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CONTENTS

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Methods for Preprocessing and Freezing of Shrimps : A Critical Evaluation

M. CHANDRASEKARAN

Centre for Biotechnology,
Cochin University of Science and Technology, Cochin - 682 022, India.

Shrimps, widely accepted as safe health food, rank first in the international seafood trade. Increasing demand for shrimps has led to shrimp farming for enhancing production throughout the world. Shrimps, unlike other foods, undergo rapid spoilage and thereby lead to wastage of raw material, unless processed adequately. Freezing, the method largely used to preserve shrimps, usually involves a long time gap between capture and processing. This necessitates adequate and efficient preprocessing measures to preserve freshness till processing. In the present review, the various methods of preprocessing viz., cleaning, beheading, peeling, cooking, icing, dip treatment in preservative solutions as well as additives, dressing of shrimps are presented and evaluated for their merits and demerits. The methods of freezing such as conventional contact freezing, quick freezing, slow freezing, brine-immersion freezing, cryogenic freezing and individual quick freezing are also dealt with critically.

Keywords : Shrimps, Preprocessing, Beheading, Peeling, Icing, Preservative, Freezing, Individual quick freezing, Methodology evaluation.

Among the seafoods, which are considered as the safest, in the context of increasing health consciousness in the modern world, shrimps rank first in the fishery trade. Shrimps, which are mainly high quality proteins with individual amino acids in amounts and proportions as required for human nutrition, constitute 20% of the total sales (Chandrasekaran 1993). The growing demands for shrimps have resulted in their production through shrimp farming in almost in all countries especially in Asia, by employing traditional, extensive, semi-intensive and intensive methods of shrimp culture. Although about 100 species of shrimps are reported from aquatic environments, only a few are commercially important (Table 1).

TABLE 1. SHRIMPS OF COMMERCIAL IMPORTANCE

Penaeid		Non-Penaeid
Penaeus indicus	*Metapenaeus dobsoni*	*Acetes indicus*
P. monodon	*M. affinis*	*Palaemon indicus*
P. semisulcatus	*M. monoceros*	*Macrobrachium rosenbergii*
P. merquiensis	*M. ensis*	*Hippolysmata ensirostris*
P. chinensis	*M. bennettae*	
P. penicillatus	*M. brevicornis*	
P. japonicus	*M. joyneri*	
P. latisulcatus	*M. stebbingi*	
P. marginatus	*M. macleayi*	
P. uncta	*Parapenaeopsis stylifera*	
P. stylirostris	*P. hardwickii*	
P. vannames	*P. sculptilis*	
P. azeticus	*Solonocera indica*	

Generally, shrimp fishery is a day fishery and shrimps are caught all along the coastline and the magnitude of catches varies from region to region (Kurian and Sebastian 1976). Catches made by both country boats and trawlers from different locations are transported to processing centers for appropriate preservation and marketing (Govindan 1985).

Shrimps, being proteinaceous in nature, undergo rapid spoilage (Velankar and Govindan 1959) and hence require immediate and adequate preventive measures to avoid wastage of raw materials due to spoilage (Pillai et al. 1985). They are preserved for consumption by adopting any one of the following methods viz., sun-drying, pickling, smoking, canning and freezing (Chandrasekaran 1985). Among these, freezing is extensively used as the method of preservation (Govindan 1985) and frozen shrimps dominate the seafood export in several developing countries, including India (Chandrasekaran 1993).

Since shrimps occupy an envious position as "Breathing Dollar" among the food items in the economic front, besides being considered as health food, an attempt is made in this review to present the various methods employed for preprocessing to safeguard freshness of the shrimps, in addition to the freezing techniques adopted for efficient preservation for ensuring quality in the final product.

What are shrimps?

The term "prawn" used in India is identical with the term "shrimp" used in Western countries (Kurian and Sebastian 1976). The standardization

of terms was the subject of discussion at the "Prawn Symposium of the Indo-Pacific Fisheries Council" held at Tokyo in 1995. The decision arrived at was that the word "Prawn" should be applied to the *Penaeids*, *Pandaeids* and *Palaemonids* and "Shrimps" to the smaller species belonging to other families (Kurian and Sebastian 1976). In the present paper, the term "Shrimps" is applied to indicate all the prawns and shrimps, as the preprocessing and freezing methods for them are same.

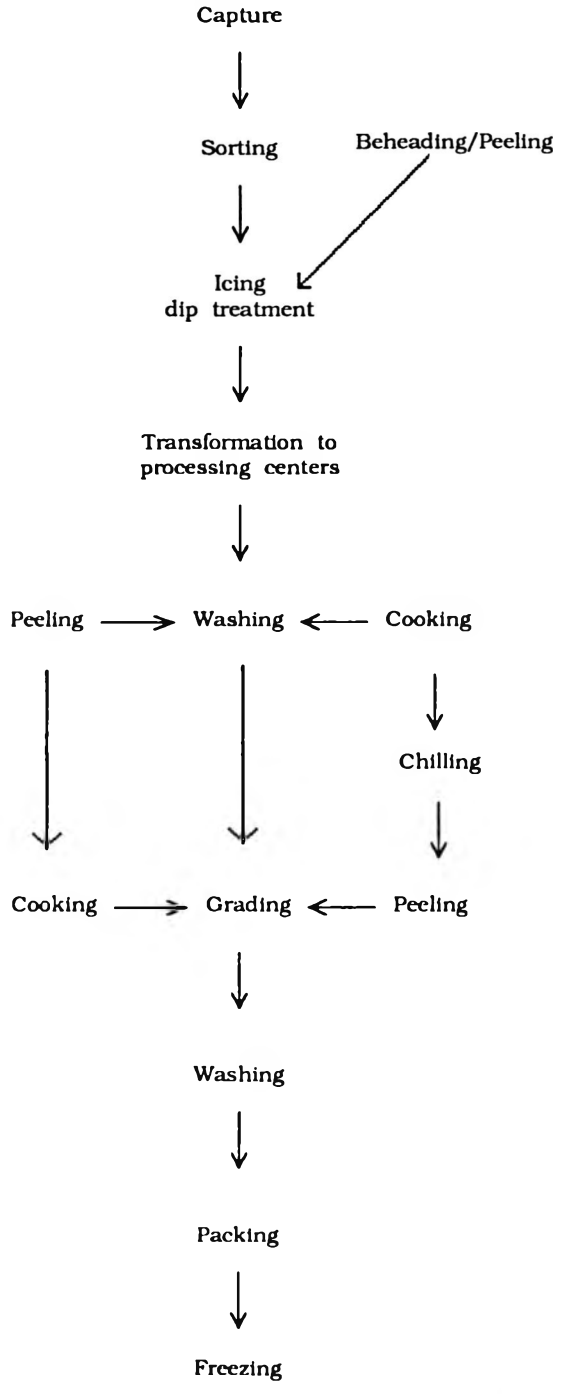

Fig. 1. Various stages of preprocessing shrimps before freezing.

Preprocessing of shrimps

Need for preprocessing : Shrimps, unlike other crustaceans and fin fishes, die very soon on capture (Velankar and Govindan 1959), thereby leading to early deterioration, caused by the commensal microflora, and/or the contaminating microflora from the deck, slime or oxidized intestinal contents of fish caught along with the shrimp (Fieger and Novak 1961). According to Thatcher and Clark (1968), fish is considered to have a short potential shelf-life or may even to be at incipient spoilage, when it harbours more than x 10^6 microorganisms per g or cm^2. Chandrasekaran (1985) observed that fresh *Penaeus indicus* carried a commensal bacterial load of x 10^7/g and spoiled rapidly within 4-8 h at ambient temperature (28-30°C), besides recording high deterioration in the proximate components, when not iced or preprocessed.

Bacterial spoilage and melanosis (blackening) are the two major causes for the enormous waste of shrimps at the site of collection and before they are received at the freezing plants (Velankar et al. 1961). Further, the cephalothorax of shrimps, containing the digestive system, undergoes immediate spoilage and results in loosening as well as blackening of heads. These undesirable factors which lead to raw material wastage, enunciate the need for adopting effective and proper preprocessing measures for the maintenance of freshness in the captured shrimps in terms of organoleptic and biochemical quality, until efficient preservation is effected.

The various steps followed from the time of capture to freezing during preprocessing are presented in Fig. 1.

Sorting : A shrimp trawl contains an assortment of shrimp, fish, crabs and often mud or debris. Some shrimps are killed immediately after entering the trawl and get badly damaged, if large quantities of fish are also caught. Before preprocessing, the shrimps, which are fresh, are separated from the trash and cleaned with a jet of sea water to remove dirt, mud and debris adhering to their body (Cobb et al. 1977).

Peeling of prawns : Beheading, peeling and deveining are primary processes in the preprocessing stage. These processes are carried out in interior villages near the landing places, if freezing factories are located at distant places, or in the freezing factories, if these are located nearby (Pillai et al. 1961). These operations are carried out rapidly, since the yield, quality, wholesomeness and

shelf-life of the product are affected in the absence of these processing steps.

In hand peeling, shrimps are beheaded and the shell (exoskeleton) on the shrimp is removed without loss or damage to the meat. A cut or incision, along the entire length of the mid-dorsal line, exposes the digestive tract, which can be pulled out by hand (Govindan 1970).

Machine peeling of shrimps, which is also practised, offers some advantages like rapid movement of product, shorter exposure to ambient temperature, and if conducted in hygienic manner, reduced level of contamination (Reddy 1975). All parts of the automatic peeling equipment are to be periodically monitored throughout the processing day, since badly adjusted peeling machines reduce yield and may cause damage to the shrimps. Shells leaving the machine are checked for incomplete removal of meat and the presence of unshelled or broken pieces of shrimps. After shelling and before packing, the peeled shrimp is examined for left-out pieces of shells, antennae, entrails, and other parts of exoskeleton, before washing thoroughly and chilling, preferably by a spray of cold potable water or weak brine solution (Chakraborty and Iyer 1971). To save time and improve the peeling operation, prototype mechanized peeling tables have been developed (Chakraborty and Iyer 1971). In this method, bacterial contamination was much less, compared to that in the conventional method of hand peeling (Chakraborty and Iyer 1971).

Yanagimoto et al (1982) have suggested an agitation method for the separation of head and viscera of shrimp. Although several peeling methods like machine peeling (Chakraborty and Iyer 1971), enzyme (Reddy 1971) and chemical peeling (Reddy 1975) have been developed, hand peeling is still considered to be the best method in India, perhaps because the yield is higher, the method is cheaper and labour is easily available (Althaf Alikhan 1986). A skilled worker can peel 2.5 to 3 kg of whole shrimp in an hour with an average yield of 46% raw meat from whole shrimp, while a single machine can perform the job of peeling as much shrimp as that by 16 workers (Dellino 1986).

According to Holmes and McCleskey (1947), peeled shrimp has two advantages over the unpeeled product. Firstly, it is more economical from the point of view of storage space, because the shells are eliminated, and secondly consumer appeal is increased as a result of its "total ready condition". Velankar and Govindan (1959) demonstrated that darkening of shrimps, which is a major commercial problem, could be minimized by beheading. Fieger (1950) observed reduction of bacterial counts to 75% on removal of head. According to Velankar et al (1961), peeled shrimps keep better than whole shrimps and headless shrimps. Probably, the difference in keeping quality is due to the cephalothorax and the exoskeleton, which are the sources of surface and gut spoilage, since the peeled shrimps keep better than the headless-shell on shrimps (Velankar et al. 1961).

Many of the peeling sheds, which are scattered along the coastal area, are sub-standard in design as well as construction and lack primary facilities like potable water supply as well as hygienic conditions (Govindan 1972). In another process called "Hut peeling", the prawns are peeled at home by the peelers and the raw materials suffer much contamination of the meat with sand and microorganisms (Govindan 1966). In this type of hand peeling, there is every possibility for the transfer of contaminating bacteria of public health significance and other bacteria from the personnel to shrimps, if strict hygienic measures are not practised. It is also reported that the leaching of total volatile nitrogen, non-protein nitrogen and α-amino nitrogen constituents are likely to be more in case of peeled and deveined material because of the fact that larger flesh surface is in contact with the ice and water (Pillai et al. 1961).

Dressing of prawns

The raw materials are usually prepared in any one of the following styles for freezing (Govindan 1970).

Whole-head on : Shrimps, as a whole, are dressed without removing the head (cephalothorax or exoskeleton-shell). This style is in demand in some overseas markets and obviously, this type is meant for reprocessing into speciality products (Chandrasekaran 1985).

Headless-shell-on/raw headless (HL) : The heads of the prawns alone are removed leaving the entire shells and tails intact. The intestine is pulled out as far as possible from the severed head portion. Only larger size grades of fresh shrimps are generally utilized in this method. This type fetches the maximum price, as retention of flavour and nutrients is maximum due to the protection offered by the shell (Govindan 1985).

Peeled and undeveined (PUD) : Head, shell and tail are removed, but the intestine is retained. Small

size shrimps alone are used for this type (Unnithan et al 1975).

Peeled and deveined (PD) : Head, shell, tail and the vein (intestine) passing through the dorsal side of the abdomen are completely removed and the edible meat alone is used. This method is used, when the shrimps are slightly stale, is indicated by the fading of the fresh colour of the shell, black spot formation, etc., and when the size is smaller (Unnithan et al. 1975; Govindan 1985).

Fan-tail/butterfly : This is a special type where the head, shell and vein are removed, retaining the last segment and tail. In some cases, the meat portion is dorsally split and spread out before freezing. Medium sized shrimps are usually used for this type of dressing (Govindan 1985).

Cooked, peeled and deveined (CPD) : After blanching, as described above, the individual prawns are peeled and deveined, and only the edible meat is used. Only the small size grades are dressed by this method (Govindan and Perigreen 1972).

Peeled, deveined and cooked (PDC) : Shrimps are peeled, deveined and blanched as described above (Govindan and Perigreen 1972).

Cleaning : The shrimps, prepared in various styles as described above, are repeatedly washed with potable water containing 1 ppm of available chlorine and less than 100 bacteria/ml. The slime and extraneous matter, such as pieces of shell and veins, are got rid of scrupulously, before draining the water (Govindan 1985).

Size grading : The shrimps are graded into various sizes (Govindan 1985), designated by the number of individuals required to weigh 45 g either manually or with the help of a grading machine (Govindan 1985). Different size grades like 0-10, 10-15, 16-20, 21-30, 31-50, 51-70, 71-90, 91-110,110 -up and at times 130-up are employed in headless shell-on, peeled and deveined and fan-tail types. The last two or three size grades are met within P&D only. Still smaller ones are used in CP, PUD and CPD types, grades being designated as 200-300, 300-500 etc., (Govindan 1985).

Refrigeration

In tropical countries like India, where the ambient temperature in many months is conducive to quick spoilage, the best approach to retard it is to reduce the environmental temperature.

Icing : The simplest of all the refrigeration techniques is to mix the shrimp with ice (Velankar and Govindan 1959). Ice lowers the temperatures of the shrimps and does not allow most of the mesophillic bacteria to grow, while retarding the proliferation of the psychrophiles and controlling the enzymatic and oxidative changes to a large extent (Govindan 1962).

In the matter of icing of shrimp in India, the amount of ice, required to bring down the temperature of a particular quantity of shrimp from an ambient temperature of 30°C to about 0°C, is 40% of the weight of the fish/shrimp, assuming that there are inside losses of the cooling capacity (Govindan 1985). But, in actual practice, considerable quantities of ice are required to cool down the temperature of the container, and to compensate for the heat absorbed from the surrounding atmosphere. Hence, the proportion of ice required to bring down the temperature of the shrimp to near about 0°C itself works out to 60% of the weight of the shrimps, and further quantities are required to maintain this temperature depending upon the storage period, insulation characteristics of the container, ambient temperature, etc., (Govindan 1985).

Method of icing determines the efficiency of cooling (Pillai et al. 1961). A close contact of ice with prawns imparts cooling of the latter. The best way to achieve this, is to pack the shrimps and ice in alternate layers in the container, with the bottom and top ice layers. The total height of the shrimp-ice mixture, so stored, should not exceed one meter, as otherwise, the shrimps in the lower layers will get crushed due to the weight. Crushed ice and thin layers of shrimps would effect intimate contact and rapid cooling (Govindan 1966). From the point of view of efficiency of cooling, flake ice is preferred to crushed block ice, as it makes better contact with the shrimps without bruising their bodies by the sharp corners of the broken lumps of the latter, melts more quickly, and is also economical (Waterman 1966).

According to Velankar and Govindan (1959), extensive use of ice for preserving prawns is necessary not only for providing good quality raw material for the processing plants engaged in the export trade, but also for increasing consumption in the domestic market. Pillai et al (1961) have indicated that icing of the prawns at the peeling site helps in the transport from the peeling centers to the factories, which sometimes exceeds 24 h. In their opinion, the success of icing largely depends on the amount of ice used and how it is applied.

The comparatively long shelf-life of iced shrimp

from tropical waters has been reported by Carroll et al (1968). Cann (1977), in his review on tropical shrimp, indicated that penaeid shrimp from the Gulf of Thailand remained in acceptable condition for 12-16 days in ice, whereas non-tropical shrimps such as *Pandalus* sp and *Nephrops* sp were totally spoiled after 8-10 days. The difference is again attributed to the bacterial flora on the shrimp, which is not active at 0°C, in the case of tropical catches, and little spoilage occurs, until a psychrophilic flora develops.

According to Cobb et al (1973), the activities of tissue enzymes and microorganisms can be kept to a minimum, if shrimps are handled on-board under sanitary conditions, and are iced promptly and properly. Melting ice possibly washes away some bacterial load on the external surfaces of iced shrimps (Green 1949; Velankar et al. 1961; Afsar 1980). Mathen and Thomas (1988) observed that whole prawns stored in ice upto 2 days gave the maximum yield without loss of nutrients, while at the same time making the peeling process easier.

Several disadvantages are also observed in the iced storage of shrimps. Velankar and Govindan (1959) have noted changes in the flavour and palatability of prawns during ice storage. They have suggested that deteriorative changes occurring in ice storage are related to the duration of storage, and are also influenced by the treatment received by the shrimps before icing, and the environment from which they are caught. Leaching of water-soluble proteins and other nitrogenous extractives, especially amino acids, by melting ice (Govindan 1962; Susamma et al. 1962), reductions in non-protein nitrogen and protein nitrogen (Wilaichon et al. 1977) are also reported. The rate of change of nitrogen loss in shrimps stored in ice increases in the order of whole, headless shell-on, and P and D shrimps (Anon 1966). Leaching losses are minimum, when the shrimps are stored in round condition, more in headless and most in peeled and deveined states (Velankar and Govindan 1959; Pillai et al. 1965). Leaching also increases with decreasing size of the shrimps in all cases, indicating that the surface area of the exposed flesh plays a very important role in the phenomenon (Govindan 1985). The effect of leaching is more pronounced in shrimps due to their small size and comparatively higher contents of water-extractable constituents (Velankar et al. 1961). The amounts of materials leached out depend upon the quantity of water formed in the melting of ice, duration of holding in ice, total surface area exposed to ice, etc., (Govindan 1985).

Ice has been proved to be a major source of contamination, depending on the nature of water used for its manufacture (Govindan 1966), and it is reported to contribute to the increase in the bacterial load as well as faecal organisms (Iyer and Chaudhury 1966). Prawns subjected to delayed icing showed an increase in total bacterial count, till it reached the processing hall, followed by reductions after peeling and freezing (Althaf Alikhan 1986). Bacterial contamination of ice also occurs due to the saw dust, gunny bags, etc., which are used to cover it during transportation (Govindan 1966). Other sources include the surfaces with which it comes into contact, like factory floors, metal tubes etc., (Gopalakrishnan and Chaudhury 1965). Hence, ice has to be manufactured from potable water and should be carefully handled to prevent further contamination. Ice, left over from one fishing trip, is also not recommended for use on subsequent trips (Govindan 1966). Ice manufactured and handled without observing these precautions has been found to be highly contaminated with bacteria (Unnithan et al. 1975; Govindan 1985).

Use of chilled sea water

The principle employed here is to hold the fish or prawn in natural or artificial sea-water cooled to -1°C (Woolrich and Novak 1977). In the early stage of development of this process, cooling of the brine or sea-water was effected by adding blocks of ice (Singh 1978). Of course, this method had the disadvantage of diluting the brine, besides the problems involved in carrying large quantities of ice required for a whole fishing trip as well as additional labour and space requirements (Govindan 1985). Now, mechanical refrigeration is employed for cooling the brine, and the temperature is maintained slightly above the freezing point of the fresh shrimps, which otherwise undergoes a process of slow freezing with accompanying problems, like denaturation of the proteins, etc., (Banks et al. 1977).

