B. Pant University of Agriculture and Technology, Pantnagar - 263 145, India.

Six varieties of potato, 'K. Badsha', "K. Bahar', 'K. Jyoti', 'K. Chandramukhi', 'JI 4486' and 'JH 222', were studied for physical characteristics of their flours. Data revealed maximum yield of flour for variety 'JH 222'. The values of water uptake were highest for variety 'K. Chandramukhi', which also had finest particles as evidenced by particle size index. Ultrastructure studies revealed varietal variations in shape of the starch granules and free starch. More free starch was visible in 'K. Chandramukhi'.

**Keywords** : Potato flour, Physical characteristics, Water uptake, Particle size index, Ultrastructure, Starch granule shape, Free starch.

A large quantity of potatoes get wasted even after cold storage (Nanda and Khanna 1988). In Indian climate, the losses in weights of potatoes vary from 20 to 70% from the month of April to September (Burton 1989). The post-harvest processing into appropriate dehydrated products will be useful to extend the storage life and assure supply during off-seasons (Nanda and Khanna 1988). Potato flour has wide applications along with cereal products (Chandrasekhara and Shurpalekar 1983, 1984a, 1984b). The present study was undertaken to evaluate the physical characteristics of potato flour, when made from six different potato varieties.

Potato varieties 'K. Badshah', 'K. Bahar', 'K. Jyoti', 'K. Chandramukhi', 'JH 222', and 'JI 4486' were obtained from Horticulture Research Centre of the University. Two potatoes of each variety, weighing approximately 100 g, were labelled, pressure-cooked in 1 l water at 10 lb/cubic inch for 22 min and cooled under running water to room temperature within 3 min. (Chubey and Mazza 1983). Boiled potatoes were classified into different texture categories, i.e., floury, waxy, close or watery as described by Burton (1989). About 1 kg tubers of each variety were boiled, peeled and crumbled. The crumbled potatoes were dried at 60°C for 24 h in a cabinet dryer and ground to flour using a laboratory mill. The colour of the flour was compared with Munsell soil colour chart and the matching hue value and chroma were recorded using Munsell soil colour chart. Water absorption was determined according to the method of Smith and Circle (1972).

Particle size index (PSI) was determined according to the method of Bedolla and Rooney (1984) by sieving 10 g flour through a series of 60 (250 µm), 70 (210 µm) and 80 (177 µm) mesh standard sieves in ro-tap type sieve shaker for 15 min. Weights of samples remaining on 60, 70 and 80 and through 80 mesh sieves were recorded and PSI was calculated as :

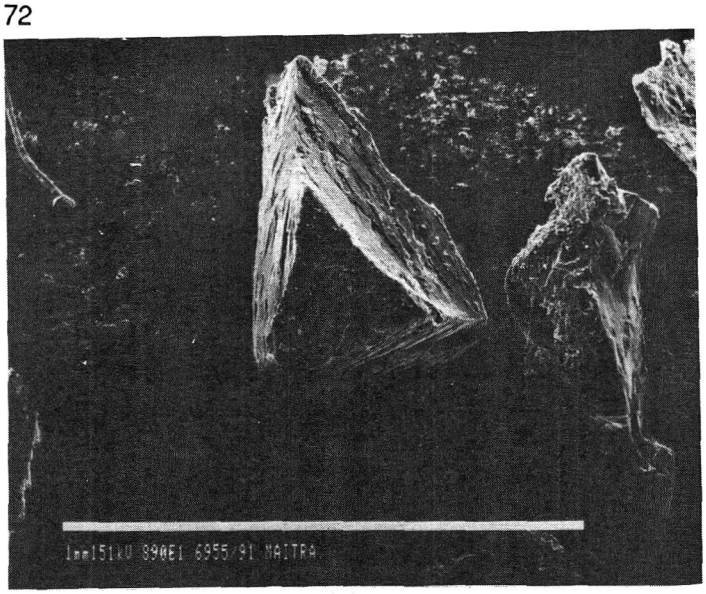
PSI = (0.1) (% retained on 60 mesh) + (0.4) (% retained on 70 mesh) + (0.7) (% retained on 80 mesh) + (1.0) (% passing through 80 mesh).

Shrinkage ratio was determined by the method of Ranganna (1979), as weight of the raw potato to that of final dried product, to assess the yield of flour. Scanning electron microscopy of the flour of different potato varieties was done by the method of Hansen and Jones (1977). Sample was crumbled on a double sticky tape on metal strip and excess flour blown-off. Gold coating was applied to make sample electrically conductive and enhance emission of secondary electrons. Samples were tilted at 45° on the SEM. (5 EOLCO Philips SEM model 515, UK,) and imaged with 25 KV electron beam.

The results of the present study (Table 1) revealed that all the varieties were waxy, except 'K. Chandramukhi' and 'K. Jyoti'. The variety 'K. Chandramukhi' was floury and 'K. Jyoti' was close as per the description of texture by Burton (1989). The results of flour characteristics (Table 1) revealed that flour of all the varieties was pale yellow, except that of 'K. Bahar', which was yellow in colour.