Tornes (1972) found that the exclusion of air by keeping the shrimp completely submerged in 3% brine or sea water at 0°C inhibited the formation of black spots. Further, he also observed that chlorination of chilled sea water and beheading of shrimp imparted better preservative effect in spite of deterioration in texture. Barnet et al (1978) found that holding shrimp (*Pandalus* sp) in brine - CO_2

mixture inhibited bacterial and enzymatic changes, and extended shelf-life of shrimp by 3 days, compared to iced samples. Afsar (1980) observed that the bacterial counts in chilled sea water shrimp were lower than those of iced shrimp, when shrimps were stored in the ratio of 1:5 between shrimp and chilled sea water, prepared by chilling two parts of sea water with one part of ice. This may be due to the lower temperature attained in chilled sea water (Spencer and Baines 1964), and also the anaerobic environment in chilled sea water, which inhibited the growth of obligate aerobes (Shewan 1977). There was no black spot formation in both chilled sea water-stored shrimps and shrimps in 3.5% NaCl in both "headless and head-on" styles upto a period of 30 days (Afsar 1980).

Refrigerated sea water (chilled sea water) is much more efficient, compared to ice, in effecting rapid cooling. It also imparts a thorough and intimate contact of the cooling medium with the shrimps. Moreover, whatever heights the tanks may be filled, the prawns do not get pressed or crushed (Govindan 1985). There is a better control of temperature in this storage, as it is generally maintained at 1°C. This eliminates the difficult task of icing, and there is considerable saving of labour, and ice storage space on board (Govindan 1985). However, there is some disadvantage too. The system accumulates heavy bacterial load, especially if the exterior of prawn is not scrupulously cleaned before storage in the tank. So, frequent changes of sea water are needed. This technique is not commercially practised in India though widely used abroad (Govindan 1985).

Use of dry ice and liquid nitrogen

Dry ice (solid carbon dioxide) and liquid nitrogen are considered useful for preservation of free shrimps (Banks et al. 1977). Dry ice has a sublimation temperature of -78°C and liquid nitrogen has a boiling point -196°C (Banks et al. 1977; Govindan 1985). As these temperatures are extremely low, they are not brought in direct contact with the prawn, in order to avoid the blemish called "freezer burn" caused on the latter (Govindan 1985). Dry ice is generally held in perforated trays suspended from the roof of the vehicle or insulated chamber, and liquid nitrogen is allowed to fall in fine spray from above. Both gases being heavier than air at their low temperatures, flow down the load of shrimps, thereby cooling them in the process. Being of inert nature, these cooling agents also help in keeping down oxidative reactions in meats caused by atmospheric air. Since both the agents are costly, compared to other techniques, these are not used in India, though adopted by developed countries (Govindan 1985).

Use of preservatives

In spite of adequate icing of prawns and use of chilled sea water storage, there is a steady increase in the bacterial counts and consequent spoilage (Fatima Shaikmahamud and Magar 1965). This led to additional processes of either incorporation of preservatives in the ice itself during manufacture or a dip of shrimps in solutions prepared using preservatives, mainly to retard the rate of multiplication of bacteria, especially psychrophiles (Fatima Shaikmahamud and Magar 1965; Govindan 1985).

Incorporation of antibiotics

Antibiotics, such as penicillin (Fatima Shaikmahamud and Magar 1965) aureomycin (Farber 1954; Govindan 1985), terramycin and tetracycline (Afsar 1980; Govindan 1985), chlorotetracycline (Surendran and Iyer 1971) oxytetracycline (Govindan 1985) were recommended by technologists for increasing the shelf-life of shrimps during refrigerated storage and transportation either by incorporation in ice or as a dip treatment before storage in ice. The principle involved in their use is that they destroy the microorganisms that cause spoilage (Surendran and Iyer 1971; Singh 1978). Of late, the use of antibiotics is considered not only unwanted, but undesirable for several reasons (Govindan 1985). Hygienic handling, thorough cleaning of the fish with potable water chlorinated to a level of 5 ppm of available chlorine, and use of ice prepared from water of the same quality are sufficient by themselves to impart reasonable shelf-life to the shrimps. Antibiotics are costly and hence increase the cost of fish. Antibiotics are not universally accepted as food preservatives (Govindan 1985). It is feared that indiscriminate use of antibiotics and their consequent ingestion by human would lower natural resistance, and kill useful bacterial flora in the human gut (Chandrasekaran 1985). Moreover, it is difficult to disperse antibiotics uniformly in water and ice (Govindan 1985).

Use of chemicals

Several chemicals such as EDTA, benzoic acid, polyphosphates, ascorbic acid, sodium chloride, etc., are used in shrimp processing industry either

by incorporation in ice or as dip treatment of shrimps. This has been the subject of study for at least five decades, owing to the lack of efficiency of icing method in the prevention of microbial proliferation, and consequent spoilage especially by psychrophiles (Fatima Shaikmahamud and Magar 1965; Singh 1978; Afsar 1980; Govindan 1985).

Afsar (1980) conducted a detailed study on the use of paraben and EDTA-incorporated ice in extending the shelf-life of shrimps. To avoid separation of paraben during ice formation, 0.02% carboxy methyl cellulose was added to the prepared paraben solution (0.05% final concentration in potable water), and kept at -20°C for 48 h to get paraben ice. This ice when mixed with shrimps at 1:1 ratio extended the shelf-life probably due to the profound effect of paraben on bacterial growth during the log phase as reported by Shiralkar (1971). In contrast, EDTA ice, prepared using 1.0% of EDTA, showed little effect on the bacterial counts, compared to paraben ice (Afsar 1980). According to Van Cleemput et al (1980), the normal practice of increasing shelf-life of cooked and peeled shrimps is the addition of benzoic acid. Surendran et al (1985) reported that shrimps stored in ice at a ratio of 1:1, following a dip in 1% solution of sodium salt of EDTA for 10 min., enhanced the shelf-life by at least 8 days over the untreated control. EDTA, absorbed by the muscle of prawn during dip in sodium salt of EDTA solution is not completely removed during their ice storage of 25 days.

According to Fieger et al (1956), if the shrimps have to be kept in wholesome condition, the use of chemical inhibitors, such as sodium bisulphite is necessary for prevention of the melanosis. Effective control of melanosis is possible by a dip in 1.25% solution of sodium metabisulphite, for 1 min before icing, or in1% ascorbic acid; when using sodium metasulphite, it is important to note that a shorter duration of dip is ineffective, while a longer dip produces discoloration (Fieger et al. 1956). Ogawa (1987) suggested that reducing substances such as sulphide, ascorbic acid and cysteine are useful in the control of blackspot in shrimp, because of their ability to immediately reduce 0-quinone, and retard melanin formation in frozen shrimps.

Peeled and deveined prawns, frozen and glazed with a 2.5% solution of sodium alginate containing phosphates of sodium and calcium, and citric acid were found better in frozen storage with respect to bacteriological and chemical characteristics, compared with control samples glazed with water alone (Pillai et al. 1965). Polyphosphates are commonly used to increase the water-holding capacity of meat (Mathen 1972). The role of polyphosphates in altering the water-holding capacity of flesh has been attributed to various factors such as pH, ionic strength changes and specific ion induced effects (Hamn 1971). According to Mathen (1968), effectiveness of phosphate decreased in the order:sodium tripolyphosphate, sodium pyrophosphate, sodium hexametaphosphate, and sodium dihydrogen phosphate. The last two were ineffective. He observed that, in spite of reduction in thaw dip,the organoleptic quality of the thawed as well as cooked product was unsatisfactory, discoloration being the major defect. He further noted that a solution of 12% sodium tripolyphosphate and 8.6% sodium dihydrogen phosphate or 2% citric acid in water, when used for dip treatment, reduced the drip loss, improved the cooking yield and organoleptic quality, without adversely affecting the biochemical characteristics. In another method, developed for reducing cook drip loss from prawn meat, the well washed meat is drained for 3-5 min., then mixed well with a solution of 12% sodium tripolyphosphate, 4% potassium dihydrogen phosphate and 16% sodium chloride, at the rate of 90 ml solution/kg shrimp meat (Mathen 1972). The treated shrimp is cooked for 45 sec. in a boiling solution of 5% sodium chloride. The cooked meat is cooled immediately in ice-cold water, drained and then packed as usual. The method was tried commercially and found to be highly effective (Mathen 1972).

Mathen and Pillai (1970) claimed that the treatment with 12% sodium polyphosphate and neutralization with 4% potassium dihydrogen phosphate is effective in preventing drip loss in frozen prawns. The method is not only costly, but the treatment with high concentration, extraction of polyphosphate is also supposed to encourage processing of spoiled raw materials, as the thick slurry masks the original characteristics of the shrimp to a great extent. Export inspection agency of India has, therefore, discouraged the use of polyphosphates in freezing of shrimps. Hebber (1979) observed that shrimps, frozen after dip treatment with preservatives like 0.2% sodium citrate, 1% propylene glycol, a mixture of 0.35% sodium ascorbate and citric acid at 2:5:1 ratio, 2% glycol, 1% sodium bicarbonate singly and along with 7% NaCl and 0.5% SO_2 as pre-dip treatment

separately showed that sodium bicarbonate was most effective in reducing drip and weight losses. Shrimps had acceptable taste and texture even after six months of frozen storage (Hebber 1979). Moreover, pretreatment with sodium chloride increased the effectiveness of sodium bicarbonate and glycerol (Hebber 1979). Sulphur dioxide pretreatment increased the effectiveness of sodium citrate, glycerol and sodium bicarbonate (Hebber 1979).

Hansen (1969) suggested that the material to be frozen, may be pasteurized by immersing in a current of water at 80°C (176°F) for a few minutes before packaging, and chilling rapidly in cold water before freezing. This assures the yield quality of the meat. Loosening of head from tail could be controlled by a dip treatment in 0.2% solution of sodium metabisulphite (food grade quality) for 2 min., as suggested by CIFT (1984).

Freezing

Although freezing of fish is known since 1865 in USA, the method did not assume importance, until the development of the mechanical refrigeration systems, which could subject refrigerants to compression, expansion and evaporation (Enochian and Woolrich 1977). Ammonia refrigeration machines were used in USA in 1880, and freezing of fish became an important industry by the end of the 19th century (Enochian and Woolrich 1977). However, this method picked up only from the mid-1950s' in India, and is now the most widely followed single method of preservation of shrimp (Govindan 1972).

Freezers used : Various types of freezers are used for freezing. These include horizontal plate freezer, vertical contact plate freezer, tunnel/air blast freezer and shelf freezer (Banks et al. 1977). Among these, horizontal plate freezer, air blast freezer and fluidized bed freezer are used for freezing of shrimps (Banks et al. 1977; Govindan 1985). In horizontal plate freezer, which takes minimum time for freezing (about 3 h.), the plates are cooled to -40°C by the refrigerant (Freon 12). This is particularly suitable for freezing shrimps in trays in the form of rectangular blocks (Govindan 1972; 1985). The tunnel/air blast freezer consists of an insulated tunnel into which the material to be frozen is arranged on trolleys, and the air at a temperature of –35°C to -40°C is blown at speeds ranging from 5 to 7 meters/sec. (Govindan 1985). In this freezer, freezing is slow, and it takes 5 h. In the fluidized bed freezer, the material to be frozen is fed in small individual pieces on to a conveyor moving inside a closed insulated tunnel, and cooled air at high velocity is blown from below (Banks et al. 1977). The material moves in a fluidized manner almost floating in the cooled air and freezing is very rapid. This freezer is suitable for individually quick frozen (IQF) shrimps (Govindan 1985).

Conventional method : In commercial practice, shrimps are frozen in different forms : Whole, headless shell-on; peeled and deveined; cooked and peeled; peeled and cooked; peeled, deveined and cooked (Govindan 1972). The dressed shrimps are washed, drained and frozen in trays at –35°C to –40°C in contact-plate freezers/tunnel freezers. Later, it is dipped in ice water to give a thin coating of ice (glazing), wrapped in polythene paper, and packed in waxed duplex cartons (Govindan 1972). The shelf-life of block frozen glazed prawns at- 20°C will vary from 24 to 40 weeks, depending upon the species and type of pack (Hebber 1979).

During freezing, when the time taken for getting over the critical temperature range of 0 to –5°C is 30 min. or less, it is considered as quick freezing, and when the time is higher than 30 min, the process is considered as slow freezing (Banks et al. 1977). Quick freezing is preferred, as it results in very small ice crystals. and yields a product of better quality (Banks et al. 1977), whereas, in slow freezing, large crystals are formed, rupturing the cell walls and causing larger drip losses, when the frozen product is thawed (Althaf Ali Khan 1986). The seafood industry employs the contact-plate freezers and belt freezers (tunnel) for quick freezing of shrimps (Govindan 1985).

Brine freezing (immersion freezing) : It is used for freezing shrimps on board fishing vessels. In this method, there is an intimate contact between the surface of prawns and freezing medium, thereby ensuring efficient and quick heat transfer (Banks et al. 1977). The shrimps are usually beheaded to reduce the bulk, washed with sea water and frozen in small batches by immersion in agitated chilled sodium chloride brine at –15°C. Once frozen, the material is transferred to the vessel's cold storage. The excessive salt penetration occurring during brine freezing could be reduced by using a mixture of glucose syrup and salts as the freezing medium (Banks et al. 1977). The glucose-salt freezing solution will give a protective glaze to the frozen product, and the frozen material will not stick together (Banks et al. 1977).

Carton freezing : In carton freezing, which is now employed widely, the prepared material with added glaze water is arranged in duplex cartons of the required size with a polythene film lining, placed in trays and loaded into the freezer (Govindan 1970). In this case, the reglazing is not necessary (Govindan 1970). Further, this method eliminates human handling of the frozen blocks, which otherwise might lead to probable microbial contamination of the product (Govindan 1970).

Cryogenic freezing : In cryogenic freezing (Banks et al. 1977), the material is frozen with liquefied gases at extremely low temperatures such as liquid nitrogen (–196°C), liquid air (–194.2°C), and liquid carbon dioxide (–71°C). The liquid is carefully sprayed in required quantities on the material passing on a conveyor belt. Liquid nitrogen frozen prawns are reported to be biochemically and organoleptically superior to conventional plate freezer, up to 59 days at–18°C. (Chakraborty and Chaudhury 1987).

Jet freezing : A process called "jet freezing" has been developed to effect quick freezing and stop bacterial and enzymic actions so that the taste of frozen shrimps will be very close to that of natural ones (Althaf Ali Khan 1986). In this process, cryogenic nitrogen (–320°F) is circulated in a multi-zoned freezer at a speed of 7000 ft./min. The cooling vapours penetrate quickly and efficiently and freeze the product to the core in a few minutes. Large ice crystals produced by slow freezing are absent (Althaf Ali Khan 1986).

In spite of the superiority of freezing, as compared to other methods of long term preservation, it almost invariably produces some detrimental effect like drip loss, toughness, protein changes, short weight, etc., (Govindan 1972). The severity of these effects depends on the product, the nature of the raw material and freezing technique (Govindan 1972). Defects in frozen shrimps cannot be detected, until thawing has taken place, whereas unfrozen products can be assessed rapidly at any stage (Merritt 1987).

Frozen prawns lose weight due to drip loss on thawing. The weight loss in 2 weeks may vary from 7 to 12% in peeled and deveined, 5 to 7% in headless and about 7% in cooked and peeled prawns normally (Unnithan et al. 1976). Usually, the weight loss is compensated by the addition of excess quantity of the material in the range of 10-15% (Geetha 1984). Drip loss is undesirable for several reasons. Large quantities of drip loss make the appearance to prawns unattractive (Unnithan et al. 1975). Since the thaw drip is usually discarded, the large portions of soluble proteins, minerals and flavour components, dissolved in the liquid phase, are lost (Unnithan et al. 1975). The tissue of thawed prawn that has lost considerable drip, becomes dry, woody, fibrous and tough in texture (CIFT 1984).

Loosening of head in frozen whole shrimps is another common defect observed, and careful handling of whole shrimps from the stage of hauling is an essential step to reduce this defect (CIFT 1984). Shrimps subjected to slow freezing showed continuous darkening during frozen storage, but drastically discolorized on thawing (Ogawa 1987). Another defect noticed within frozen shrimps and detectable only in the frozen state, is the presence of white patches due to dehydration, referred to as "freezer burn" (Govindan 1970).

Individual quick freezing (IQF) : It is defined by the International Institute of Refrigeration as the freezing of individually separated food units of small sizes such as berries, peas, shrimp etc. IQF products represent quality, and are usually attractive to the customers (Nicholson and Johnston 1993). IQF can be achieved with air blast, contact, immersion/spray freezers, and some recent examples are combinations of these methods (Nicholson and Johnston 1993). All types of dressed shrimps used in the convention method of freezing are individually quick frozen. There is good demand for individually quick frozen prawns in international markets (Nicholson and Johnston 1993). Disadvantage of this method is that it necessitates accurate measurement of the correct freezing time for each product, since even 1 min. excess process time for a product, which freezes in 10 min. results in a considerable loss in throughput and extra energy use. Further, it is very difficult to glaze IQF shrimps, and the chances of desiccation are more, when packed without glazing. During storage and transportation too, utmost care has to be taken to maintain the proper temperature, otherwise the material will get partially thawed, and will stick together, forming a lump (Nicholson and Johnston 1993).

Factors influencing quality

Quality of final product, ready for consumption, depends largely on the nature of handling and type of processing exercised from the time of capture of shrimps to freezing and storage (Velankar and Govindan 1959). In this long process, several

factors, such as biochemical, bacteriological and environmental, directly and indirectly, influence the overall quality of shrimps in spite of the types of preprocessing and processing methods employed (Velankar and Govindan 1959; Velankar 1965). Shrimps contain greater amounts of free amino acids than fish, which facilitate rapid bacterial, growth and result in quicker spoilage (Pillai et al. 1965). The head portion which holds the digestive system, undergoes rapid autolysis in shrimps, compared to fishes. Hence, shrimps spoil within 4-6 h. immediately after catch (Velankar 1965; Chandrasekaran 1985).

Shrimps, in their natural environment, carry a commensal bacterial load, the composition of which may be governed by such factors, as their feeding and living habits, the geography of the area, the seasons, and the temperature and quality of the water in which they exist (Cobb et al. 1973; Chandrasekaran 1985). The bacterial loads on catches of shrimps, therefore, vary considerably and quality and wholesomeness of the catch will depend on the numbers and types of bacteria present and on how they are controlled during processing and marketing (Cann 1977). Among the commensal flora, species of *Vibrio* and *Pseudomonas* dominate in fresh and dressed prawns at 28±2°C and at 2-4°C, respectively (Chandrasekaran 1985).

Primary contamination of shrimps is attributed to the mud, debris, feed and nature of water in the environment (Cobb et al. 1973). Secondary contamination of heterotrophic spoilage bacteria and bacteria of public health significance are contributed by the gear, boat deck, shrimp holds, wooden boxes and bamboo baskets used for storing shrimps on board (Gopalakrishnan and Chaudhury 1965; Cann 1977). The latter harbour heavy bacterial load, unless properly cleaned and disinfected after each operation (Gopalakrishnan and Chaudhury 1965; Govindan 1985). Beheading and peeling processes, where hands of personnel are involved, are potential stages of contamination of bacteria of public health significance. Bacterial load of the water used for the preparation of ice and washing of dressed prawns and other utensils govern the level of contamination (Gopalakrishnan and Chaudhury 1965). Nature of ice (flake ice, crushed ice, cube ice, etc., and the quantity used, method of application and duration of delay in icing influence proliferation of contaminating flora, nature of deterioration and development of melanosis (Govindan 1985). Frozen shrimps, that were iced immediately after catch, showed minimum amounts of drip and weight losses, compared with those iced after 2 h and thereafter (Hebber 1979). Duration period of transportation of shrimps held in ice from landing or peeling centers to freezing factories, also influences the keeping quality of frozen shrimps, as the temperature of the containers, melting of ice and re-addition of ice during transportation directly affect the freshness of shrimps (Pillai et al. 1965). Further, filth, flies, ants, droppings of rodents, hair and other debris, associated with frozen shrimps during cold storage, also influence the final quality of the shrimps marketed (Govindan 1966; 1970).

The quality of shrimps is assessed in terms of bacterial load, total volatile bases (TVB), total volatile nitrogen (TVN), ammonia (NH), total volatile substances (TBR), trimethylamine (TMA), α-amino acid nitrogen, water extractable nitrogen, volatile acid number (VAN), indole, hydrogen sulphide, hypoxanthine, orthophosphate, pH, weight, moisture content and organoleptic scores (Chandrasekaran 1985).