Flour of variety 'JH 222' had lowest water absorption (35.33%), whereas 'K. Chandramukhi' had the highest (46%), and the difference between the varieties was significant (P ~ 0.5). This could be attributed to varietal differences in free starch

* Corresponding Author

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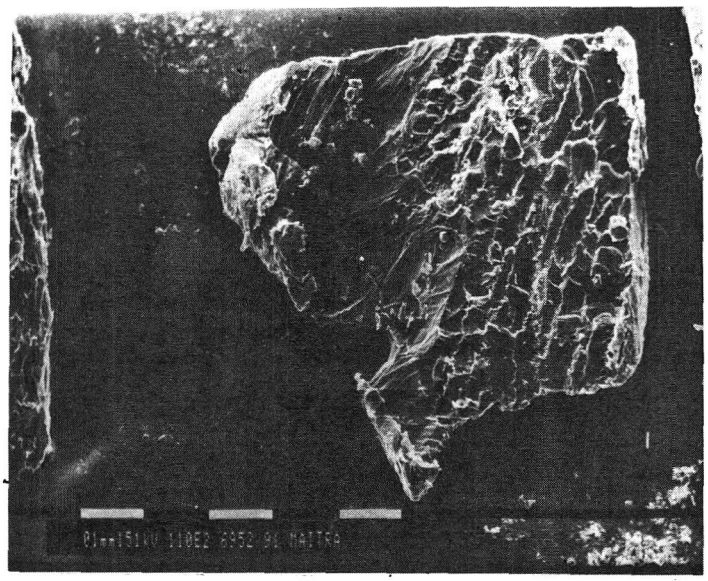

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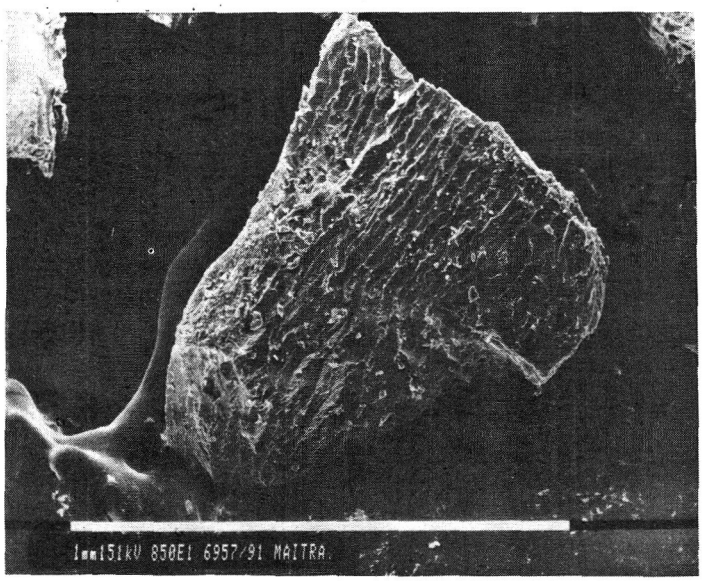


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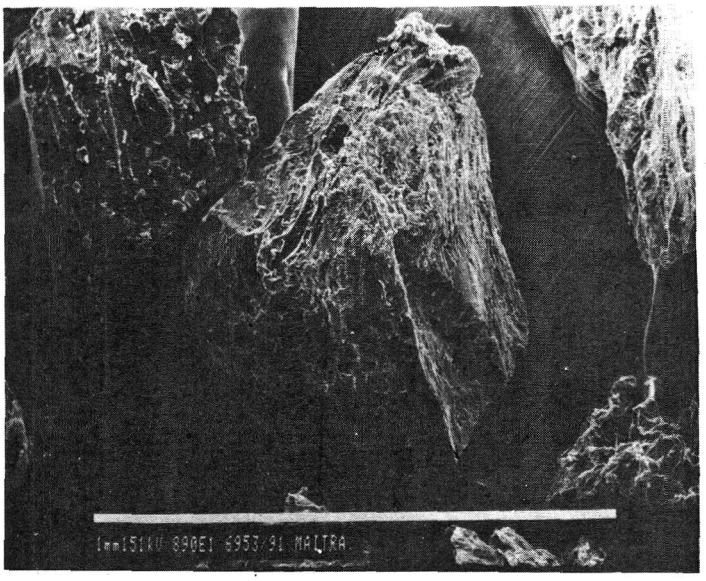