Conclusion

Significant progress has been made over the years in developing suitable methods for preprocessing and freezing of shrimps, However, in the present context of increased awareness and demand for shrimps in the developed and developing countries, the methods practised to maintain freshness in the product in India are not adequate. Especially, in the wake of the need to meet the more rigid and striingent concepts of hazard analysis critical control points (HACCP) and ISO 9000, the standard the stipulated for seafood in the international market, it is imperative to evolve and adopt efficient and fool-proof methods that can ensure freshness in the frozen shrimps.

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Effect of Divalent Metal Ions and Temperature on the Properties of Proteins from Prawn (*Metapenaeus dobsoni*)

B.A. SHAMASUNDER AND V. PRAKASH*

Department of Protein Technology,
Central Food Technological Research Institute, Mysore-570 013, India.

Effect of divalent metal ions, i.e., calcium and magnesium chlorides at 1% level, and exposure at 95°C for different durations on the properties of proteins from prawn during frozen storage, have been investigated. The extractable nitrogen decreased by 33% in magnesium chloride treated sample at the end of 150 days of storage. Fluorescence emission spectra showed a blue shift by 2 nm in calcium chloride treated sample, thereby revealing an aggregation process. Sedimentation velocity pattern and gel filtration profile also indicated aggregation process. Viscosity at zero protein concentration indicated no major change as a result of storage. Thermal denaturation profile showed 66 ± 1°C transition temperature of total protein from native to denatured states, with storage at -18°C for 60 days. The effect of heat for different periods on the extracted proteins indicated complete dissociation, as revealed by sedimentation velocity and polyacrylamide gel electrophoresis.

Keywords : Prawn proteins, Divalent metal ions, Physico-chemical properties, *In vitro* heating.

In the food processing industry, divalent metal ions have played a major role, especially, in maintaining the texture of certain vegetables and fruits during processing (Meyer 1960). Similar treatments using divalent metal ions were attempted by a number of workers, to understand the storage stability of fish as well as the effect of these metal ions on the fish proteins (Snow 1950; Sikorski et al. 1976; Nakayama et al. 1986; Saeki et al. 1986; Yoshioka and Arai 1986; Shomer et al. 1987).

The structure of protein decides many properties, both in solution, and interaction behaviour with other molecules (Tanford 1962). Changes such as pH, temperature, ionic strength, nature of buffer ions and protein concentration result in shifting equilibrium from native to denatured states and *vice versa* (Kauzmann 1959). Such an equilibrium process also gives information on the stability of protein over a range of temperature, both in native state as well as in partly dissociated and denatured states due to ligand binding (Wyman 1964). Hence, monitoring of such a process of denaturation, through temperature increments of protein solution, can give an insight into the mechanism of interaction of protein with other constituents in solution. The effect of divalent metal ions, in the form of calcium and magnesium chlorides independently, as a pre-freezing dip treatment of prawns, on the prawn proteins has been studied from the view point of drip loss, nitrogen extractability, and physico-chemical properties during frozen storage.

* Corresponding Author

Materials and Methods

Treatment with divalent metal ions : Peeled and deveined prawns (*Metapenaeus dobsoni*) were dipped in 1% calcium chloride ($CaCl_2.2H_2O$) solution for 5 min. The volume of calcium chloride solution was twice the quantity of peeled and deveined prawn. The solution was drained, excess fluid removed from the prawns by gentle squeezing, the prawns were packed in polyethylene bags, and frozen. In case of magnesium chloride ($MgCl_2.6H_2O$), the concentration of the salt in dip solution, dip time and other conditions for treatment were the same as in calcium chloride treatment.

As a control batch for all the above, peeled and deveined prawns were dipped in distilled water for 5 min, using 2:1 ratio of water to prawn. Further processing was the same as described earlier (Shamasunder and Prakash 1994 b). The frequency of the withdrawal of frozen samples varied for different analyses, ranging from 0 to 150 days with three sampling.

Thermal denaturation curves : The proteins from untreated and treated prawns were extracted in 0.03 M phosphate buffer (pH 7.8) containing 1 M sodium chloride and the concentration of protein was determined as described earlier (Shamasunder and Prakash 1994 b). A known protein concentration of this solution was equilibrated to 25°C in special thermal melting cuvettes and melting curve was followed at 287 nm in the temperature range of 25-95°C at 1°C interval, using Gilford Response II Spectrophotometer (Cleveland, Ohio). The curves were smoothened using software supplied with instrument and melting

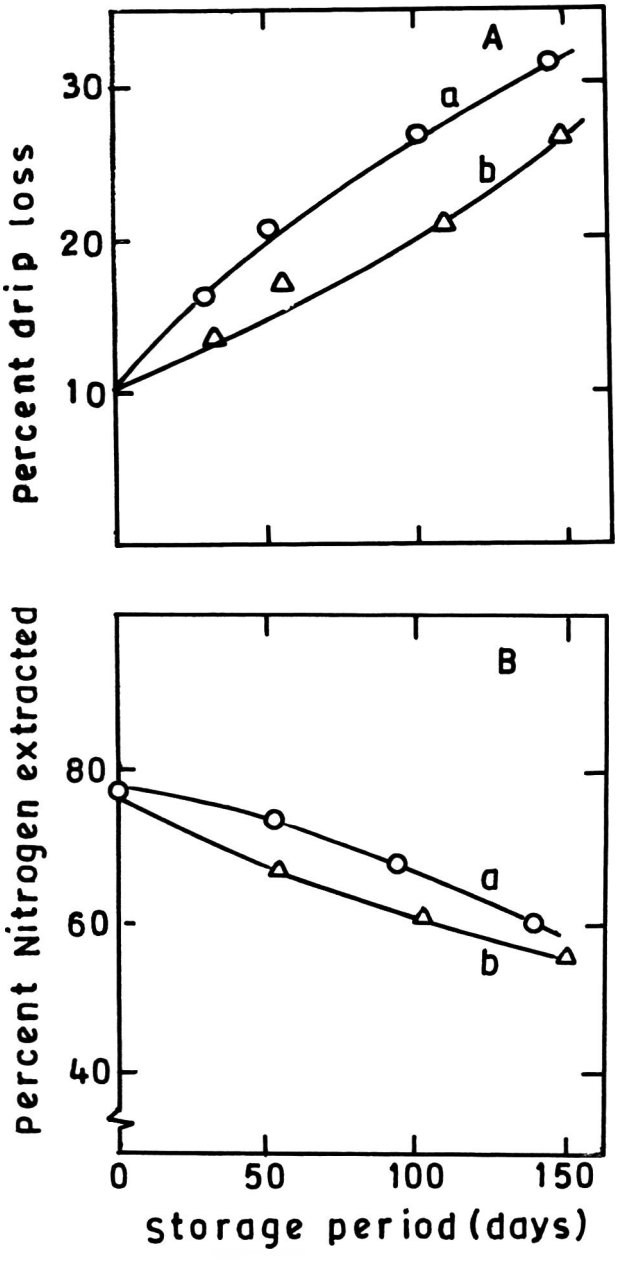

Fig.1 **A.** Effect of storage period of prawns frozen and stored at -18°C on the amount of drip collected during the process of thawing and expressed as percentage of drip loss based on frozen weight of prawn. **a.** Prawns dipped in 1% $CaCl_2$ solution for 5 min. before freezing, frozen and stored at -18°C. **b.** Prawns dipped in 1% $MgCl_2$ solution for 5 min. before freezing, frozen and stored at -18°C.

B. Effect of storage period on the nitrogen solubility profile of prawns stored at -18°C. **a.** Prawns dipped in 1% $CaCl_2$ solution for 5 min. before freezing, frozen and stored at -18°C. **b.** Prawns dipped in 1% $MgCl_2$ solution for 5 min. before freezing, frozen and stored at -18°C.

temperature was obtained. The other methodologies followed for parameters such as gel filtration, fluorescence, nitrogen extractability and drip loss were as described elsewhere (Shamasunder and Prakash 1994 a, b, c, d).

Results and Discussion

Drip loss : Per cent drip losses, as a function of storage period of prawns in the presence of both calcium and magnesium chlorides, are reported in Fig.1A. It increased from an initial value of 10% to a value of 26% in calcium chloride treated prawns during the frozen storage studies (150 days). However, in case of magnesium chloride treated prawns, the value was higher throughout the storage period, as compared to calcium chloride

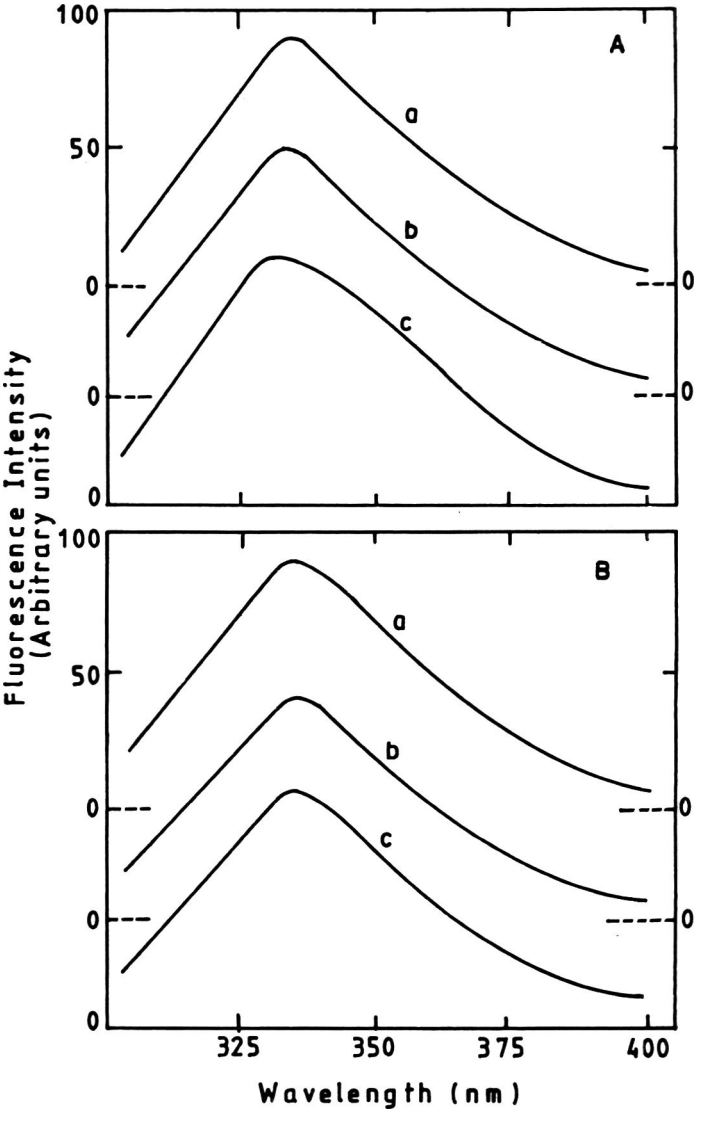

Fig.2 Effect of storage period of frozen prawns at -18°C on the fluorescence emission spectra in the range of 300-400 nm of total proteins extracted from untreated, $CaCl_2$ treated and $MgCl_2$ treated prawns.

A. $CaCl_2$ treated prawns **a.** Fresh untreated; **b.** 45 days storage and **c.** 128 days storage

B. $MgCl_2$ treated prawns **a.** Fresh untreated **b.** 50 days storage and **c.** 135 days storage. In order to compare the fluorescence emission spectra (qualitatively) the ordinates are shifted such that there is no overlapping of individual spectrum. The 'Y' axis hence is shown in arbitrary units.

treated prawns, and the drip loss was 31% at 148 days. The control sample showed a drip loss of 18.5% during the same period (Shamasunder and Prakash 1994 c), thereby indicating that the loss is higher in presence of salts than in control. Calcium or magnesium salts are known to affect the solubility, association- dissociation phenomenon and can denature proteins in a few cases (Jencks 1969; Von Hippel and Hamabata 1973). Hence, further investigations were carried out to see the effect of these salts on the physico-chemical properties of prawn proteins.

Nitrogen solubility : Nitrogen solubility profile as a function of storage period is shown in Fig.1B. It decreased with increase in storage period in the presence of both calcium and magnesium chlorides. Such trend was also reported by Jencks (1969). The decrease in nitrogen solubility was nearly two-fold upto 150 storage days, as a result of aggregation of proteins, which is mediated by salt linkage by calcium or magnesium ion (Jencks 1969). If such a process of aggregation should occur, the fluorescence changes should be seen especially in the emission maxima of these proteins.

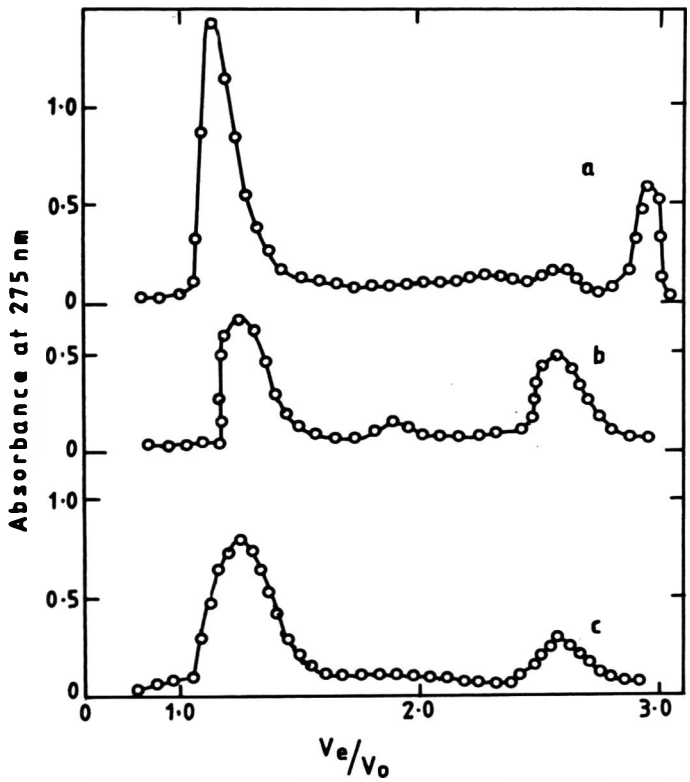

Fig.3 Effect of storage period at -18°C on gel filtration profile in Sepharose 4B gel of the proteins extracted from prawn dipped in 1% $CaCl_2$ solution for 5 min before freezing. The 'X' axis represents the ratio of elution volume (V_e) of each fraction of void (V_0) of column. The absorbance of the fractions were monitored at 275nm **a.** Untreated fresh sample at 0 day; **b.** Sample stored for 45 days and **c.** Sample stored for 135 days.

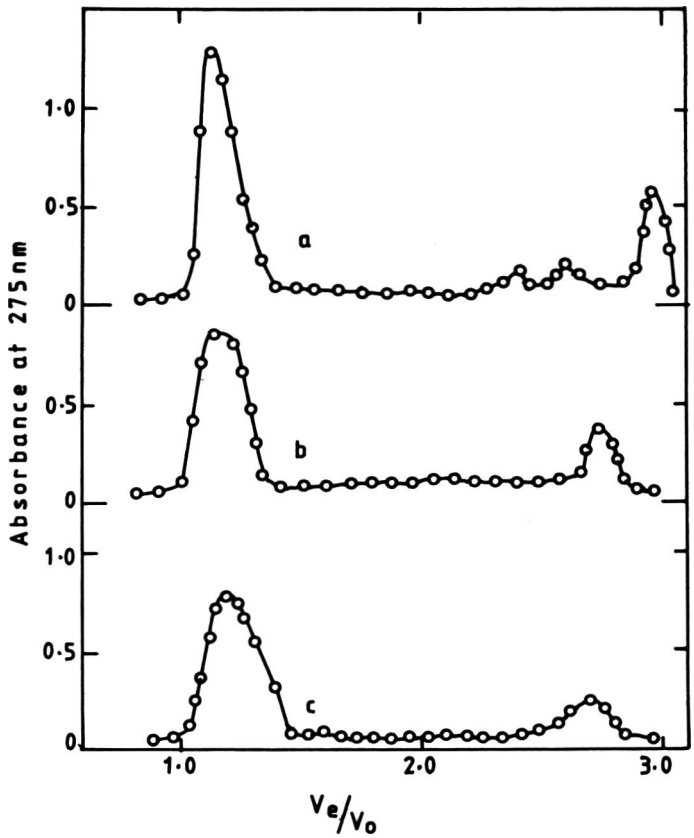

Fig.4 Effect of storage period at -18°C on gel filtration profile in Sepharose 4B gel of the proteins extracted from prawn dipped in 1% $MgCl_2$ solution for 5 min before freezing. The notations for axes are as in Figure 3. **a.** Untreated fresh sample at 0 day; **b.** Sample stored for 55 days and **c.** Sample stored for 145 days.

Fluorescence spectra: Representative fluorescence emission spectra of fresh, treated with calcium chloride at 1% level and stored at -18°C are shown in Fig. 2A. The fluorescence emission maximum of the fresh untreated sample, was 335 nm (Fig. 2A). In the presence of calcium chloride after 45 days of storage,the emission maximum was blue shifted to 333 nm, while it was at 330 nm at 128 days of storage, indicating that the tryptophanyl residues in the protein are now in a more non-polar environment or in a lower dielectric constant medium, as compared to control protein. This can happen only, if there is an aggregation of protein, wherein the exposed residues of tryptophan are entrapped in the associated molecule resulting in such changes (Chebbrert et al. 1991). The fluorescence emission maxima of proteins, extracted from magnesium chloride treated prawns, suggest that the process of aggregation has not occurred, since the spectrum was identical with that of the untreated sample, even after 135 days of storage (Fig. 2Bc).

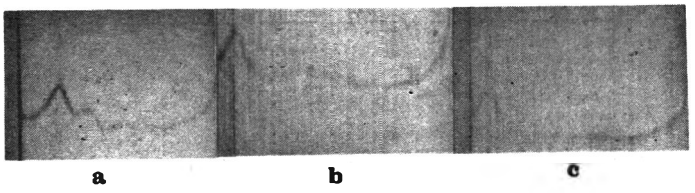

Fig.5. Effect of storage period on representative sedimentation velocity pattern of $CaCl_2$ and $MgCl_2$ treated prawn total proteins extracted in phosphate buffer (0.03M, pH 7.8) containing 1M NaCl. The sedimentation proceeds from left to right. The photographs were taken 60 min. after reaching two thirds maximum speed at a bar angle of 55°. **a.** Fresh untreated prawn. **b.** Fresh prawns dipped in 1% $CaCl_2$ solution for 5 min before freezing and stored at -18°C for 65 days and **c.** Fresh prawns dipped in 1% $MgCl_2$ solution for 5 min before freezing and stored at -18°C for 65 days.

Gel filtration : The gel filtration patterns of proteins extracted from both calcium and magnesium chloride treated prawns, as a function of storage period, are shown in Fig.3 and 4. It is evident that both calcium and magnesium chlorides bring about changes in the association-dissociation of the protein. The peak eluting at V_e/V_0 of 2.70 at 128 days of storage in the calcium chloride treated sample, whereas there was a broad peak of lower concentration in the magnesium chloride treated sample, as compared to untreated sample at the same period. This is in agreement with the results observed from fluorescence experiments. In calcium chloride treated sample, the peaks eluting at a V_e/V_0 of 1.12 has increased in area, thereby suggesting that protein in prawn is now aggregated in the presence of calcium chloride during frozen storage. Even though there is a marginal increase in the area under the peak eluting at a V_e/V_0 of 1.12 in case of magnesium chloride treated sample, the difference is not significant enough to draw any major conclusion.

Sedimentation velocity : Data on sedimentation velocity pattern of total proteins, extracted from prawns treated with calcium and magnesium chlorides at 65 days of frozen storage, are shown in Fig.5. Magnesium chloride treated sample showed 9S and 6S components. However, the pattern was dominated by 6S component, with minimum of oligomers of S values greater than 14S. In case of calcium chloride treated sample, it is possible that the aggregated proteins are not soluble, as a result of which only the 6S component is predominantly present in the system. Protein molecules in prawn, which associate especially with actin and myosin, can orient themselves into linear polymers, resulting in lower sedimentation coefficient values (King 1966). Such an aggregation process is dominated by frictional coefficient rather than the size of the molecule (Schachman 1957).

Viscosity : The effect of calcium and magnesium chlorides on the viscosity profile of prawn protein is shown in Fig. 6A and B. During the period of storage, the viscosity value increased at the same concentration of protein over a period of 135 days, as shown by the increasing slope of the line both in calcium and magnesium chloride treated samples. The value, extrapolated to zero protein concentration indicates that there is no gross change in shape factor of protein after treatment with these two salts and storage. However, because of the complexity and nature of total proteins, it is difficult to interpret such results in heterogeneous proteins and caution has to be exercised in interpreting such results.

Thermal denaturation : Effect of temperature from 25° to 85°C on the absorbance at 287 nm of proteins from untreated prawns at four different concentrations is shown in Fig.7. This profile indicated two important results. The extent of denaturation, as shown by the plateau region, increased with increase in protein concentration, while the transition temperature (mid point of transition) did not vary with increase in protein concentration. The transition temperature of

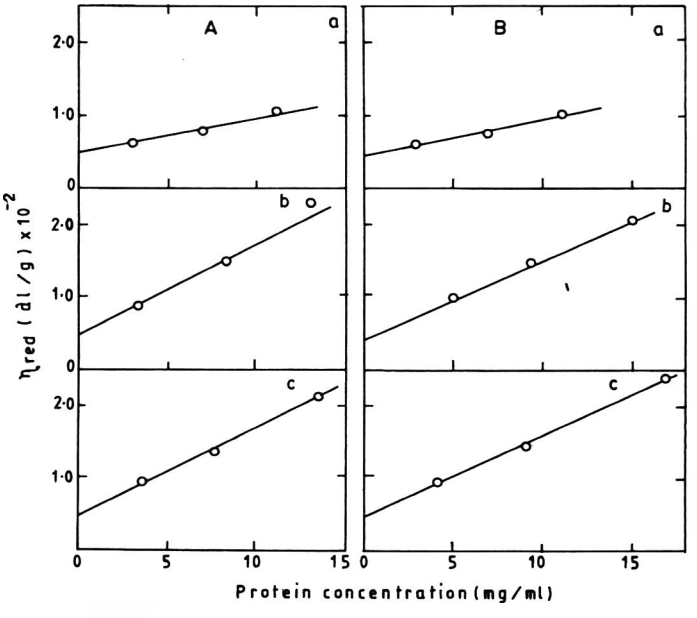

Fig.6. Effect of storage period on the apparent reduced viscosity of the proteins extracted from prawns dipped in 1% $MgCl_2$ or 1% $CaCl_2$ solution for 5 min. before freezing and stored at -18°C for different periods of time. A. $MgCl_2$ dipped prawns. **a.** Fresh untreated **b.** 45 days storage and **c.** 135 days storage.
B. $CaCl_2$ dipped prawns **a.** Fresh untreated **b.** 45 days storage and **c.** 135 days storage.

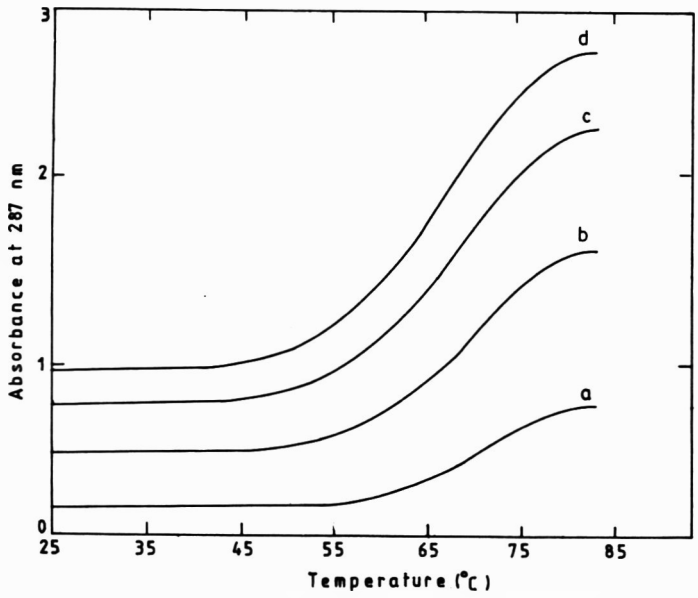

Fig.7. Effect of temperature on the proteins extracted from untreated prawns as a function of protein concentration. The rise in temperature was from 25-90°C at 1°C increment over a period of 48 min. The concentration of the protein solution was **a.** 0.35 mg/ml. **b.** 0.58 mg/l. **c.** 0.78 mg/l and **d.** 1.00 mg/ml.

untreated protein from native to denatured state, as measured at 287 nm as a function of temperature, was 66°±1°C (Fig.8). With storage at -18°C for 60 days, the transition temperature of untreated protein decreased to 57°C. These results confirm that the proteins are susceptible to thermal denaturation during storage at -18°C, due to the changes in conformation and dissociation (Shamasunder and Prakash 1994d).

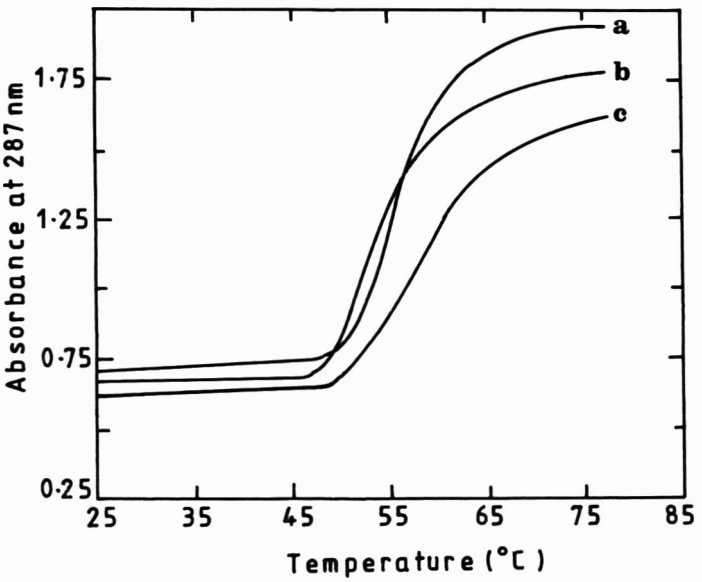

Fig.8. Effect of temperature on the proteins extracted from untreated prawn stored for different periods at -18°C. The rise in temperature was from 25-95°C at 1°C increment over a period of 48 min. **a.** 60 days; **b.** 130 days and **c.** 170 days.

Blanching of extracted total proteins : The *in vitro* studies on blanching of extracted total proteins from prawn were investigated to understand the mechanism of the heat denaturation of prawn of proteins. The various physico-chemical properties of heated proteins were evaluated by sedimentation velocity and polyacrylamide gel electrophoresis (PAGE) experiments. Effect of blanching for 15, 30 60 and 180 sec on the extracted total proteins from

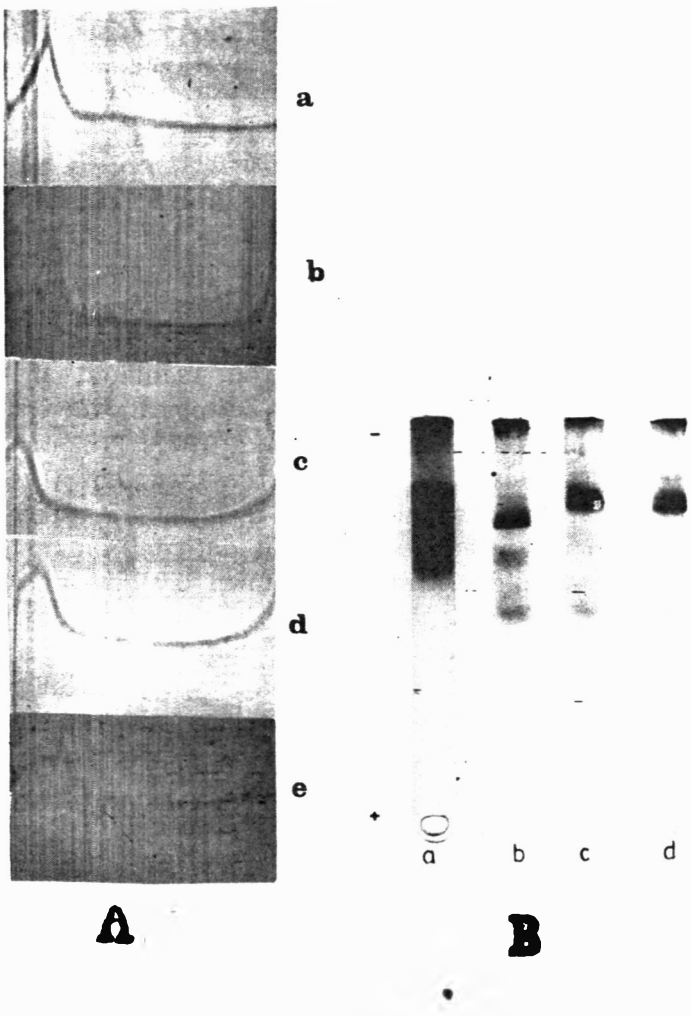

Fig.9A. Effect of heating for different periods of time on the sedimentation velocity pattern of total proteins extracted from untreated prawns. The protein solution was dialysed versus phosphate buffer (0.03M, pH 7.8) and used for sedimentation velocity experiment after heating for different periods of time at 95°C. The sedimentation proceeds from left to right. The photographs were taken 60 min after reaching two third maximum speed at a bar angle of 55°. **a.** Fresh untreated (unblanched-Reproduced from Shamasunder and Prakash 1993b). Protein solution was heated at 95°C for **b.** 15 sec; **c.** 30 sec; **d.** 60 sec and **e.** 180 sec.

B. Effect of heating for different periods of time on the polyacrylamide gel electrophoresis pattern of the total proteins extracted from untreated prawns. The concentration of the protein loaded was 50 micrograms. **a.** Untreated; **b.** 30 sec; **c.** 60 sec and **d.** 120 sec.

prawn on sedimentation velocity is depicted in Fig. 9A. These results showed that the process of blanching induced dissociation in total proteins with concomitant decrease in high molecular weight sedimenting components, such as 20S, 14S and 11S.

The PAGE pattern of proteins extracted from prawns and heated to 90°C for 30, 60 and 120 sec is shown in Fig. 9B. The pattern showed a fast moving component at all the three blanching periods as well as with constant loss of slow moving components compared to untreated sample. Such dissociation of protein components might involve disrupting the hydrophobic interaction between the aromatic amino acid residues (Jencks 1969).

These results suggest that the process of heating does favour dissociation of proteins as seen in sedimentation velocity experiments, gel filtration profile and PAGE pattern. However, the process of aggregation leads to precipitation of proteins, as a result of heating to different periods, and such a process of aggregation can be mediated by hydrophobic interaction, which is entropically driven (Jencks 1969; Prakash and Nandi 1977; Prakash 1982). These results support the fact that the divalent metal ions, calcium and magnesium, at the same concentration, bring about different extents of association-dissociation in prawn proteins and is a salt-dependent phenomenon. The *in vitro* study of temperature effect suggests that the proteins aggregate at elevated temperature by entropically driven hydrophobic interaction, as shown by fluorescence studies and results in polymer formation, as shown by sedimentation velocity experiments, ultimately leading to precipitation of proteins.

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Properties of the Proteins from Drip of Frozen Prawn (*Metapenaeus dobsoni*)

B.A. SHAMASUNDER AND V. PRAKASH*

Department of Protein Technology,
Central Food Technological Research Institute, Mysore-570 013, India.

Nature of proteins in drip exudate from frozen prawns has been investigated. The total solids and proteins in the drip were 4.25±0.20% and 4.8±0.25%, respectively. The ultraviolet absorption spectra did not show a defined maximum. The fluorescence excitation maximum and the emission maximum were at 282 and 334 nm, respectively, which could be attributed to tryptophan residues. Amino acid composition of drip indicated that it is rich in lysine, aspartic acid, glycine, valine and aromatic amino acids. Sedimentation velocity pattern showed a single broad peak with $S_{20,w}$ value of 2.5 ± 0.2S, suggesting the presence of predominantly low molecular weight proteins. Gel filtration profile showed a major peak eluting near the total volume of the column. Several proteins in the drip had a molecular weight range of 11,000 to 24,000, as seen by SDS - polyacrylamide gel electrophoresis. The proteins of the drip showed aggregation with increase in frozen storage period of prawns at -18°C.

Keywords : Drip, Prawn proteins, Physico-chemical properties, Aggregation, Frozen storage of prawns.

Process of freezing brings about crystallization of the free water present in muscle (Bose 1969), and the distribution of these ice crystals depends upon the nature and conditions of the muscle as well as the rate of freezing (Love 1960; Bose 1969). The freezing of water can bring about changes in an ordered water structure around the protein molecule (Kauzmann 1959). The process of water released from intracellular matrix during the process of thawing generates loss of fluids from the muscle, which is predominantly seen in large, if the method of freezing is not rapid and efficient (Love 1960). Such fluid is normally referred as 'drip', while the loss of fluid is generally termed in literature and in prawn industry as 'drip loss'.

The drip loss in prawns is a serious problem and accounts for 20-30% of fluid loss during the process of freezing, storage at frozen temperature and subsequent thawing before use (Godavaribai et al. 1987). The nature of drip is reported to be flavouring components; nitrogenous materials like small peptides and free amino acids; proteins and nutrients such as vitamins; and some soluble carbohydrates (Bose 1969; Godavaribai et al. 1987). However, no detailed study is available on the constituents of drip, especially, the proteins that are present in it.

Hence, a detailed study was undertaken to understand the nature of proteins, their physico-chemical properties such as sedimentation behaviour, fluorescence emission spectra, molecular weight, number of protein components present in drip collected from prawn muscle, as a result of varying periods of storage at -18°C. The results of this investigation will have a bearing on the qualitative and quantitative losses of proteins of drip from frozen prawns. The data may help to develop a rationale to prevent drip loss.

Materials and Methods

The methodologies followed for procurement, processing and storage of prawns are as described elsewhere (Shamasunder and Prakash 1994b).

Amino acid analysis : It was determined in LKB amino acid analyzer (Model 9-151 Alpha Plus, Upssala, Sweden), equipped with a programmer and integrator. The drip was diluted with distilled water (1:1), lyophilized and used for amino acid analysis. The sample preparation for amino acid analysis was according to Moore and Stein (1963). The amino acid composition was calculated from the readings of standards obtained from the integrator and expressed as percentage. Tryptophan in the protein extract was estimated by N-bromosuccinimide method (Spande and Witkop 1967).

Total hydrophobicity : It was calculated from the amino acid composition of the drip. The values of frequency of non-polar side chains (NPS), the ratio of the volume occupied by polar residues to that occupied by non-polar residues (P), and average hydrophobicity (HQ) were based on Tanford's free energies of transfer of amino acid side chain from an organic environment to aqueous environment, from the literature (Bigelow 1967).

The other methodologies, like fluorescence

* Corresponding Author

emission spectra, sedimentation velocity, gel filtration and polyacrylamide gel electrophoresis are as described elsewhere (Shamasunder and Prakash 1994 a, b, c, d).

Results and Discussion

Composition : The total solids in the drip were 4.25 ± 0.20% and the pH was 7.0 ± 0.1. The nitrogen content of drip was 0.76 g/100 g drip. Non-protein nitrogen constituted nearly 30% of the total nitrogen. The protein content of the drip was nearly 4.8 ± 0.25%.

Fluorescence spectra : Fluorescence excitation and emission spectra of drip showed that the fluorescence excitation maximum is at 282 nm (Fig.1a) and the emission maximum at 334 nm (Fig.1b). These results indicate that the drip proteins/peptides are rich in aromatic amino acids, especially tryptophanyl residues, and partly exposed to bulk solvent (Shifrin et al. 1971; Teale 1960).

Amino acid composition : The amino acid composition of drip is shown in Table 1. The drip was rich in lysine, aspartic acid, glycine, valine and aromatic amino acids. From the amino acid composition, the hydrophobicity, NPS and P value were calculated. The average hydrophobicity of the drip proteins was 1108 ± 90 cal/residue. The frequency of non-polar side chain (NPS) and ratio of volume occupied by polar residues to that of non-polar residues (P) were 0.31±0.05 and 1.04±0.08, respectively. The higher P value indicates more of polar amino residues (Bigelow 1967).

Sedimentation velocity : The sedimentation velocity pattern of drip (Fig.2) showed a single broad peak with S_{20},w value of 2.5±0.2, indicating the presence of a group of low molecular weight proteins. The origin of these proteins could be either from the dissociated proteins of prawn muscle or the low molecular weight group of proteins present in the prawn itself, which get solubilized and leached during the process of freezing, storage and thawing.

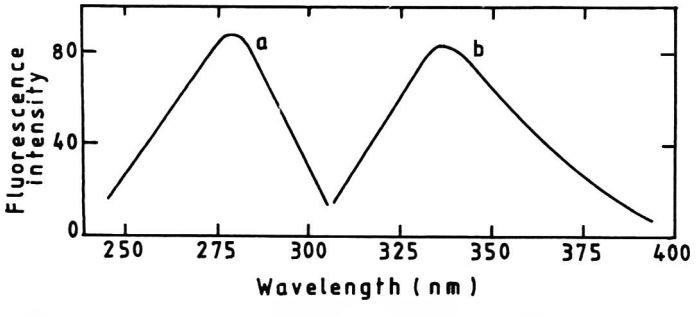

Fig.1. Fluorescence excitation and emission spectra of the drip. The concentration of the protein was 0.10 mg/ml. **a.** Fluorescence excitation spectrum of drip was obtained by fixing the emission maximum at 334 nm. **b.** Fluorescence emission spectrum of the drip fixing the excitation at 282 nm. The fluorescence units are expressed in arbitrary units.

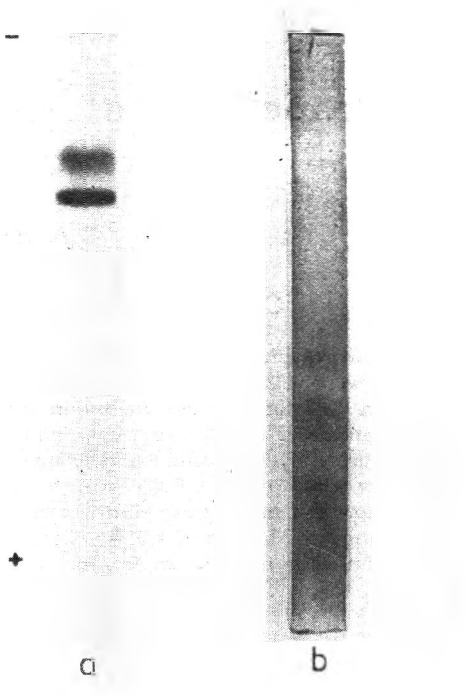

Fig.2. Sedimentation velocity of drip from fresh frozen prawn stored for 15 days at -18°C. The protein concentration was 10mg/ml. Sedimentation velocity proceeds from left to right. The photograph was taken 70 min after reaching two third maximum speed at a bar angle of 60°.

Gel filtration : The gel filtration profile of the

TABLE.1. AMINO ACID COMPOSITION OF DRIP FROM UNTREATED FROZEN PRAWN

Amino acid	g/100 g protein*
Lysine	7.42
Histidine	1.88
Arginine	9.66
Aspartic acid	11.03
Threonine	4.58
Serine	4.08
Glutamic acid	12.65
Proline	7.87
Glycine	7.72
Alanine	5.40
Valine	4.95
Methionine	1.93
Isolcucine	3.91
Leucine	6.75
Tyrosine	3.03
Phenylalanine	6.01
Tryptophan[a]	0.47

[a] Tryptophan was estimated by the method of Spande and Witkop (1967). * Mean of three estimations.

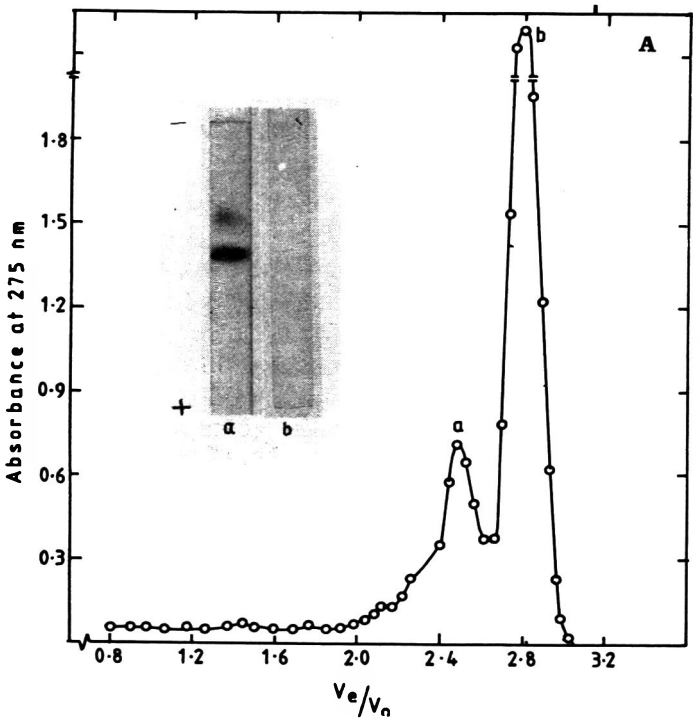

Fig.3A. Gel filtration pattern of drip from fresh prawn stored for 15 days at -18°C, on Sepharose 4B gel. The drip obtained from thawed frozen prawn is directly loaded onto the column. The protein concentration was 30 mg. 'X' axis represents the ratio elution volume (V_e) to void volume (V_o) of the column. The absorption of each fraction was monitored at 275 nm. (Inset) PAGE pattern of the above pooled fraction (a) and (b).