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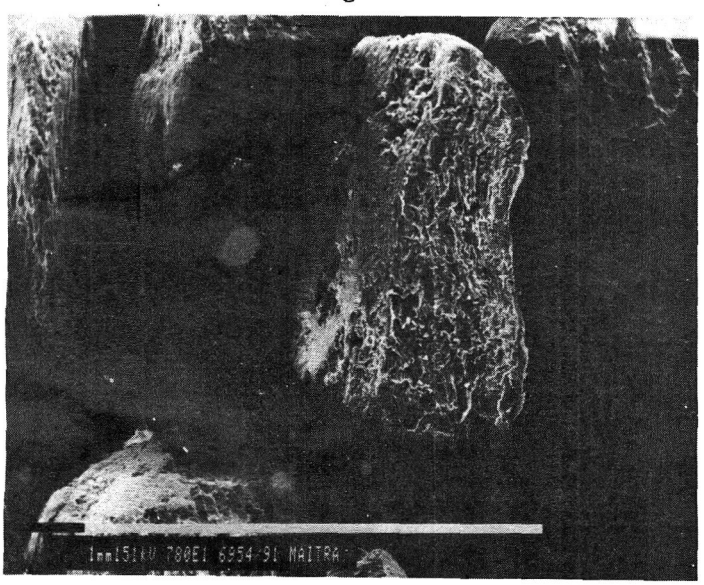


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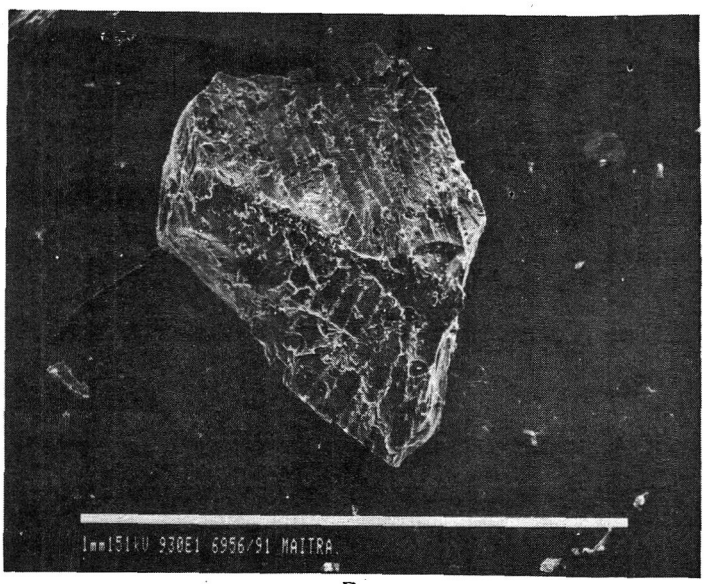


F

Fig. 1. Scanning electron microscopy of the potato varieties A: 'K. Chandramukhi', B: 'K. Badshah', C: 'K. Bahar', D: 'K. Jyoti', E: 'JH 222' and F : 'JI 4486'.

TABLE 1. PHYSICAL CHARACTERISTICS OF BOILED POTATO TUBERS AND THE POTATO FLOURS OF DIFFERENT VARIETIES

| Variety | Texture of boiled potato tuber | Water absoption, % | Colour | Shrinkage ratio, % | Particle size index |
|---|---|---|---|---|---|
| 'K. Badshah' | Waxy | 39.33 | 5y (8/4), pale yellow | 22.32 | 34.83 |
| 'K. Bahar' | Waxy | 40.33 | 2.5y (7/8), yellow | 20.62 | 35.02 |
| 'K. Jyoti' | Close | 37.67 | 5y (3/4), pale yellow | 24.65 | 32.52 |
| 'K. Chandramukhi' | Floury | 46.00 | 5y (8/4), pale yellow | 21.52 | 36.02 |
| 'JH 222" | Waxy | 35.33 | 2.5y (8/4), pale yellow | 19.15 | 34.17 |
| 'JI 4486' | Waxy | 39.00 | 5y (8/4), pale yellow | 20.26 | 32.08 |

levels and the composition of starch (Purves and Scrively 1975). Similar trend is evident from SEM of the flour (Fig. 1, A to F). The ultra-structure of the starch granule is almost similar in case of 'K. Badsha', 'K. Bahar', and 'JI 4486'. These also showed almost similar starch damage. 'K. Jyoti' and 'K. Chandramukhi' had almost similar shape of starch granule. Though more free starch was visible in 'K. Chandramukhi', 'JH 222' showed the starch granules of different shapes and no free starch may be attributed to the amylose and amylopectin ratio, which also affect water absorption (Greenwood 1976).

Particle size index, which is inversely related to fineness of flour, also affects water absorption capacity (Khan et al. 1982). The results showed that 'K. Chandramukhi' had finest flour. A significant positive correlation (r=0.894) was found between water absorption and particle size index. The shrinkage ratio, a measure of yield, ranged from 19.75 to 24.65% in different varieties, minimum, being in 'JH 222'.

It could be concluded that almost all the varieties could be processed for flour making. However, suitability of such flours from different varieties needs to be evaluated for use in different products.

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# Survey of the Quality Characteristics of *Tandoori* Dough and *Tandoori Roti*

**D.C. SAXENA AND P. HARIDAS RAO***

Milling and Baking Technology Department,
Central Food Technological Research Institute, Mysore, India.

The flour, dough and *tandoori roti* samples, collected from different sources, were assessed for various quality characteristics. In general, survey indicated the use of the mill *atta* in preparing *tandoori roti* and the use of salt in the formulation. The dough was of softer consistency due to higher amount of water (75-80%) added. The shape of the *tandoori rotis* was found to be circular, with a diam of about 18 cm and thickness of 4.5 mm. However, texture of *tandoori rotis* varied widely, as indicated by the shear value ranging from 16.9 to 52.5 N and sensory textural score from 3.3 to 6.5. The *tandoori rotis* prepared from whole wheat flour had softer texture and better flavour. The moisture content in *tandoori roti* ranged from 31.7 to 40.2 %, which is higher than that of *chapati* (25-30 %).

**Keywords** : *Tandoori roti*, Consistency, Texture, Mill *atta*, Salt.

Over 80% of the wheat produced in India is utilized for the preparation of various traditional products like *chapati, phulka, tandoori roti, nan, poori* and other products, which are mainly consumed as meal items. Whole wheat flour, obtained by grinding wheat in a plate mill, is used for the preparation of these products. Among these traditional products, *chapati, phulka* and *poori* are prepared both in households and commercial establishments. However, *tandoori roti* is mainly prepared in hotels, restaurants and road-side '*dhabas*', as it requires a typical oven - *tandoor*, which is an inground oven, built with refractory bricks around an earthen pot. The oven is heated from the bottom side by burning wood or charcoal. *Tandoori roti* is prepared by mixing whole wheat flour, water and salt into the form of a dough, flattening a piece of dough by palms into a circular shape of about 150 mm diam and thickness of about 3 mm, pasting it on to the heated wall of the oven and baking for 1 to 2 min, before taking out with the help of two rods.

Scientific information, like suitability of wheat variety, quality of wheat, effect of grinding method, rheological characteristics of dough on the quality of the product, is available for the traditional products like *chapati, nan* and *phulka*. But, no information is available on *tandoori roti*. Such information is required for mechanisation of the manufacture of this traditional product. In order to initiate studies on various technological aspects of the preparation of *tandoori roti*, quality assessment of dough and *tandoori roti* from different sources was carried out and the results are presented in this communication.

* *Corresponding Author*

Flour, dough and *tandoori roti* samples were collected from different restaurants in and around Mysore city. The dough used for the preparation was brought to the laboratory, after packing it in an air tight container. *Tandoori roti*, immediately after baking, was allowed to cool, before packing in polypropylene pouches. On enquiry, it was found that most of the *tandoori rotis* were made using mill *atta*. Moisture, ash, proteins and damaged starch contents of different flour samples as well as the overtailings of 10XX (130 μ) were estimated by standard AACC (1983) procedures. The dough characteristics were measured after a time lapse of exactly 1 h from mixing stage. The farinograph characteristics of the dough were measured, as per the standard procedure (AACC 1983). The consistency of the dough was measured as the time required (sec) to extrude the dough by one cm in a Research Water Absorption Meter (RWAM) (Henry Simon Ltd., Cheadle Heath Stockport, U.K.). The compliance and elastic recovery of the dough (2.2 cm diam x 1 cm height) were measured in a penetrometer (FPN 3, Trumpet Manufacturers (India) Pvt. Ltd., Bombay, India) using a flat plunger (4.5 cm diam, having a dead weight of 32 g), as per the method described by Sai Manohar and Haridas Rao (1992). The height of the dough before and after compression for 10 sec was noted. Then the dough was allowed to recover for 60 sec and the height of the recovered dough was again noted. From these values, percentage compliance and recovery were calculated.

*Tandoori roti* was evaluated for various physical parameters like thickness, weight and length of major and minor axes. The force required to shear a piece ($3 \times 8$ cm$^2$) of *tandoori roti* was measured

TABLE 1. QUALITY ATTRIBUTES OF *TANDOORI ROTI*

| Characteristics | Desirable | Undesirable |
|---|---|---|
| Colour | wheatish yellow | burnt, dark brown |
| Appearance | uniformly distributed small blisters | large blisters |
| Handfeel | soft and pliable | brittle, hard |
| Texture | soft and slightly chewy | leathery and highly chewy |
| Mouthfeel | clean | doughy, sticky |
| Taste and aroma | typical, pleasant, wheaty aroma | residual taste, charred |

Rating : Excellent-7, Very good -6, Good-5, Satisfactory-4, Fair-3, Poor-2, Very poor -1.

using a texturometer (Universal Testing Machine, model 4301, Instron Corporation, Canton, Massachusetts, UK) at 100 mm/min plunger speed, V-type cutter and 500 kg load cell. The moisture and fat in samples of *tandoori roti* were determined as per the standard AACC (1983) procedures and the range and average values are reported. The *tandoori roti* samples were also evaluated for sensory attributes like colour, appearance, handfeel, texture, mouthfeel, taste and aroma by a panel of 12 members familiar with the product, following a seven point scale system as per the proforma (Table 1). The desirable and undesirable attributes of *tandoori roti* were arrived at, after discussion with panel members, who were familiar with the product.