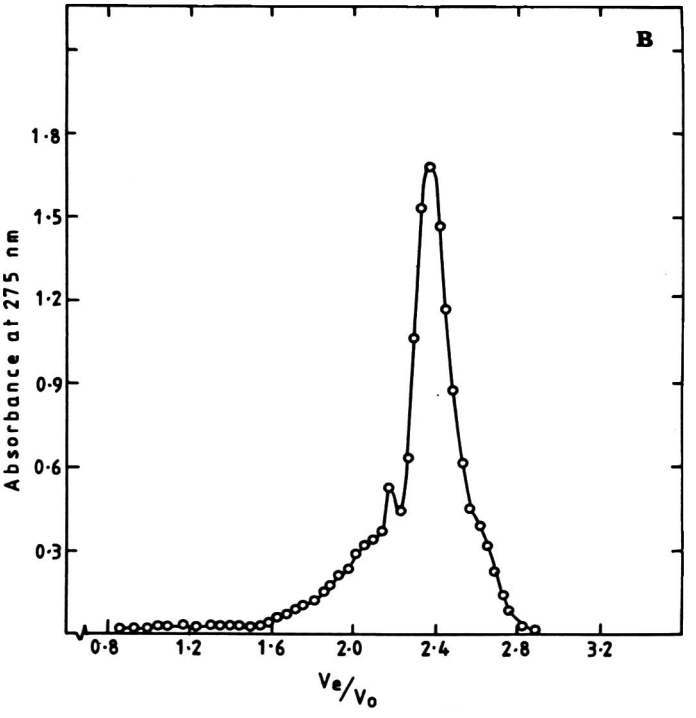

Fig.3B. Gel filtration pattern of dialysed drip from fresh untreated prawn stored for 15 days at -18°C, on Sepharose 4B gel, the drip obtained from thawed frozen prawn was dialysed versus EB for 20 hr at 4°C then loaded to the above column. The protein concentration was 25 mg.

drip on Sepharose-4B gel showed a major peak eluting at V_e/V_0 of 2.8 and a minor peak eluting at V_e/V_0 of 2.45, as shown in Fig. 3A. The proteinaceous nature of the drip, which accounts for 70% of the nitrogenous material, was confirmed by trichloroacetic acid precipitation of the drip. Dialysis of drip exhaustively versus phosphate buffer (0.03 M, pH 7.8) containing 1M sodium chloride and subsequent loading of drip on the Sepharose 4B gel column showed a single major protein fraction eluting at V_e/V_0 of 2.40 (Fig.3B), indicating that the fraction eluting at V_e/V_0 of 2.80 (Fig.3A) was mostly free amino acids and small peptides, which get removed during dialysis. This was further confirmed by the analysis of pooled fractions a and b (Fig. 3A, inset) by polyacrylamide gel electrophoresis (PAGE). The fraction eluting at V_e/V_0 of 2.8 did not take up the protein stain in PAGE pattern (Fig.3A, inset b).

Electrophoresis : The PAGE, sodium dodecyl sulphate - PAGE (SDS-PAGE) patterns of the drip are presented in Fig.4. The PAGE pattern (Fig.4a) showed two bands, while the SDS-PAGE showed four bands (Fig.4b) with molecular weights in the range of 11,000 to 24,400, indicating that the drip consisted mostly low molecular weight proteins.

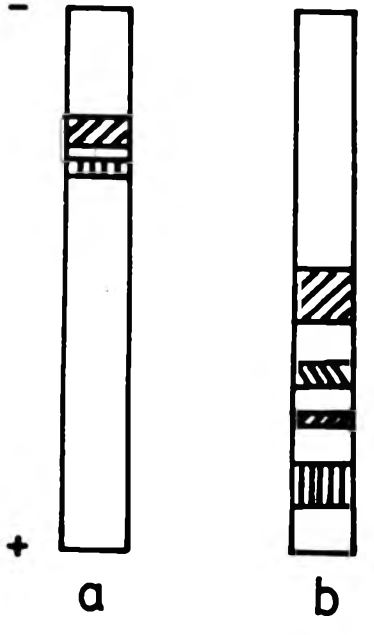

Fig.4. Line drawings of PAGE pattern of drip from fresh untreated prawns stored for 15 days at -18°C. **a.** Under native conditions in phosphate buffer 0.03 M, pH 7.8 containing 0.1% 2-mercaptoethanol. 50 microgram of protein was loaded onto the gel. **b.** Sodium dodecyl sulphate-PAGE of the drip equilibrated to a final concentration of 0.1% SDS and 0.1% 2-mercaptoethanol. 50 microgram of protein was loaded onto the gel.

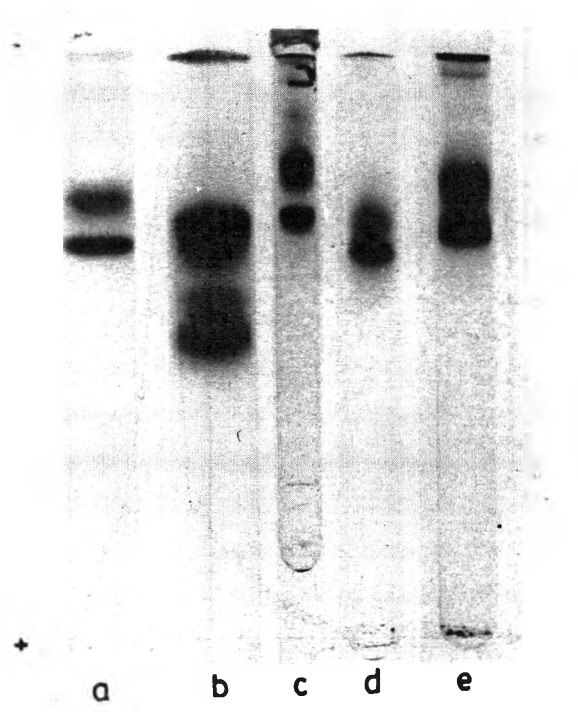

Fig.5. PAGE pattern of proteins of drip obtained from fresh prawn stored at -18°C for different periods of storage in phosphate buffer (0.03M, pH 7.8). The protein concentration loaded in each gel is 45 micrograms. **a.** 0 day stored, **b.** 40 days stored, **c.** 160 days stored, **d.** 208 days stored and **e.** 260 days stored.

The PAGE pattern of drip, collected from prawn stored at -18°C for different periods of time, are shown in Fig. 5. There appeared to be an increase in the number of components, especially the slow moving components, with progressive storage at -18°C. At 208 day of storage, two fast moving bands along with two slow moving bands were observed (Fig.4). Such aggregation processes are also reported in fish muscle (Connell 1959; Lowey and Holtzer 1959; King 1966; Buttkus 1970). These results indicate that the dissociated proteins that are leached out from frozen prawns are low molecular weight proteins, in addition to the leaching of some of the associated proteins at longer periods of storage at -18°C (Shamasunder and Prakash 1994 a, b, c, d).

The analysis of drip suggests that it is made up of 70% proteinaceous nitrogen. The drip showed fluorescence excitation maximum at 282 nm and emission maximum at 334 nm, suggesting a higher proportion of tryptophanyl residues. The drip was found to be rich in aromatic amino acid residues as well as in arginine and lysine (Table 1). The various hydrophobicity parameters calculated from amino acid composition suggests that drip was highly hydrophobic. The free amino acids, peptides and other non-protein nitrogenous materials constituted nearly 30% of nitrogen in drip, as has been seen before and after dialysis as well as in trichloroacetic acid precipitate of undialyzed sample, as compared to dialyzed sample. The drip consists of a group of low molecular weight proteins as evidenced from sedimentation velocity experiments having S_{20},w value of 2.5±0.2.

These results reveal that the drip is composed of low molecular weight proteins, which undergo changes, as a result of frozen storage and can possibly be used as an index for the storage behaviour of frozen prawns as well as to monitor deteriorative changes that take place during frozen storage.

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Thermal Process Requirements for Beans, Gourds and Curried Vegetables

L. SAIKIA[1], SIDDALINGU AND S. RANGANNA[2]*
International Food Technology Training Centre,
Central Food Technological Research Institute, Mysore-570 013, India.

Thermal resistance (D Value), sterilization values (F_0) and process time (Pt) have been determined using *Clostridium sporogenes* for 4 types of beans, 3 types of gourds, curried *pathra* and stuffed brinjal in oil. F_0 values very near to or corresponding to 5 D, wherein D had been determined using $^{-10^3}$ and not $^{-10^4}$ spores, rendered the canned product microbiologically safe. Pt values calculated using D values, found by the modified fraction negative method, were significantly high ($P<0.05$), as compared to those without modification, but not F_0 in canned vegetables in brine. In curried vegetables, F_0 as well as Pt values were high ($P<0.01$). For canned vegetables, in which heat transfer was by convection, and in curried vegetables, in which heat transfer was by conduction, process time calculated by Ball's formula method, using revised fh/U:g values, were similar to those found by improved graphical method.

Keywords : Thermal processing, *Clostridium sporogenes*, Canning, Low-acid foods, Commercial sterilization, Beans, Gourds, Curried vegetables.

Thermal process requirements for canned drumstick, okra, elephant yam, potato and orange segments have been discussed earlier (Saikia and Ranganna 1992). Some studies on acidified vegetables and fruits (Nath and Ranganna 1979; Azizi and Ranganna 1993), and also on tomatoes (Mudhar et al. 1986) have been made. Similar studies carried out with four types of beans, three types of gourds and two types of curried vegetables, which are canned in India to meet the local and export requirements, are reported in the present paper.

Materials and Methods

Preparation of vegetables : Fresh vegetables were purchased from market at Bulsar. Field bean (*Dolichos lablab*) and green pigeon pea (*Cajanus cajan*), also called redgram or congo pea, were shelled. Broad bean (*Vicia faba*) and cluster bean (*Cyamopsis tetragonoloba*) were trimmed. Bitter gourd (*Momordica charantia*, Linn) (Indian name: *karela*) was cut into 1.0 cm thick pieces, after trimming the ends. The two ends of ivy gourd (*Coccinia cordifolia*) (Indian names: *kundri, tindora*) and pointed gourd (*Trichosanthes dioica*) (Indian names: *parval, parwar*) were trimmed. The prepared materials were blanched for 3 min in boiling water and used for canning.

Determination of thermal resistance (D) and °C required for the thermal resistance curve to traverse one log cycle (z-value), conversion of D-value corresponding to experimental z-value in terms of another or classical z-value of 10°C, measurement of heat penetration into the canned products; process time (Pt) calculation, and inoculated pack studies were carried out as described earlier (Saikia and Ranganna 1992). The pH of the canned products was determined using Toshniwal-Polymetron pH meter and glass electrode, standardized using pH 4.0 buffer and counter-checked with pH 7.0 buffer.

To prepare stuffed brinjal (*Solanum melongena*), salt (2.2%), turmeric powder (0.2%), spice mixture (1.6%), potato (6.5%), coriander powder (1.5%), chilli powder (7.5%) and gram flour (6.5%) were added to heated refined oil (10%), mixed, cooked to a paste consistency, and cooled. After removal of stem and calyx, small round-shaped brinjals (65.0%) were partially slit, and blanched for 3 min. Curry was stuffed between the slits. The stuffed brinjal (420 g) was filled into Al Tall (78 x 119 mm) cans, covered with 45 ml of oil, exhausted, sealed and processed. To prepare *pathra*, curry paste, prepared using powdered jaggery (7.0%), lentil flour (30.0%), green chilli (3.0%), salt (1.6%), spice mixture (0.4%) and water (27.0%), was mixed with heated oil (1.0%). The paste was smeared on the dorsal side of colocasia leaves (30.0%). The smeared leaves were layered, rolled to make a bundle (75 mm in diam), steamed, stuffed into 78 x 119 mm cans (450 g/can), exhausted, sealed and processed.

Stuffed brinjal was blended with 3 parts, and canned *pathra* with 4 parts of sterile water, for preparing spore suspensions of *Clostridium sporogenes* (PA-3679, ATCC 7955) and for determination of thermal resistance, as described

* *Corresponding Author. Present Address : [1]Department of Horticulture, Assam Agricultural University, Jorhat-785 013; [2]63, Ananthashram Road, Jayalakshmipuram, Mysore-570 012, India.*

earlier (Saikia and Ranganna 1992). Preliminary heat penetration studies on canned curried vegetables indicated that the heat transfer was by conduction, and the geometric center was the cold point. Hence, thermocouples were positioned at the geometric centre, in the subsequent heat penetration studies. Process calculations were made by the graphical method, and by Ball's formula method and its modifications, as in canned vegetables in brine (Saikia and Ranganna 1992).

Values of g for different values of fh/U were obtained by five procedures. Procedure I used an algorithm developed by Vinters et al (1975) for the calculation, while procedure II depends upon modification of the algorithm of Vinters et al (1975) by Steele et al (1979) for z of 10°C, to account for the errors in original fh/U:g table of Ball and Olson (1957). Procedure III used algorithm of Kao et al (1981) for the calculation, so as to account for varying jc and z, while procedure IV is same as procedure III, except that fh and fc were obtained from linear fit curves. The procedure V is also same as procedure III, but z of 10°C is used in place of experimental z. Procedures III, IV and V are similar to procedures A, B and C as described in the previous paper (Ranganna and Saikia 1992).

Statistical methods, followed for determining arithmetic mean ($\bar{x}$), standard deviation (σ), coefficient of variability (cv), and paired t-test to compare the different methods of process calculation were those described by Ranganna (1986). Pt. found by improved graphical method (IGM), was used as the baseline for comparing different formula methods by paired t-test. The mean ($\bar{x}$) between the two methods was calculated using the formula :

$\bar{x}$ = (Σx of reference method + Σx of test method)/2N

$_{\sigma}d$ = standard deviation by difference

N = number of observations

Results and Discussion

pH changes : On canning of vegetables in brine, the pH was invariably lowered and the extent of decrease was higher in commercial samples, due to the practice of addition of small quantity of citric acid during canning (see footnotes of Tables 1 and 5). In curried stuffed brinjal, the pH increased, but it decreased in *pathra*.

D-value for Cl. sporogenes *in plant materials :* Graphical and formula methods of correction for heating lag made no significant difference in the calculated D-values (t = 0.05±0.04 min). Hence, formula method was used in effecting the correction. The overall mean come up time for the vegetables studied, including those reported earlier (Saikia and Ranganna 1992), was 2.68±0.48 min (n=54), of which only 31.8±6.8% contributed to lethality. The respective values for the curried vegetables were 4.65±0.57 min and 39.1±3.3%. The death of bacteria, when subjected to moist heat at lethal temperatures, is considered to be logarithmic, though exceptions have been reported (Cerf 1977). Berry and Bradshaw

TABLE.1. EXPERIMENTAL D AT 121.1°C AND z VALUES OF Cl. sporogenes AND D IN TERMS OF CLASSICAL z VALUE OF 10°C.

Medium	pH*	Spores/ tube (A)	Experimental D min	Experimental z °C	D when z=10°C min	Spores/ tube (A)	Experimental D min	Experimental z °C	D when z=10°C min
Vegetable in brine									
Beans									
Field bean	6.5	11280	0.62	9.3	0.68	1185	1.27	10.9	1.12
Pigeon pea	6.4	15800	0.65	9.6	0.68	1650	0.98	9.8	1.10
Broad bean	6.5	14000	0.72	10.1	0.64	1040	1.22	10.8	1.06
Cluster bean	5.8	11200	0.60	9.5	0.68	1380	0.92	9.9	1.04
Gourds									
Bitter gourds	5.8	11200	0.74	10.0	0.76	1380	1.09	10.4	1.04
Pointed gourd	5.6	11200	0.50	8.8	0.62	1280	0.78	9.1	0.90
Ivy gourd	4.6	11200	0.39	8.6	0.50	1185	0.69	9.4	0.74
Curried vegetables									
stuffed brinjal	4.3	9200	1.23	11.6	0.96	920	2.19	12.3	1.60
Pathra	5.9	10800	0.68	10.0	0.70	1080	1.68	12.2	1.30

Experimental D : corrected values; calculated by the method of Stumbo et al (1950). D corresponding to 10°C was found by interconversion via process calculation. Correlation coefficient (0.9731-0.9988) of thermal resistance curves were highly significant (P<0.01). *The pH value reported is for laboratory made canned product.

(1982) made a significant observation that the D-value of *B. stearothermophilus* increased by more than three-fold, as the spore concentration reduced to $1x10^3$ or lower. To determine whether *Cl. sporogenes* behaved as above in terms of D-values, the spore concentrations of $\sim10^4$ (termed A) and $\sim10^3$ (termed B) were made use of.

D and z values in plant material : Thermal resistance curves, drawn using the observed values, exhibited a high degree of correlation ($P<0.01$) (Table 1). In each group, the D-value decreased with pH, except in curried vegetables. The D-value was highest for stuffed brinjal, due to the protective action of oil (Hersom and Hulland 1980), in spite of the inhibitory action of added spices. The value in curried *pathra* was lower, than that in stuffed brinjal, probably due to citric acid added in industrial processing.

The D-values found using a spore concentration of $\sim10^3$ (B) were 1.5 to 2.5 (mean value : 1.75±0.3) times higher than the values found using $\sim10^4$ spores (A). With an increase in the D-value, the z value also increased. For vegetables in brine, the z-value for spore concentration of A was 9.4±0.5°C and that of B was 10.3±0.65°C. The respective z-values with curried vegetables were 10.8±0.8°C and 12.2±0.04°C. Malmborg (1983) explained the tailing of survival curves of bacteria - which occurred at conditions other than high cell counts and with short times and/or low temperature - on the basis of the Casalori model in which the D-value increased with falling cell count.

In order to assess the extent of increase in D in terms of one z, conversions were made via process calculation (Saikia and Ranganna 1992) in terms of classical z value of 10°C (Table 1). The converted D-values of B were 1.55±0.14 times higher than the values of A, but the extent of increase for *Cl. sporogenes* was lower than that reported by Berry and Bradshaw (1982) for *B. stearothermophilus*. When the experimental z-value was lower than the classical z-value (10°C), the corresponding D-value, on conversion in terms of the classical z-value, was higher, and vice versa, in the case of an experimental z-values measured at a temperature higher than 10°C.

Improved general method (IGM) : Thermal resistance studies resulted in two D-values and two z-values for each vegetable (Table 1). Earlier, it was shown that the F_0 and Pt (operators processing time) corresponding to $5D_2$ of A and experimental z was nearer to the statutory requirement of F_0 of 3.0 min (Saikia and Ranganna 1992). The values evolved in this way, or by two segment approach of Berry and Bradshaw (1982), i.e. F=D of A (log a-3.0)+D of B(3-log bx) did not render the canned product microbiologically safe from *Cl. sporogenes*. Two segment approach, intended to reduce the spore concentration to less than one, [i.e., F=D of A (log a – log a_x) + D of B (log a_x– log 1) or F=1D or A + 4D of B] yielded F_0 and Pt values nearer to 5 D values of B, which were the highest. To ensure that the product is microbiologically safe, process time corresponding to values nearer to or equal to 5 D of B is required. The pattern was similar for beans, and curried vegetables, with the exception of ivy gourd, in which 5 D of A was less than the statutory minimum of F_0 of 3.0 min, because of lower pH (4.5), which resulted in lower experimental D-values.

In some instances, z-values were higher than 10°C (Table 1). In such cases, the resulting F_0 and Pt were higher, when the process calculation was made assuming z to be 10°C, thereby contributing further to the safety of thermal process. This is not so, when experimental z was lower than 10°C. Calculations made assuming z as 10°C, in such cases, result in lower F_0 value, which may increase chances of spoilage.