Some physico-chemical characteristics of flour samples collected from different sources are given in Table 2. Out of the 19 samples collected, 15 samples were of mill *atta*, while the remaining were whole wheat flour. This indicated that many of the restaurants are using mill *atta* for the preparation of *tandoori roti*. Even in mill *atta* or whole wheat flour, there is a considerable variation in the quality characteristics due to the method of processing and type of wheat used. In general, mill *atta* samples contained high moisture and low damaged starch content, as compared to whole wheat flour ground

TABLE 3. VARIATION IN THE QUALITY CHARACTERISTICS OF *TANDOORI* DOUGH AND *ROTI*

| Characteristics | Range* | Average* |
|---|---|---|
| **Dough** | | |
| Consistency, sec | 35.0 - 56.0 | 42.8 ± 6.60 |
| Compliance, % | 35.2 - 53.4 | 45.7 ± 5.73 |
| Elastic recovery, % | 12.2 - 19.3 | 15.6 ± 2.46 |
| **Roti** | | |
| **Physical** | | |
| Thickness, mm | 3.7 - 5.2 | 4.5 ± 0.50 |
| Weight, g | 48.4 - 81.3 | 59.8 ± 11.54 |
| Major axis, cm | 16.6 - 21.0 | 18.5 - 1.38 |
| Minor axis, cm | 15.7 - 19.2 | 17.2 ± 1.43 |
| Shear value, N | 16.9 - 52.5 | 25.6 ± 10.36 |
| **Chemical** | | |
| Moisture, % | 31.7 - 40.2 | 35.6 ± 2.77 |
| Fat, % | 1.3 - 1.8 | 1.6 ± 0.14 |
| **Sensory** ** | | |
| Colour | 5.3 - 6.6 | 5.6 ± 0.52 |
| Appearance | 3.4 - 6.1 | 4.8 ± 0.92 |
| Handfeel | 4.2 - 6.3 | 5.1 ± 0.82 |
| Texture | 3.3 - 6.5 | 4.9 ± 0.93 |
| Mouthfeel | 3.4 - 5.4 | 4.3 ± 0.59 |
| Taste and aroma | 4.2 - 6.3 | 4.7 ± 0.67 |

* of 19 samples

** Rating : Excellent -7, Very good-6, Good-5, Satisfactory-4, Fair-3, Poor-2, Very poor-1.

in *chakki*. Similar observation was reported earlier (Haridas Rao 1982). The dough and *tandoori roti* characteristics are given in Table 3. The dough characteristics as assessed by handfeel, indicated that the consistency was much softer than that of the *chapati* dough. This was confirmed by extrusion studies, as the extrusion time was very low (about 10 sec) for all doughs obtained from different sources, as compared to 50 to 80 sec for *chapati* dough (Haridas Rao 1982). The very low extrusion time posed problems in accurate measurement and hence, the dead weight of 2.0 kg, provided along with the instrument, was changed to 1.5 kg to bring the values within measurable range (40-80 sec). The colour of the dough was wheatish, indicating that the dough was either prepared from mill *atta* or by blending whole wheat flour with refined wheat flour (*maida*). It was confirmed that 15 out of 19

TABLE 2. PHYSICAL AND CHEMICAL CHARACTERISTICS OF WHOLE WHEAT FLOUR AND MILL *ATTA*

| Characteristics | Mill *atta** | | Whole wheat flour** | |
|---|---|---|---|---|
| | Range | Average± deviation | Range | Average± deviation |
| Overtailings of 10XX,% | 58.2 - 78.4 | 68.3 ± 10.1 | 21.8 - 31.3 | 26.5 ± 3.3 |
| Moisture, % | 11.0 - 13.4 | 12.2 ± 1.2 | 6.0 - 8.4 | 7.5 ± 0.97 |
| Ash, % | 0.78 - 1.39 | 1.09 ± 0.31 | 1.5 - 1.73 | 1.61 ± 0.09 |
| Proteins (N x 5.7), % | 8.8 - 10.4 | 9.34 ± 0.57 | 9.46 - 10.45 | 9.93 ± 0.41 |
| Damaged starch, % | 5.9 - 10.2 | 7.1 ± 1.6 | 13.3 - 16.1 | 14.4 ± 1.1 |

* Range and average of 15 samples

** Range and average of 4 samples

TABLE 4. EFFECT OF SALT ON THE RHEOLOGICAL CHARACTERISTICS OF *TANDOORI* DOUGH

| Salt,% | Farinograph characteristics | | | | R W A M |
|---|---|---|---|---|---|
| | Peak, BU | Dough development time, min | Stability, min, | Mixing tolerance index, BU | Extrusion time, sec |
| 0 | 420 | 2.0 | 1.0 | 80 | 42 |
| 1.0 | 380 | 2.5 | 2.0 | 60 | 61 |
| 1.5 | 370 | 3.0 | 2.5 | 60 | 69 |
| 2.0 | 360 | 4.0 | 3.5 | 50 | 77 |

samples collected were made using mill *atta*. The compliance of different doughs showed wide variations (35.2 to 53.4 %), while elastic recovery ranged from 12.2 to 19.3 %, the latter indicating that the extent of gluten development was low in *tandoori* dough.

Most of the *tandoori roti* samples had desirable wheatish yellow colour, with uniformly distributed light brown spots. The *tandoori rotis* were soft and pliable. The sensory texture of *tandoori roti* varied considerably, as indicated by the wide range of the textural score. *Tandoori rotis* made from whole wheat flour scored higher for texture. This is in agreement with the shear values measured using Instron texturometer. In general, *atta* having higher damaged starch content yielded product with soft texture, which was also reflected by the lower shear value. All the *tandoori roti* samples had salty taste, indicating the presence of salt in the formulation.

The shape of the *tandoori roti* was found to be almost circular, as indicated by an equal length of major and minor axes. The thickness of *tandoori roti* ranged from 3.7 to 5.2 mm with an average of 4.5 mm, as against 2.0 to 2.5 mm of *chapati* (Haridas Rao 1982). The weight of *tandoori rotis* ranged from 48.4 to 81.3 g, which is more than double the weight of *chapati*. The shear value of *chapati* has been correlated to the sensory texture (Haridas Rao 1982) higher shear values leading to harder texture of the products. The shear value measured for *tandoori rotis* (16.9 to 52.5 N) indicated a large variation in the texture of *tandoor roti* made in different hotels and restaurants. In general, the *tandoor rotis* made from mill *atta* had higher shear values (30.1 to 52.5 N) than those made from whole wheat flour (16.9 to 28.7 N). Moisture content (31.7 to 40.2 %) in the *tandoori roti* was higher as compared to 25 to 30% observed in the *chapati* (Haridas Rao 1982). The moisture content in samples made from whole wheat flour was higher (35.1 to 40.2%), evidently because of its high damaged starch content and the consequent high water absorption capacity. The fat content of 1.3 to 1.8% revealed that fat was not used in the preparation of *tandoori roti* dough.

Table 4 shows the effect of salt on the rheological characteristics of *tandoori* dough. Incorporation of salt at increasing levels, gradually reduced the peak height of the farinogram and increased the dough development time of the dough. The stability increased from 1 to 3.5 min, when salt concentration was 2% in the dough. Similar increase in stability, on incorporation of salt was reported earlier (Srivastava et al. 1994). The extrusion time, which reflected the consistency of dough, increased with the increase in level of salt, probably due to increase in the number of ionic bonds in proteins (Pomeranz 1988).

The study indicated that the consistency of *tandoori* dough is much softer than that of *chapati* dough, and the formulation invariably contained salt. The *tandoori roti* has on an average 20 cm diam and 4.5 mm thickness, with wide variation in the texture of samples collected from different sources. In general, *tandoori rotis* made from whole wheat flour had better texture and flavour as compared to those made from mill *atta*.

The first author acknowledges the senior research fellowship, provided by Council of Scientific and Industrial Research, New Delhi.

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***Seafoods*: Chemistry, Processing Technology and Quality. Edited by F. Shahidi and J.R. Botta Published by Blackie Academic & Professional, an imprint of Chapman & Hall, Wester Cleddens Road, Bishopbriggs, Glasgow G64 2NZ, UK. 1994, pp 342, Price £ 79/-**

Seafoods such as fish, crustaceans and molluscs form excellent sources of proteins and lipids rich in polyunsaturated fatty acids. Unlike other animal foods, seafoods are prone to rapid deterioration, unless adequate steps are taken to process and preserve them. A knowledge of physical and chemical changes, which occur in seafoods after harvest and during processing and storage is of vital importance in ensuring the quality of seafoods. This book provides an extensive and valuable information on the nuances of chemical and sensory qualities of seafoods. However, one would rather get disappointed, while looking for the principles and techniques of conventional methods of processing such as freezing, canning, smoking, salting and drying.

Following a very brief overview in chapter 1, the chemistry of seafood components such as proteins, lipids, flavour and taste-active components is dealt in chapters 2 to 8. The chemical nature, composition and importance of various protein and non-protein fractions in seafoods have been dealt with concisely in chapter 2. The role of proteases in contributing to the quality of seafoods, problems involved in identification of enzymes, factors affecting the amounts of enzymes, extraction and activity of proteases, viz., trypsins and chymotrypsins, cathepsins, alkaline and neutral proteases, collagenases and modori-inducing proteases are elaborated in the chapter on protein hydrolysis in seafoods.