Calculation of D by the method of Stumbo *(1948):* In the determination of D (designated as D_2 in this study), Stumbo et al (1950) have estimated the number of survivals using the equation of Halvorosan and Ziegler (1932). On the other hand, Stumbo (1948) considered the number of positive tubes, as the number of organisms surviving lethal heat, in the calculation of D-value (designated as D_1 in this study). The D_2 values were higher as compared to D_1 by 0.028±0.015 min, when the spore concentration used as A, and 0.050±0.03 min, when the spore concentration was B. F_0-values, found by using D_2, were insignificantly higher, than those found using D_1. However, the resulting differences in Pt values were significant ($P<0.05$)

TABLE 2. EFFECT OF METHOD OF CALCULATIONS OF D ON F_0 AND Pt VALUES

	Vegetables in brine		Curried vegetables	
	F_0	Pt	F_0	Pt
N	33	33	8	8
$\bar{x} \pm_e d$	3.9±0.2	19.1—0,6	5.6±0.45	49.3±0.6
cv	0.041	0.029	0.081	0.017
t-value	1.95^{NS}	2.41^{*}	3.86^{**}	3.64^{**}

* P<0.05, ** P<0.01, NS : not significant

TABLE 3. HEAT PENETRATION PARAMETERS OF CANNED VEGETABLES IN BRINE AND CURRIED VEGETABLES.

Particulars	IT °C	Cut, min	jh	fh min	fc min	jc
Vegetables in brine processed at 115.6°C						
Field bean						
Eye fit	67	6	0.59	7.00	12.4	1.14
Linear fit	67	6	0.63	6.73		1.05
Pigeon pea						
Eye fit	57	3	0.72	5.40	6.4	1.00
Linear fit	57	3	0.69	5.42		0.97
Broad bean						
Eye fit	57	9	1.60	5.00	13.5	1.00
Linear fit	57	9	1.47	5.00		1.00
Cluster bean						
Eye fit	61	7	0.47	6.75	6.0	1.21
Linear fit	61	7	0.44	6.51		1.05
Bitter gourd						
Eye fit	61	6	0.833	13.10	7.0	1.23
Linear fit	61	6	0.710	14.22		1.17
Ivy gourd						
Eye fit	68	7	1.186	5.10	7.7	1.25
Linear fit	68	7	1.030	5.25		1.14
Pointed gourd						
Eye fit	60	7	0.52	6.50	10.5	1.00
Linear fit	60	7	0.52	6.38		1.00
Curried vegetables processed at 121°C						
Stuffed brinjal						
Eye fit	72	14	1.48	39.00	84.5	1.00
Linear fit	72	14	1.41	39.70		1.00
Pathra						
Eye fit	57	14	1.61	44.50	88.0	1.06
Linear fit	57	14	1.61	44.13		1.03

• Measurement and calculation made in °F and then converted to °C. Cooling water temperature : 29-32°C, Linear fits of the heat penetration curves were highly significant (r=0.9824-0.9988; P<0.01).

(Table 2). In curried vegetables, differences in F_0 as well as Pt were highly significant (P<0.01). Hence, the method of Stumbo et al (1950) should be used in the calculation of D-value, and the values (i.e. D_2) so found (Table 1) should be used in evolving the thermal process schedules.

Process calculation by formula method : The advantages and disadvantages of IGM have been discussed earlier (Saikia and Ranganna 1992). Formula method developed by Ball (1923) overcame these disadvantages to some extent. Merson et al (1978) have examined the method critically. In developing tables of fh/U:g for calculation by formula method, Ball and Olson (1957) assumed (i) fc=fh, (ii) jc = 1.41, (iii) no break in the semi-log plot of heating curves, and (iv) the retort come-up time was sudden. Semi-log plots of heat penetration data, obtained in this and earlier studies, showed no breaks, in heating curves. However, there was lag in the retort come-up time, but fc was not equal to fh, and jc was less than 1.41 (Table 3). In spite of these variations and other limitations, Ball's method has been in use in the canning industry for the last 70 years, and is also approved by FDA (Lund 1984), as the fh/U:g values listed in the tables lie within a very narrow range and thus minimise the errors.

Values of g for different values of fh/U were calculated by five different procedures. Smith and Tung (1982) have compared Ball's formula method (Ball 1923; Ball and Olson 1957) and its modifications for conduction heating foods, considering z as 10°C, and numerical IGM as reference method. They found that the largest error occurred, when g (unaccomplished temperature difference in the can) was large and the height to diam ratio was close to unity. In the present and earlier (Saikia and Ranganna 1992) studies, the heat penetration data collected under actual commercial processing conditions at intervals of 1 min showed that the heating was by convection for 11 canned vegetables in brine, and by conduction in the 2 curried vegetables.

Canned vegetables - heat transfer by convection : The height to diam ratio of A1 Tall (78x119 mm) cans was 1.53 and the maximum fh/U was 1.32, corresponding to the g-value of 0.85. For the statutory requirement of F_0 of 3.0 min, Pt calculated by procedures I and II of Ball's formula method, were not significantly different from the values found by IGM (Table 4). Similar was the trend, when F_0 was greater than 3.0 min. In contrast, Pt calculated for F_0 of 3.0 min by the methods, involving the use of eye fit curve (procedure III) or linear fit curve (procedure IV), were significantly higher than the values found by IGM. Pt, calculated assuming z as 10°C by procedure V, was highly significant (P<0.01), as compared to the value found by IGM.

Curried vegetables - heat transfer by conduction: In cans with a height to diam ratio of 1.53, the maximum fh was 44.5 min in *pathra* at retort temperature (RT) of 121°C. When F_0 was 3.0 min, the fh/U value was 14.83, and the corresponding g was 10.32, which was the maximum value noted in the present studies. The process time calculated by procedure I was significantly lower (P<0.01), but

TABLE 4. SIGNIFICANCE OF DIFFERENCE BETWEEN PROCESS TIME FOR A1 TALL CANS AS CALCULATED BY FORMULA METHODS WITH GRAPHICAL METHODS

Sl. No.	F_0, min	IGM vs test Procedure	N	Process time, min: IGM x±σ	Process time, min: Test procedure x±σ	2N	Results of comparison: x±σ	cv	t-value calculated
				Vegetables in brine; convection heating					
1	3.0	I	4	19.0 ± 3.30	19.04± 3.04	8	19.20 ± 0.64	0.033	(-) 1.0^{NS}
2	3.0	II	5	17.8 ± 3.80	18.50± 3.60	10	18.10 ± 0.70	0.038	(-) 1.94^{NS}
3	3.0	III	11	15.9 ± 3.37	16.65± 3.24	22	16.27 ± 0.71	0.043	(-) 2.68*
4	3.0	IV	11	15.9 ± 3.37	16.67± 3.27	22	16.28 ± 0.74	0.045	(-) 3.88**
5	3.6+0.75	I	9	23.0 ± 4.90	23.30± 5.10	18	23.20 ± 0.56	0.024	1.27^{NS}
6	3.7±0.07	II	15	21.3 ± 5.10	21.80± 5.20	30	21.50 ± 0.78	0.036	1.87^{NS}
7	4.1±1.16	V	29	20.1 ± 5.60	20.80± 5.60	58	20.40 ± 0.93	0.048	(-) 2.86**
				Curried vegetables; conduction heating					
8	5.9±1.5	I	8	47.8 ± 5.90	46.90± 6.10	16	47.30 ± 0.83	0.018	2.65**
9	5.9±1.6	II	8	47.8 ± 5.90	48.60± 6.20	16	48.20 ± 0.87	0.018	(-) 2.84*
10	6.5±2.75	V	10	50.8 ± 6.30	51.80± 7.00	20	51.30 ± 1.08	0.021	3.75**

* Significant at 0.05, ** significant at 0.01, NS : Not significant, Analysis includes data of vegetables in brine reported earlier (Saikia and Ranganna 1992), besides the seven vegetables reported in this paper.

it was higher in case of procedure V (P<0.01), and there was no difference in process time in case of procedure II. Irrespective of these differences, the mean (x) of the values between the IGM and the test procedure, as also standard deviation by difference ($^{\sigma}$d) and coefficient of variation, indicated that the procedure II is not likely to cause gross under-processing- nor will procedure III cause gross over-processing - within the limits of this study.

Inoculated pack studies : Based on limited laboratory studies, the F_0 and process time found adequate, as also the pH of the fresh and canned products are given in Table 5. The F_0 values found adequate are either nearer to or equal to 5 D of B. It is emphasized that the requirement may vary under commercial processing conditions.

TABLE 5. pH OF FRESH VEGETABLE AND CANNED PRODUCT AND RECOMMENDED F_0 AND Pt VALUES OF VARIOUS VEGETABLES FOR 78X119 MM (301X411) CANS.

Vegetables	pH: Vegetable	pH: Canned product*	F_0, min	Processing time, min
	Vegetables in brine			
Field bean	6.5	6.0	5.0	22.3
Pigeon pea	6.4	5.7	4.6	18.5
Broad bean	6.5	5.3	5.1	21.3
Cluster bean	5.8	4.3	5.1	23.0
Bitter gourd	5.8	4.1	3.8	26.0
Ivy gourd	4.6	4.6	3.7	16.0
Pointed gourd	5.6	4.8	4.5	19.0
	Curried vegetables			
Stuffed brinjal	4.3	4.7**	5.8	45.0
Pathra	5.0	5.3	6.5	57.0

* The pH reported is for commercial product ** pH of vegetable portion in the canned product. Processing of vegetables in brine and curried vegetables was done at 115.6° and 121°C, respectively.

Conclusions

The method of Stumbo et al (1950) is recommended for calculating D-values. The concentration of heat resistant spores in the prepared material is likely to be only a few per g of product, or container. Since D and z values are higher with ~10^3 spore concentration, the thermal process evolved, using the values so found, represents the conditions prevailing in the canned product, and adds to the microbiological safety of the processed product. This is also borne out by the inoculated pack studies. F_0 and Pt values, calculated by Ball's formula method, using fh/U:g values revised by Steele et al (1979), are not significantly different from the values found by IGM.

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Studies on Rheological Properties of Rice-Soya Crackers

M.V. PRINCE, P.K. CHATOPADHYAY* AND R.K. MUKHERJEE

Post-harvest Technology Centre, Agriculture Engineering Department,
Indian Institute of Technology, Kharagpur-721 302, India.

Rice-based soya crackers were made with different levels of soya flours, and the rheological properties of the paste, dough and crackers were determined. A definite relationship could be established between the rheological parameters of the finished product and its dough. Soya at 20-30% level in the mix was found to be optimum from the textural point of view of the final product, dough rheology, sensory quality and paste as well as product rheology.

Keywords : Rice-soya cracker, Dough rheology, Dough workability, Paste rheology, Product rheology, Sensory quality.

Several investigators have indicated the possibility of using rice-legume mixes for the production of snack foods, including rice-soya crackers of good quality, in terms of nutritional value and consumer acceptability (Baker and Hin 1984; Chauhan and Bains 1985; Bhole 1992), apart from a variety of supplementary foods based on rice-pulse for infants (Naikare and Mabesa 1993; Swaminathan 1980) and rice legume foods (Sowbhagya et al. 1991) A blend of rice and soya not only reduces the cost, but also gives a product of better quality (Bhole 1992); it is also used for the preparation of weaning foods (Malleshi et al. 1986). However, the important physico-chemical and mechanical properties of raw materials and of the mixes and products have not received due attention. This paper presents the results of a study conducted on rice-soya crackers, with the object of optimizing the proportion of rice and soya flour mix, based on the rheological properties of doughs. Also, a relationship was sought to be established between dough and product rheology, in order to obtain a better textured product. In addition, sensory evaluation was conducted to supplement the findings of the objective evaluation.

Materials and Methods

Milled, raw rice flour (Cv. "IR36") and full-fat soya flour (Cv. "Bragg"), having particles size 100 µ, and moisture contents of 12.6 and 8% (db) respectively, were used. The amylograph peak viscosity, cooked paste viscosity, cooled paste viscosity and apparent gelatinization temperature were determined for 0, 10, 20, 30 and 35 or 40% soya flour in the mix, by the method of Halick and Kelly (1959), using a Brabender visco-amylograph. The plastic limit and liquid limit for the different rice-soya mixes were determined, using the crack test and the Cassagrande liquid limit in apparatus, in accordance with ISI (1970). The plasticity index, flow index, toughness index and consistency index were calculated, using the following equations:

$$\text{Plasticity index} = \text{Liquid limit} - \text{Plastic limit},$$

$$\text{Flow index} = \frac{W_1 - W_2}{N_2/N_1}$$

where W_1 and W_2 are the moisture contents in % (db), corresponding to N_1 and N_2 drops.

$$\text{Toughness index} = \frac{\text{Plasticity index}}{\text{Flow index}}$$

$$\text{Consistency index} = \frac{\text{Liquid limit} - \text{Natural moisture content}}{\text{Plasticity index of dough}}$$

The tensile loads required to cause rupture of dough sheets, of length 10 cm, width 2 cm and thickness 0.1 cm, made with different proportions of flour in the mix, and with an average moisture content of 68% (db), were determined according to Mohsenin (1970), using an Instron universal testing machine (model 1101), with a chart to cross-head speed ratio of 10:1.

Rice-soya crackers were made with 0 (control), 10, 20, 30 and 35 or 40% soya flour in the mix, by adopting the process outlined by Li and Luh (1980). The crackers with average dimensions of 2 x 1.5 x 0.1 cm (length x width x thickness) were analyzed for hardness and fracturability, using the Instron universal testing machine, as above (Mohsenin 1970). Crispness was measured by the steepness of the force-deformation curve. To test this property, rice-soya crackers, with different proportions of soya flour in the mix, were mounted over a ring of 2 cm dia; a pin of 0.625 cm dia was used to punch the chip. Also, the area under force-deformation curve is a measure of work done during the test, which characterizes the crunchiness of the cracker. Sensory evaluation of the rice-soya crackers, containing different levels of soya flour in the mix, was conducted on the basis of a

* Corresponding Author

composite scoring test, by a 7-member test panel (ISI 1971).

Results and Discussion

Results of amylographic paste viscosity characteristics of rice-soya flour mixes and tensile load on dough sheets of rice-soya dough mixes are presented in Fig.1. It is observed that the amylographic peak viscosity, cooked paste viscosity as well as final cooled paste viscosity showed a decreasing trend with the increase in soya level in the mix, possibly due to the decreased starch content of the mix, as the quantity of soya increased. The reduction was particularly sharp, when the soya level increased from 10 to 30%. In fact, no peaks were observed for 40 and 100% soya mixes, which resulted in little or no starch in the mix. It was observed that the apparent gelatinization temperature tended to increase, albeit only slightly, from a value of 60°C for 0% soya (only rice) to 62.25°C for 40% soya in the mix. This small increase might be due to a possible effect of the presence of non-starch material (soya) in the mix, on the apparent gelatinization temperature of the starch.

Tensile tests of the dough sheet of different dough mixes showed an increasing trend in tensile load with increase in the level of soya in the mix

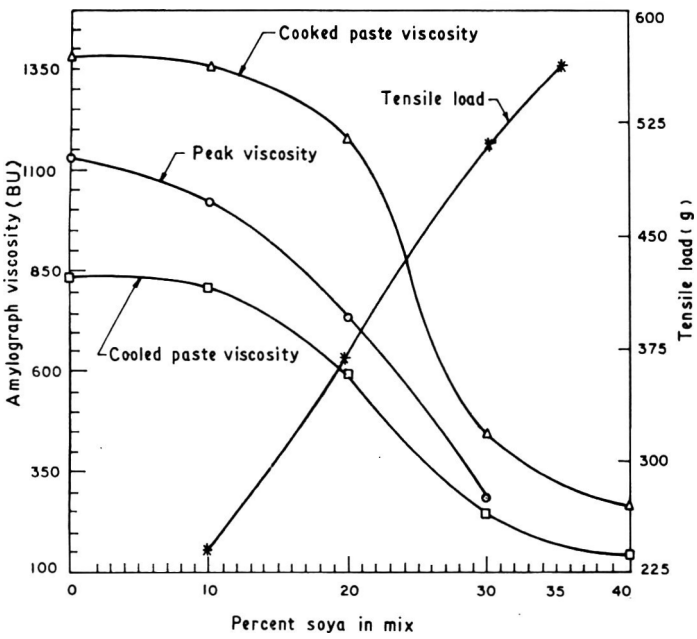

Fig.1. Relationship between soya level in the mix with amylograph viscosity and tensile load. -0—0- : peak viscosity; -Δ—Δ- : cooked paste viscosity; -□—□- : cooled paste viscosity; -*—*- : tensile load.

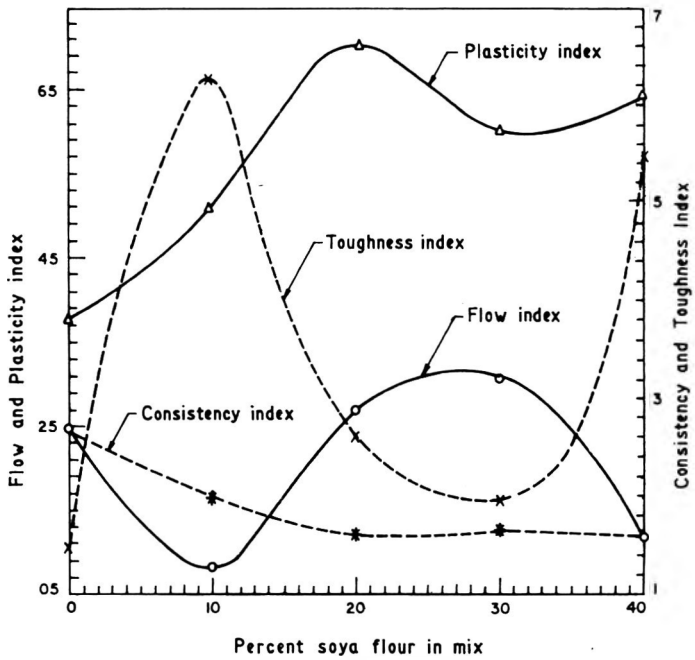

Fig.2. Relationship between soya level in mix with indices for flow, plasticity, toughness and consistency. -0—0- : flow index; -Δ—Δ- : plasticity index; -x—x- : toughness index, -*—*- : consistency index.

(Fig.1). This was probably due to an increase in the protein content in the mix, due to higher proportion of soya. Between 10 and 20% soya in the mix, the tensile load of the dough sheet was reduced by 35.1%, in contrast to 8% between 30 and 35% soya in the mix. Thus, the tensile strength of the dough sheet declined sharply beyond 30% soya in the mix.

The variations in the indices for flow, plasticity, toughness and consistency are shown in Fig.2. Flow index values were higher for 20 to 30% soya in the mix; but they declined on either side of this range, for which no possible explanation can be offered at present; therefore, a further thorough study is needed. However, the values for plasticity index increased with increase in soya level and the maximum value was recorded for 20 to 30% soya in the mix. Toughness index exhibited a somewhat erratic trend with sharp decline, when the mix contained 20 to 30% soya. The reasons for this irregular trend are not fully clear, since toughness index is inversely related to the flow index, which itself had some irregular trend with reference to proportions of soya in the mix. The consistency index exhibited a more reasonable behaviour, with a decreasing trend upto 20% soya in the mix; it became constant thereafter. Because of limited experimental data, it is difficult to identify the precise soya level in the mix for dough

TABLE 1. HARDNESS, FRACTURABILITY, CRISPNESS AND CRUNCHINESS VALUES FOR RICE-SOYA CRACKERS WITH DIFFERENT PROPORTIONS

Attribute	Level of soya in mix, %			
	10	20	30	35
Hardness, g	5190.0	4720.5	3062.0	2290.0
Fracturaility,g	2000.0	1926.0	1911.6	1272.0
Crispness	2630.0	2460.0	2134.0	2068.0
Crunchiness	98.5	243.8	491.7	511.5

characterization. Nevertheless, 20 to 30% soya in the mix would seem to be a desirable proportion for better workability of the dough, in view of the lower toughness and consistency indices, as well as relatively high flow and plasticity indices, measured at these levels of soya in the mix.

The force-deformation analyses, and punch tests of the prepared rice-soya cracker showed that the hardness, fracturability and crispness values decreased, but crunchiness increased, as the percentage of soya in the mix increased (Table 1). These trends remained sharp upto 30% soya in the mix, and then slowed down. Thus, a relationship seems to exist between the rheological parameters of the finished product and its dough. The sensory evaluation of the prepared rice-soya crackers (Table 2) supported the findings of the objective evaluation of their textural attributes.