Chapters 4 and 5 are devoted to the study of lipids. Seasonal changes in the lipid content of fish and shellfish, variation in PL and NL contents in lean and fatty fish and the effect of processing on the FA composition are elucidated with the help of figures and tables (Chapter 4). Oxidation of lipids is an important cause of quality deterioration in seafoods, owing to the relatively high amounts of PUFA found in organisms from the marine environments. Cellular catalysts of lipid oxidation, the potential role of transition metals and lipoxygenases in catalyzing oxidation of fish muscle lipids, the role of inhibitors in preventing initiation and propagation reactions and some of the bottlenecks in developing strategies for lipid oxidation in seafoods are highlighted in chapter 5.

Flavour components of fish, shellfish and Kamaboko, form the main theme of chapters 6 and 7. Occurrence of off-flavour components in fresh water fish, marine fish and shellfish such as shrimp, prawn, lobster, oyster, crayfish and clam are discussed. The chemical nature of flavour compounds of fish and shellfish, their occurrence, the pathway for formation, the role of lipoxygenase and deterioration of fish flavours due to TMA production, lipid oxidation and formation of sulphur compounds are emphasized. Chapter 8 critically reviews taste-active components of specific seafoods with special reference to umami substances, contribution of low molecular weight compounds to taste and their identification.

The quality of seafoods with respect to their freshness, preservation, microbiological safety and sensory attributes are discussed in chapters 9 to 13. Location and season of harvest, method of catching and handling of seafoods have a great bearing on the quality. Useful chemical indicators of organoleptic quality such as total volatile bases, ATP breakdown products and biogenic amines and the instrumental methods of assessing appearance, colour, texture, odour and flavour are reviewed in chapter 9. A detailed account of subjective and objective methods of sensory evaluation of seafoods is given in chapter 13.

The initial quality of seafoods depends on the species, seasonal and biological changes, culture conditions, and techniques of fishing. Post-harvest handling, standard of hygiene during landing, storage, processing and preservative treatments are known to alter the quality attributes. The changes in quality of both fresh and processed fish are authoritatively discussed in the chapter on preservation of seafood quality.

Microbiological aspects of seafoods and marine toxins are discussed in the next two chapters. Microbiological quality of seafoods is an important factor in determining their edibility and safety for human consumption. Contamination of seafoods by virus, bacteria and parasites, and the application of molecular biological technique such as gene probes and PCR for the detection of microbial contaminants are outlined in chapter 11. The organisms enumerating toxins, the nature and chemical structure of toxins causing PSP, ASP, DSP, scombroid poisoning and ciguatera toxins find

a place in chapter 12.

The last three chapters deal with further processing of raw material and highlight processing of surimi from lean and fatty fish, and preparation of by-products and value-added products from processing discards. Factors affecting the quality, yield, and frozen storage stability of surimi are discussed. Some functional properties are highlighted. However, information on the role of cryoprotectants in improving functional properties of surimi is lacking.

Some aspects of the subject matter such as flavour components, lipoxygenase and water washing of surimi have been repeatedly dealt with and needed editorial attention. In an attempt to be both concise and all encompassing, the editors leave the reader looking for more information, though this has not affected the overall clarity of presentation. Extensive and up-to-date bibliography is provided at the end of each chapter. This book should serve as a valuable reference to scientists, technologists and researchers working in the area of chemical, microbiological and sensory qualities of seafoods and post-graduate students in Fish Processing Technology.

L.N. SRIKAR
COLLEGE OF FISHERIES,
MANGALORE-575 002

***"Greek Vegetarian Cooking"* published by Wiley Eastern Ltd. 4835/24, Ansari, Road, Daryaganj, New Delhi-110002 © Sawan Kirpal Publications, Delhi, 1993. by Evy Caradja, Victoria Caradja, Maria Symeonidou. pp 123, Price: Rs. 350/-**

The goodness of vegetarian diet has long been proven in many Indian vegetarian dietaries. The present generation also finds the welcome change of non-vegetarians to vegetarians even in Western countries. Added to this, the palate of younger generation favours variety and introduction of new types of foods and dishes in everyday meal. Hence this book is welcome on the shelf of a housewife or for that matter anybody interested in trying out their culinary talents.

The book starts with a brief introduction of traditional Greek vegetarian dishes by the author, which is very much in place and goes on to describe many other benefits of vegetarianism apart from health. The recipes are divided into various sections like pasta and grains, vegetables, pulses, pies and pizzas, salads, sauces and dressings, soya soups, deserts and interspersed with colourful photographs. The recipes are written well with clear and concise instructions making it easier to follow. Printing is of very good quality. Proof reading is done excellently with no mistakes.

Since these dishes are traditional Greek dishes and to start with, an Indian may not be familiar with either the look or taste of it. I do envisage some problems, while using the book. For example, there are many unfamiliar foods, which are mentioned in various recipes, which may not be available everywhere, for e.g., vegetables like stalk rocket, zucchini, farfalle, scallions, leeks etc., varieties of cheeses like gruyere, roquefort, fete, kefalotiri, parmesan etc., ingredients available in Greek speciality stores like trahana or mustus syrup and Greek phyllo pastry sheets. Certain important details regarding amounts of ingredients are left out in certain recipes. Recipe on page 2, Spaghetti with roquefort cheese mentions using 1 container fresh cream. The size of container is not given. Fassaloda (Bean soup) on page 34 does not mention the amount of olive oil used. For preparation of Piroski (cones) on page 38, the amount of wheat flour is not given. These details are required for a beginner. Most of the recipes mention salt and pepper to taste. May be a mention of approximate amounts in teaspoon could have been better, since the recipes are usually for 4-6 servings. Many specific details are missing in some recipes. For some of the baked dishes, baking time and temperature are not given (page 18). Recipe on page 2, Sphaghatti with garlic does not mention boiling sphaghatti in water, which is an essential step.

Dishes, in general, use a very high amount of oil/butter/cheese, which might undo the good vegetarianism its doing. The amounts added make them very high calorie foods. Personally, I do not recommend using such high amounts of oil/butter/cheese in everyday meal. Selection of foods from different sections like pasta, vegetables, pulses, sauces, dressings etc., still will turn out a very rich meal. Also, since olive oil used for edible purposes is not available locally, we may have to substitute that with other refined oils. The effort of making reader familiar with nutrients in the last section of the book is appreciated, but it is very brief and partial. This section could have been made more comprehensive by adding compositional details of vegetarian foods. Information on herbs, preparation of sprouts and good eating habits make interesting reading.

Although the cost of the book is prohibitive, it is recommended for anybody who is interested

in different types of recipes. Most of the recipes can be tried out with minor substitutions in terms of available ingredients.

JAMUNA PRAKASH
MANASAGANGOTHRI, UNIVERSITY OF MYSORE
MYSORE-570 006.

***Nutrition in the 90's* Current Controversies and Analysis Volume-2, Edited by Frank N. Kotsonis and Maureen A. Mackey; Published by Marcel Dekker Inc 270, Madison Avenue, New York N.Y. 10016, USA; 1994, pp: 192, Price US $35/-**

It is a review on several controversial issues of nutrition presented in ten chapters. The topics covered are highly selective and focusses on newer concepts of diet, nutrition and health and physical exercise. The authors of all the ten chapters have done a commendable job of reviewing the research and presenting in a comprehensive and easily understandable manner. Most chapters are closely related and the subject matter dealt with, is useful for practising nutritionists to update their knowledge.

The first chapter on dietary fat, fibre and cancer is presented with epidemiological evidence about the association between cancer, dietary fat and fibre. The association between quality of fat and cancer is interesting and new. Second chapter describes the beneficial effects of complex carbohydrates and this is of current interest to all. Newer concepts such as influence of processing on reduction in digestibility of food products owing to complexes formed in processing can be advantageous in the treatment of metabolic disorders such as diabetes.

The third chapter describes altogether a different approach for treatment of cardiovascular diseases. Genetic basis for susceptibility of diseases is described. The new theory of genetic predispositions to cardio-vascular diseases and lipid metabolism is explained with experimental evidence generated through animal studies. Genetic predisposition to diseases is traced to major genes as well as polygenes. Metabolic relationship between genetic and environmental factors is interesting.

The evidence that body weight can be reduced by modestly reducing the fat content of the diet without adapting high bulk low calorie diets is useful for many obese people, who find it hard to switch over to a different diet.

There are three chapters on association between physical activity, fitness and health in adults as well as aged. The importance of regular physical exercise in maintaining the functional health status and body weight is explained with adequate data generated from several studies carried out in USA. Special attention is given to osteoporosis problem in women and the importance of nutrition and exercise in the prevention of disease. The adverse effects of smoking, alcohol and role of calcium and vitamin D, in minimising the risk of osteoporosis is comprehended well.

A chapter on "Food Health Research" traces the ethinicity of food habits and health problems prevalent in population groups. It also gives an account of the value of non-nutritional compounds such as phyto-estrogens, flavonoids, lignans and coumestans to health. Some account of cross-cultural studies relating to health opens up possibilities to find the origin of health problems to the varieties and types of foods consumed by different ethnic groups. Food acculturation due to movement of people is indicated to affect health.

A chapter on transgene technology gives an account of animal studies on transgenic animals developed to produce more milk and better meat. Recent advances in gene transfer techniques in animals and its implications in finding solutions to genetically based human diseases are highlighted. This is a new area and helps in understanding human diseases involving metabolic disorders.

The last chapter is on regulations for nutrition labelling, including health messages. It covers various aspects of health claims used in commercial food products and emphasizes the need for agreement among qualified experts on the health claims and safety of foods. How the parameters approved by FDA for new food/products present challenges to manufacturers has been elaborated.

P. GEERVANI
SRI PADMAVATI MAHILA VISVAVIDYALAYAM
TIRUPATI - 517502

# ANNOUNCEMENT

# ICFoST - 95

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

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

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

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

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

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

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

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

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

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

**Published papers/notes/reviews**

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

**Books/approved methods**

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