TABLE 2. MEAN SENSORY SCORES FOR TEXTURAL ATTRIBUTES OF RICE-SOYA CRACKERS WITH DIFFERENT PROPORTIONS OF SOYA FLOUR IN THE MIX

Textural attribute	Mean sensory score		
	Level of soya in mix, %		
	20	30	35
Hardness	15.85	14.85	12.00
Fracturability	15.28	15.14	12.57
Crispness	15.85	15.00	9.42
Crunchiness	11.71	14.28	15.28
Overall acceptability	14.42	16.00	15.20

Maximum score : 20. Effect of soya level significant at 1%, CD at 5% :3.48, at 1% : 5.005, for individual attributes.

Considering the workability of the dough, as affected by the dough rheology and textural attributes of the cracker, i.e., product rheology, and its substantiation by the sensory data, especially overall acceptability of the product, as much as 30% soya in the mix used for making rice-soya crackers was considered to be suitable.

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Essential Oil of *Decalepis hamiltonii* as an Antimicrobial Agent

N.Y. PHADKE[1], A.S. GHOLAP[2], K. RAMAKRISHNAN[1] AND G.SUBBULAKSHMI[1]*

[1]Department of Post Graduate Studies and Research in Home Science, SNDT Women's University, Bombay-400 049, India.

[2]Food Technology and Enzyme Engineering Division, Bhabha Atomic Research Centre, Bombay - 400 085, India.

Essential oil was extracted from the roots of *Decalepis hamiltonii*, using a modified simultaneous distillation and extraction technique. The essential oil inhibited *Escherichia coli*, *Salmonella typhi* and *Saccharomyces uvarum* at minimal concentration of 30 mg in case of 10^8 nos of microorganisms/5 ml of broth. It prevented microbial growth in *khoa* and tomato puree for 7 and 21 days, at refrigerated temperature, when used at concentrations of 0.16 g/100 g and 0.08 g/100 ml, respectively. The major antimicrobial component of essential oil was identified as 2-hydroxy-4-methoxy benzaldehyde.

Keywords : *Decalepis hamiltonii*, Vanillin, Antimicrobial activity, 2-hydroxy-4-methoxy benzaldehyde, *khoa*, Tomato puree

Decalepis hamiltonii (family Asclepiadaceae) is a root largely used in South India for pickling, along with curds or lime juice (CSIR 1952). It is markedly fleshy and has a strong aroma. The fresh root tastes sweet, and produces a tingling sensation on tongue. The volatile aroma constituents of the root are considered to possess antimicrobial properties (Murti and Sheshadri 1942). A remarkable decrease in microbial count of chilli pickle has been reported, when the roots are either added to the pickle, or kept in its vicinity (Bhaskaran 1992). The present study was, therefore, undertaken to isolate and identify the root volatile constituents, with a view to study their antimicrobial effect. Effect of vanillin (a structural analog of 4-o-methyl resorcylicaldehyde), one of the commonly used food flavourings (Beuchat and Golden 1989), was also investigated for comparison.

Materials and Methods

Roots of *Decalepis hamiltonii* were procured from the market in Madras (Tamil Nadu) and transported to Bombay. The roots were 4-5 days old upon arrival. They were cleaned in free flowing water for 10 min, wiped, peeled, and the non-edible portion removed. The fleshy, edible portion was cut into small pieces, using a stainless steel knife. The peeling and cutting was done in a cold room (0-4°C) and the cut portion was stored at –4°C, for use within a month. All the solvents (diethyl ether, petroleum ether, hexane, ethyl acetate, carbon tetrachloride) used were of analytical grade, and they were used only after distillation.

In each batch, 200 g of fresh root was used for isolation of the essential oil. After adding 500 ml of distilled water, the roots were subjected to a simultaneous distillation-extraction technique for 2 h, in the laboratory- modified Nickerson and Likens apparatus (Nickerson and Likens 1966) constructed with a provision for passing steam through the sample. Peroxide-free diethyl ether was used as an extracting solvent. Simultaneous condensation of the steam distillate and the immiscible extracting solvent was carried out by chilled water (4°C) circulation. The solvent was then removed by passing a slow stream of nitrogen gas to obtain the essential oil. A total of 12 samples were analysed, to determine the yield of essential oil, based on fresh weight of the root.

For TLC separation, silica gel G, spread 0.25 mm thick on 20 x 20 cm glass plates (Mangold and Malins 1960), was activated at 110°C for 90 min. One hundred mg of essential oil in ether (10%, w/v) solution as well as vanillin (10%, w/v) in ether were spotted on the plate and developed using petroleum ether (BP 60-80°C) : diethyl ether at 80:20 (v/v) ratio as a solvent system. The plates were removed after the solvent front had travelled approximately 16 cms. A preliminary odour assessment of separated components was done directly on the plate, after complete removal of solvent by flushing the plate with a slow stream of nitrogen. The spots were visualized for nature and intensity by exposing the plates to iodine vapour in a glass chamber. For isolation of the major component of the essential oil, preparative TLC was done and the sample (80 mg) in ether was applied in a band form on a 0.7 mm thick plate, before developing in the above solvent system. The developed plates were air-dried

* Corresponding Author

for 15 min, and exposed to iodine vapour to visualize the bands. The band representing the major fraction (R_f 0.623) was marked with a pin. The contents in the marked area were scraped off with a stainless steel spatula, and added directly into a wide-mouth miniature column (8 cm x 0.8 cm OD), fitted with grease-free stopcock and a small filter bed (1.5-2.0 cm in length) of celite 545 (BDH Chemicals, Poole, England) over a cotton plug. The column was previously flushed with diethyl ether, and the filter bed was always kept under diethyl ether (Gholap et al. 1971).

The eluted compound was recovered, after removing the solvent by passing a slow stream of nitrogen gas and studied further for identification purposes. Gas liquid chromatographic (GLC) analysis of the essential oil, and of the TLC separated major fraction, was performed in a gas chromatograph (GC-15A, Shimadzu, Kyoto, Japan), fitted with flame ionization detector, and a column of stainless steel (0.625 cm o.d x 180 cm) packed with 10% carbowax 20 M (Field Instruments, Surrey, UK) and supported on acid wash chromosorb W (60-80 mesh). The column temperature was programmed at 140-210°C, at the rate of 4°C/min. Initial hold was for 5 min, and final temperature hold was for 15 min. Injector and detector temperatures were maintained at 220°C. Nitrogen at a rate of 40 ml/min was used as carrier gas. Peak areas were determined through integration, by peak area normalisation, using Chromatopac data system (C-R6A, Shimadzu, Kyoto, Japan).

For high performance thin layer chromatography (HPTLC), the essential oil, TLC separated major fraction and standard vanillin (BDH Chemicals, Poole, England) in ether (1% solution) were individually applied at 2 µl quantities by Linnomat IV (CAMAG, Muttenz, Switzerland) on 10 x 10 cm HPTLC silica gel 60 F_{254} precoated plate (E. Merck, Darmstadt, Germany). The application was made by spray-on technique, in the form of a band, at a delivery speed of 10 sec/µl. A nitrogen pressure of 2 psi was maintained. The chromatogram was developed in CAMAG twin trough (10 x 10 cm) with hexane:ethyl acetate (85:15, v/v) as solvent system. Development was completed, when the solvent front had travelled 6 cm. The plate was dried in air for 5 min, and then subjected to densitometric analysis, in the UV range of 200-400 nm, using a CAMAG TLC Scanner II with LABDATA system and CATS evaluation software, volume 3.15 (CAMAG, Muttenz, Switzerland). The instrument was kept in reflectance mode with deuterium lamp, monochromator band width 30 nm, slit dimensions of 0.3 x 4 mm, and scanning speed of 4 mm/sec for recording respective spectra. Further densitometric evaluation of all samples was done at 290 nm in the same scanner.

Proton magnetic resonance spectra of the major fraction separated by TLC were recorded on a PMR spectrophotometer (A 60 A, Varian, USA), using carbon tetrachloride as solvent, and tetramethylsilane as an internal standard.

For studying the effect of the essential oil on microorganisms, a saline suspension of a 24-h growth was made on an agar slant. Different quantities of essential oil were added to test tubes containing 5 ml of sterile nutrient broth. One ml of culture suspension (cell density 0.3×10^8/ml) was added to each of these tubes, while the inoculated tube, devoid of the essential oil, served as control. The tubes were incubated at 37°C for 48 h, and the number of survivals in each tube was determined by pour plate method, using plate count agar (FAO 1979). The microorganisms studied were *Escherichia coli* (ATCC 10148), *Salmonella typhi* (NCTC 786), *Saccharomyces uvarum* (NCIM 3220) and *Bacillus cereus* (local isolate). Effect of vanillin was also determined as per the above procedure. The cultures were maintained by sub-culturing on nutrient agar slants at monthly intervals. Except for *S. uvarum*, all other cultures were obtained from the Haffkine Institute, Bombay.

Khoa was prepared from whole milk in the traditional manner, under hygienic conditions. Different quantities of the essential oil were weighed and transferred to sterile petri plates. Approximately 25 g of *khoa* was added to each plate and the entire content was mixed aseptically. A control devoid of essential oil was maintained. All the plates were wrapped in aluminium foil and stored in a refrigerator for 7 days. Total plate count (TPC) was done at the end of 7 days by pour plate method using plate count agar incubated at 37°C.

Tomato puree was prepared from ripe tomatoes, under hygienic conditions, and it was boiled for 10 min. No salt or spices were added, but corn flour, at a level of 0.1%, was added in order to avoid separation of solids from the juice during the boiling process. Fifty ml of puree was transferred to each of the sterile bottles; then, different quantities of the essential oil were added and mixed thoroughly; the opening of the bottle was covered with aluminium foil. A pair of controls, devoid of the essential oil, was maintained. All the bottles were stored in a refrigerator for 3 weeks. Aerobic and mould/yeast

TABLE 1. COMPARATIVE EFFECT OF ESSENTIAL OIL AND VANILLIN ON VARIOUS MICROORGANISMS

	Total plate count as colony forming units/ml broth						
		Essential oil, mg/5ml broth			Vanillin, mg/5 ml broth		
Organism	Control	30	40	50	30	40	50
E. coli	1.35×10^8	ND	ND	ND	3×10^8	3×10^8	3×10^8
S. uvarum	3.50×10^8	ND	ND	ND	ND	ND	ND
S. typhi	2.65×10^8	ND	ND	ND	1.16×10^5	ND	ND
B. cereus	4.80×10^7	7.5×10^8	8.2×10^6	2.5×10^7	3×10^7	1.8×10^7	1.93×10^7

ND : No survivor detected

counts were done at the beginning and end of 3 weeks storage, using the pour plate method. Incubation temperatures were 37°C for aerobic, and 27°C for yeast and mould counts. All the plate counts were analyzed by standard methods (FAO 1979).

Results and Discussion

The yield of essential oil of *Decalepis hamiltonii* varied, depending on freshness of the root (0.54% from fresh root, as compared to 0.13% from heat-treated and air-dehydrated roots). Thus, some loss of volatile components occurred during heat and air dehydration treatment. However, the essential oil in all the cases gave characteristic odour of vanillin. The essential oil got resolved into a few minor components, apart from a major one at R_f 0.623. Amongst the solvent systems used, petroleum ether : diethyl ether (80:20, v/v) was found to be the best. On TLC separation, the root essential oil was devoid of any component corresponding to the R_f value of vanillin. This indicates the possible existence of a structural variation between the TLC-separated, major fraction of the essential oil of *Decalepis hamiltonii* and standard vanillin.

GLC analysis of whole oil and the TLC-separated major fraction showed a similar pattern of separation, but with an appreciable difference in the relative proportions. The relatively lower content of the major fraction could be attributed to its loss during isolation. A similar separation of steam-distilled oil of *Decalepis hamiltonii* on GLC (OV 1 column) has been reported (Divakaran et al. 1985). In densitometric evaluation of HPTLC chromatograms, 3 peaks, at migration distances of 10.1, 43.2, 56.8 mm, were observed, both in the essential oil and in TLC-separated major fractions. Standard vanillin gave a peak at migration distance 18.9 mm, and so, its presence in the essential oil of *Decalepis hamiltonii* was ruled out. This was also observed in the analytical TLC study.

UV spectra of the essential oil of the TLC-separated major fraction, and of vanillin showed similar absorption maxima at 281 and 314 nm. This suggests the presence of a phenolic group in the molecule, and a close similarity in structure between the TLC-separated major fraction and vanillin. The presence of phenolic and aldehyde functional groups was further confirmed by NMR studies. An NMR signal at δ 3.9, corresponding to singlet of 3 H, indicated the presence of a methoxy group, while a downfield singlet of 1 H at δ 9.7 showed the presence of an aldehyde group. Presence of an aromatic ring was indicated by a singlet of 1 H at δ 6.3, and doublets of 1 H at δ 6.6 and δ 7.4. Data suggest that the aromatic ring is substituted at 2 of its 6 positions. The NMR data were comparable to those of standard 2 hydroxy-4-methoxy benzaldehyde described in the literature (Pouchart 1983).

Comparative effect of essential oil and vanillin on microorganisms is illustrated in Table 1. The essential oil was inhibitory to *S. uvarum*, *E. coli*, and *S. typhi* at concentration; of 30 mg for 10^8 nos of organisms. Vanillin was effective against *S. uvarum* at same concentration; but a concentration of 40 mg was needed for inhibitory action on *S. typhi*. Vanillin was not effective against *E. coli*. Neither the essential oil nor vanillin was effective against *B. cereus*. The essential oil was found to restrict microbial growth at the concentration of 0.16 g/

TABLE 2. EFFECT OF THE ESSENTIAL OIL ON MICRO-BIOLOGICAL QUALITY OF *KHOA* AND TOMATO PUREE STORED IN A REFRIGERATOR FOR 7 AND 21 DAYS.

	Khoa	Tomato puree	
Essential oil used, mg	Total plate count, cfu/g at 7 days	Total plate count, cfu/ml at 21 days	Yeast + mould, cfu/ml at 21 days
0 (control)	1.0×10^8	2.32×10^7	3.0×10^7
30	6.2×10^4	1.50×10^6	2.6×10^5
40	ND	ND	ND

ND : No survivor detected

100 g *khoa* and 0.08 g/100 ml tomato puree. The microbial counts were nil in refrigerated *khoa* and tomato puree samples, after 1 and 3 weeks, respectively (Table 2.)

Thus, the present study has resulted in standardizing the extraction procedure for isolation of essential oil of *Decalepis hamiltonii* and identification of its major component. The extracted essential oil is a promising antimicrobial agent. Further experiments are needed to study the specificity of the effect on particular groups of microorganisms.

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Protease Secretion by Resting Cells of *Aeromonas hydrophila* as a Possible Mode of Survival in Flesh Foods

S.N. DOKE, S.B. WARRIER, M.D. ALUR*, V. VENUGOPAL AND P.M. NAIR

Food Technology and Enzyme Engineering Division,
Bhabha Atomic Research Centre, Trombay, Bombay-400 085, India.

Washed, resting cells of *Aeromonas hydrophila* secreted protease in the absence of nutrients. The amount of protease secretion, measured in terms of tyrosine release, was found to be dependent on the level of initial cell numbers. The protease secretion was inhibited by chloramphenicol, thereby suggesting *de novo* synthesis of the enzyme. The enzyme secretion was enhanced by myosin, and more significantly, by trypsin-treated myosin. The results suggest that the major structural proteins of muscle can influence protease secretion by the bacterium. Using protease inhibitors, the proteases secreted by *A. hydrophila* were characterized as serine and metallo-proteinases.

Keywords : *Aeromonas hydrophila*, Resting cells, Protease secretion, Survival mode, Muscle foods.

Muscle foods, during post-mortem, are exposed to contamination by a variety of microorganisms (Gupta et al. 1983; Thampuran and Mahadeva Iyer 1989). The structural proteins of muscle, consisting of myosin, actin and other regulatory proteins are not permeable to bacterial cells (Gill and Penny 1977). Utilization of large muscle proteins for microbial growth is facilitated by the hydrolytic action of proteases secreted by the organisms (Thomas et al. 1987). However, secretion of these enzymes during the exponential state of bacterial growth is minimal (Glenn 1976). Thus, it is likely that, the free amino acid pool in the muscle is rapidly depleted during early stages of bacterial growth, and the cells reach a state of nutrient limitation. Thereafter, further breakdown of complex proteins will require secretion of proteases by these cells.

Aeromonas hydrophila, is a bacterium of public health significance. It has been isolated from unirradiated spoiling mackerel (Alur and Lewis 1980). During studies on the spoilage potential of some contaminant bacteria, isolated from Indian mackerel, it has been observed that *A. hydrophila* brings about faster spoilage of fish protein (Alur et al. 1989). It is of significance to note that growth of *A hydrophila* and spoilage of fish protein occur within 6-8 h (Alur et al. 1989; Paturkar et al. 1992). The growth curves of *A. hydrophila*, along with protease secretion in nitrogen fractions of proteinaceous foods have been reported earlier, (Alur et al. 1988). It has also been recognized as a widely prevalent bacterium in several muscle foods (Palumbo et al. 1985). Its pathogenicity has also been demonstrated (Buchnan and Palumbo 1985). A detailed study on protease secretion by resting cells of *A. hydrophila* is desirable to understand the role of its survival in flesh foods, and to develop appropriate methods for preventing spoilage of flesh foods.

The present work was, therefore, undertaken to study protease secretion by starving cells of *Aeromonas hydrophila* and the influence of myosin on the enzyme secretion. No attempt was made to investigate the pathogenicity of *A. hydrophila* in this study, which was confined to establishing the degradative ability of the bacterium.

Materials and Methods

Aeromonas hydrophila used in this study was isolated from Indian mackerel (*Rastrelliger kanagurta*) (Alur et al. 1988). It was grown in 0.8% (w/v) Difco nutrient broth (200 ml) on a rotary shaker for 18 h, before centrifugation at 6,000 rpm at 0°C for 30 min, to harvest the cells. The cell pellet was washed thrice with 0.01 M phosphate buffer (pH 7.2) and resuspended in the same buffer at a turbidity equivalent to an absorption of 400 units in a Klett-Summerson photocolorimeter, using red filter.

The cell suspension (10 ml) was incubated on a rotary shaker at 25°C. Aliquots (3 ml cell suspension) were withdrawn at regular intervals, centrifuged at 6,000 rpm for 15 min at 0°C, and the supernatants were used for protease assay, using haemoglobin (Difco) as the substrate. The assay system, containing 15 mg substrate in 0.1 M phosphate buffer, (pH 7.5) and cell supernatant, was incubated at 50°C for 30 min, and the tyrosine liberated was estimated as described earlier (Pansare et al. 1985). One unit of activity was expressed as μ mole tyrosine liberated per ml culture supernatant, during the incubation period, under the assay conditions.

* Corresponding Author

Myosin from white muscle of Indian mackerel was isolated according to the procedure of Shenouda and Piggot (1974). The fish muscle (30 g) was homogenized in ice-cold 0.01 M phosphate buffer (pH 7.5), containing 0.01 M pyrophosphate, 0.4 M KCl and 0.001 M β-mercaptoethanol. The homogenate was centrifuged at 10,120 x **g** for 15 min at 0°C. The supernatant was diluted to 800 ml, using chilled, distilled water. The precipitate was collected by centrifugation at 10,120 x **g** for 15 min at 0°C. The residue was dissolved in 0.4 M KCl (100 ml), and the protein content of the myosin preparation, as measured by the method described by Lowry et al (1951), was found to be 8 mg/ml. The myosin was dialyzed overnight at 0°C against 0.4 M NaCl, prior to use.

Results and Discussion

Survival of resting cells of *A. hydrophila*, during prolonged incubation at room temperature on a rotary shaker, showed no decline in the first 6 h, but did show 50% cell inactivation after 24 h.

Fig.1 depicts the data on tyrosine release as a function of initial cell density of the bacterium incubated in phosphate buffer. It is of interest to note that the cell density played a significant role in tyrosine release. All the systems exhibited cell density of 10^8 cells/ml after 18 h incubation. In order to obtain optimum protease activity of cells, cell suspensions (10^{10} cells/ml) were used for further study.

Fig.2 shows secretion of protease by washed

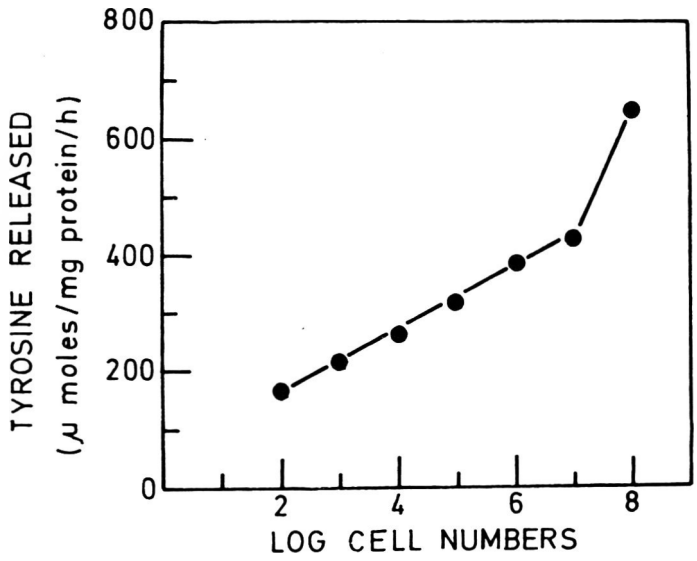

Fig.1. Tyrosine released as a function of cell numbers seeded in buffer.

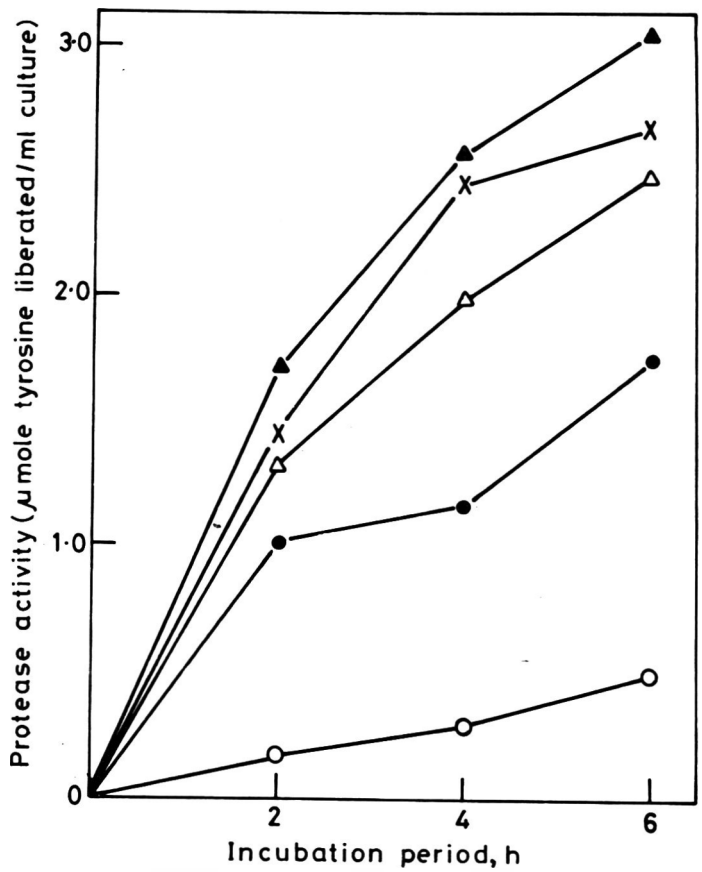

Fig.2. Secretion of protease by resting cells of *A. hydrophila*. —O— : 1 ml, —●— : 2 ml, —Δ— : 6 ml, —x-- : 8 ml, —▲— : 10 ml.

cell suspension of *A. hydrophila*. The cells secreted the enzyme in the absence of any carbon and

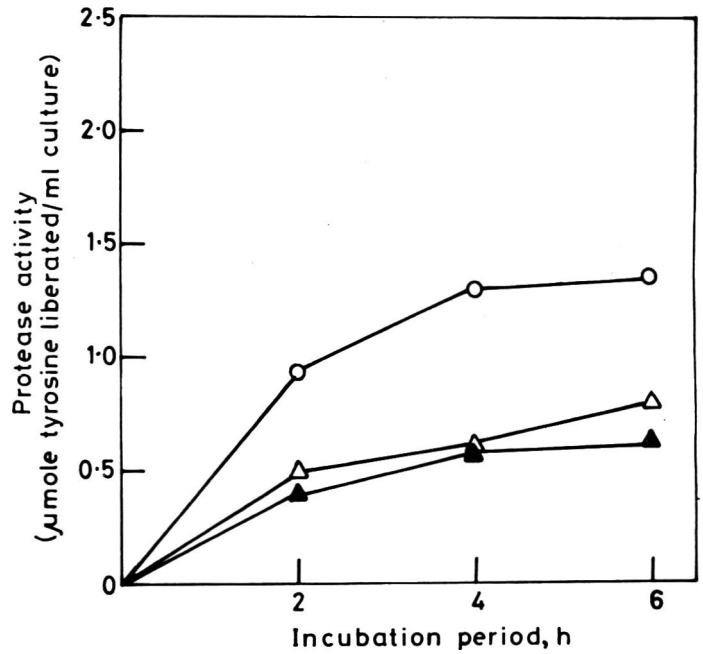

Fig.3. Effect of chloramphenicol on protease secretion by *A. hydrophila*. A volume of 10 ml cell suspension was used. Chloramphenicol concentrations —O— : 0 μg, —Δ— : 100 μg, —▲— : 1000 μg.

nitrogen sources. With increase in cell concentrations, higher amounts of the enzyme were secreted. Since resting cells secreted protease in the absence of nutrients, it was of interest to examine whether the protease was synthesized *de novo* during starvation. This was examined by measuring the enzyme secretion in the presence of chloramphenicol. The secretion was found to be inhibited by chloramphenicol at 100 and 1,000 µg/ml level, suggesting *de novo* synthesis of the enzyme by the cells (Fig.3).

The influence of myosin on protease secretion by the resting cells is shown in Fig.4. The cells secreted appreciable amounts of protease in the presence of increasing concentration of myosin. It is likely that the low amounts of protease secreted by the resting cells could hydrolyze large muscle protein, and the resulting hydrolytic products could act as a substrate that triggers the protease synthesis. This was examined by determining the effect of hydrolyzed myosin on the enzyme secretion. Myosin (8 mg/ml) was treated with 100 µg trypsin in 0.01 M phosphate buffer, pH 7.5, at 50°C for 2 h. After the treatment, trypsin was heat-inactivated

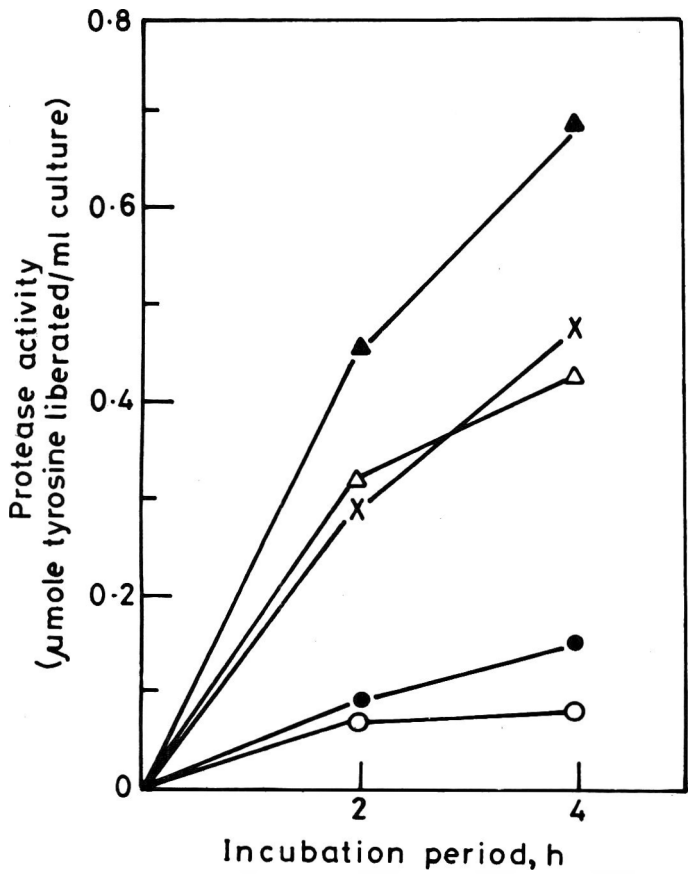

Fig.4. Effect of myosin concentration on protease secretion by *A. hydrophila*. —O— : No myosin, —●— : 8 mg protein/ml, —Δ— : 16 mg protein/ml, —▲— : 32 mg protein/ml, —x— : 64 mg protein/ml.

TABLE 1. EFFECT OF HYDROLYZED MYOSIN ON PROTEASE SECRETION

Incubation period, h	Protease activity (µ mole tyrosine released/ml culture supernatant)		
	No myosin	Non-hydrolyzed myosin	Trypsin-hydrolyzed myosin
2	0.10	0.50	0.74
4	0.20	0.75	1.03
5	0.25	0.87	1.20

Protease activity was measured using 1 ml cell suspension of *A. hydrophila* in a total volume of 10 ml containing 8 mg trypsin hydrolyzed or non-hydrolyzed myosin.

at 100°C, in a boiling water bath. The effect of hydrolyzed myosin on enzyme secretion is given in Table 1. It is evident that the protease secretion was enhanced in the presence of hydrolyzed myosin, as compared with unhydrolyzed myosin.

Further, the proteases elaborated by *A. hydrophila* were characterized by using specific protease inhibitors (Table 2). Phenylmethyl sulfonylfluoride (PMSF) and ethylene diamine tetra-acetic acid (EDTA), both at 2 mM concentrations, inhibited protease synthesis in resting cells of *A. hydrophila* to the extent of 50 and 90%, respectively. Thus, the proteinases of *A. hydrophila* could be assigned to the categories of serine and metallo-proteinases.

The results suggest a mechanism for survival of *A. hydrophila* in muscle foods. The process may have two stages. Firstly, initial synthesis and secretion of protease by the cells, during nutrient limitation conditions; and, secondly, the myosin-dependent induction of the enzyme synthesis. The initial growth is probably at the expense of low molecular weight components present in sarcoplasmic (soluble) fractions of the muscle. These have been shown to be required for supporting initial growth of spoilage and pathogenic microorganisms (Yada and Skura 1981; Dainty et

TABLE 2. EFFECT OF INHIBITORS ON SECRETION OF PROTEASES BY *AEROMONAS HYDROPHILA* DURING INCUBATION

Incubation at RT, h	Protease activity (µ mole/mg protein/h)				
	Inhibitor (2 mM) used				
	Control	Pepstatin	PCMB	PMSF	EDTA
3	2,059	1,819	2,196	1,169	274
6	2,402	2,463	2,300	1,407	274
24	2,670	2,610	2,898	572	572

PCMB: Parachloromercuribenzoate, PMSF: Phenylmethyl-sulfonylfluoride, EDTA : Ethylenediamine tetracetic acid, RT: Room temperature.

al. 1983). Subsequently, it comes under nutrition limitation stage. Bacterial cells under nutrient limitations have been shown to initiate a rapid programme of metabolic adjustments, termed as stringent response (Cozzone 1981). It is here that the second stage comes into picture and the bacterium is able to survive. The synthesis of protease under nutrition limitations has also been observed in *Bacillus subtilis*, wherein mutants are known to have higher levels of protease (Kadrekar and Rama Sarma, 1990; May and Elliott 1968). Such enzyme synthesis in *Vibrio alginolyticus* under induction by peptone was related to the accumulation of stable mRNA (Reid et al. 1980). A similar protease synthesis in *Erwinia chrysanthemi* was reported to be produced by hydrolyzed casein and not by unhydrolyzed casein (Wandersman et al. 1986). Myosin-dependent secretion of protease by the bacterium has also been harnessed in our laboratory, to prepare protein hydrolysate from waste fish (Venugopal et al. 1988).

Involvement of protease in allowing the growth of microorganisms in muscle foods has been well recognized (Gill and Penny 1977). Bacterial penetration of muscle is known to be favoured by extracellular proteases (Thomas et al. 1987). Such bacterial penetration of muscle could be enhanced by secretion of multiple forms of proteases, and also by low substrate specificities of the secreted proteases. In these respects, it is worthwhile noting that *A. hydrophila* has been recognized to secrete two proteinases and one amino-peptidase (Pansare et al. 1985). These proteinases could act on several proteins having maximum activities on myofibrillar proteins (Pansare et al. 1985). Consequently, these properties could facilitate proliferation of *A. hydrophila* and could explain its survival in several muscle foods (Palumbo et al. 1985).

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Composition and Nutritive Value of Sun-dried *Puntius sophore*

Ch. SAROJNALINI* AND W. VISHWANATH

Department of Life Sciences, Manipur University,
Canchipur - 795 003, Imphal, Manipur, India.

Proximate composition and digestibility of sun-dried *Puntius sophore*, a low-cost variety of sun-dried fish, used in a traditional fermented fish preparation (*Ngari*) in Manipur, were evaluated. Sun-dried *Puntius* contained (%) moisture 18.1, proteins 45.0, lipids 18.5 and ash 11.0. *In vitro* digestibility of protein by pepsin was 44.1% in 2 h, as against 55.7% by pepsin + trypsin in 24 h. Nitrogen intake, nitrogen output, and digestibility in feeding trials on laboratory rats were 8.8, 2.3 and 82.0, as compared to the values of 7.7, 0.7 and 92.7, respectively, for reference casein. The biological value, food conversion ratio, and protein efficiency ratio were 96.8, 3.1 and 2.0, as against the respective values of 97.1, 3.7 and 2.7 for reference casein. The α-amino nitrogen of *Puntius* was 32.6 mg/g total nitrogen. The total volatile basic nitrogen value, thiobarbituric acid number and peroxide value were within the acceptable limits.

Keywords : *Puntius sophore*, Sun-dried fish, Proximate composition, Protein digestibility, Biological value, Protein efficiency ratio, Thiobarbituric acid number.

The most important contribution of fishes to human diet is the supply of much required protein of good quality (Valanju and Sohonie 1957; Moorjani and Vasantha 1973). Small sized fishes are not preferred due to their lower meat content and bony nature (Sarojnalini and Vishwanath 1988). However, such low valued fishes offer minerals in their most edible forms and in more abundance than the large sized fishes do (Higashi 1962). In Manipur (India), the consumption of fresh *Puntius* is not common, mainly due to its poor taste (Sarojnalini and Vishwanath 1988). However, sun-dried *Puntius* is used in the preparation of a fermented fish paste 'Ngari' for human consumption (Sarojnalini and Vishwanath 1988).

Some reports have been published in India on the digestibility and nutritive value for fishes and their improvement (Sawant and Magar 1961; CSIR 1962; Rao et al. 1965; Rege et al. 1986; Ammu et al. 1986; Das et al. 1979). Though little work has been done on the nutritive value of small-sized fish, there are a few reports on the nutritive value of small sized fishes of Manipur (Vishwanath and Sarojnalini 1989; Bijen et al. 1990). In the present paper, proximate composition and nutritive value of sun-dried *Puntius sophore* are reported.

Materials and Methods

Sun-dried *Puntius sophore* (6.5 - 7.5 cm in length) was purchased from the local market. The fishes were defatted by chloroform-methanol mixture as per the standard methodology (Folch et al. 1957). Pepsin and trypsin were from Loba Chemie.

Proximate analysis : Total nitrogen, moisture, and lipid contents were estimated in sun-dried sample, following the standard methods (AOAC 1975). Total ash content was estimated by igniting the sample at 550°C in a muffle furnace (AOAC 1960). Protein values were calculated by multiplying the nitrogen values by 6.25 for fishes and by 5.7 for wheat (Osborne and Voogt 1978).

In vitro *digestibility :* Defatted samples and 0.2% pepsin solution were prepared according to AOAC (1975). Six conical flasks were taken, and 1 g of powdered, defatted sample, and 150 ml of pepsin solution were added to each flask. The flasks were then incubated at 40 ± 1°C for 2 h with constant shaking in a metabolic shaker (80 rpm). At the end of 2 h, the contents of 3 flasks were filtered. To each of the remaining 3 flasks, 0.3 g of trypsin was added, after adjusting the pH of the suspension to 8.2. Incubation was continued at 45°C for a further 22 h. and then the suspensions were centrifuged at 5000 rpm for 20 min. Digestible protein in pepsin digest and combined pepsin and trypsin digest were expressed as % total proteins of the sample. For comparison, experiments were carried out using standard casein under the same conditions.

Alpha-amino nitrogen : Alpha-amino nitrogen was estimated by formol titration as per Sorensen's method (AOAC 1984). The aliquots, after 2 and 24 h of enzyme hydrolysis, were used for α-amino nitrogen determination.

Feeding experiments : Weanling male albino rats (21 days old), weighing 25-30 g, were selected for *in vivo* digestibility studies, protein efficiency

* Corresponding Author

ratio (PER), food conversion ratio (FCR) and biological value (BV) of the sun-dried *Puntius*. For feeding purposes, food prepared from sun-dried *Puntius* (test diet) was used along with the control diet, and the protein-free diet. All the diets contained 10% protein, except for the protein-free diet. Control diet contained vitamin-free casein (ICN pharmaceuticals, Ohio), while lipid-free fish powder was added to the test diet, so as to provide 10% total protein (N x 6.25). The protein-free diet contained (g) cellulose 5, sucrose 20, starch 65, and refined groundnut oil 5 in a 100 g final diet. The other ingredients include 9 g of refined groundnut oil in control and test diets. All the diets contained 4 g of a salt mixture (AOAC 1960) and 1 g of a vitamin mixture (AOAC 1960). Wheat flour was added to make up the weight to 100%, wherever required (Bijen et al. 1990). The final protein content of the diet, which also included protein from wheat flour, was 17.8%. Urine and faecal samples were collected every day as described by Ammu et al (1986).

Protein efficiency ratio (PER) was obtained by dividing weight gain by protein intake (ISI 1982). Absorbed nitrogen (absorbed N) was calculated by subtracting metabolic nitrogen (metabolic N) from nitrogen intake (N intake) (ISI 1962). The value of food conversion ratio (FCR) was obtained by dividing food intake by weight gain (ISI 1982) and biological value was the ratio of retained nitrogen (retained N) to absorbed N (ISI 1982).

Tests for spoilage : Non-protein nitrogen (NPN) was determined by precipitating all protein by treating with TCA. Residue was taken for protein-nitrogen estimation (Cutting 1962). The value of protein N was subtracted from total N to obtain non-protein N. Water-soluble nitrogen (WSN) was determined by difference between total nitrogen and insoluble nitrogen. Sample was homogenized 2 times with distilled water and then centrifuged. Residue was taken for nitrogen estimation, which gives the value of insoluble nitrogen. Total volatile basic nitrogen (TVBN) was estimated by using TCA extract in a Conway apparatus, following the method of Morris (1959). Thiobarbituric acid (TBA) number was estimated by the method of Sinhubar and Yu (1958). Free fatty acid was analyzed following the method of Morris (1959). Oil was extracted with ethyl ether and dissolved in 95% neutral alcohol, followed by titration with 0.1 N NaOH. The value was expressed as % of oleic acid. Peroxide value was estimated, following the method of Morris (1959).

Results and Discussion

Proximate composition : Sun-dried *Puntius sophore* contained (%) moisture 18.1 ± 0.1, lipids 18.5 ± 0.01, proteins 45.0 ± 0.1 and ash 11.0 ± 0.2, as against the levels of 14.0 ± 0.22, 0.8 ± 0.04, 10.3 ± 0.21 and 0.7 ± 0.02, respectively, in wheat flour. It is emphasized that the lipids, proteins and ash are many times higher in sun-dried *Puntius*, as compared to those in wheat flour. About 16 times more ash in sun-dried *Puntius* is due to its higher bony consistency and scaly characteristics. Higher lipids and protein contents are of importance from nutritional point of view, as they constitute a cheaper source of these nutrients for the ever increasing population in the country.

Spoilage status of sun-dried Puntius : Various tests performed gave valuable insight in the spoilage status of proteins and lipids of sun-dried *Puntius*. The TVBN of sun-dried *Puntius* was 4.6 mg/g. The sun-dried *Puntius*, therefore, cannot be classified as spoiled fish, as any product with less than 10 mg TVBN/g is considered to be fresh, according to Morris (1959). Similar is the case for TBA number. According to Sinhuber and Yu (1958), a processed product having upto 3 TBA number is regarded as being in good condition. In the present case, TBA number of sun-dried *Puntius* is 0.507 mg/1000 g material, which is within the acceptable limit. Taniguchi (1988) reported that the peroxide value of lipids in fishes increased during drying, and also depended on smoking time. The low peroxide value of the sun-dried *Puntius* (4.20 millimoles/g) shows that sun-drying did not cause much oxidation of the lipids. The levels of free fatty acid (as oleic acid), water-soluble nitrogen and non-protein nitrogen in sun-dried *Puntius* were 42.86 mg/g, 3.26% and 2.60%, respectively. The data, thus, indicate minimum spoilage of proteins and lipids in sun-dried *Puntius sophore.*

In vitro and in vivo digestibilities : The digestibility values of fish samples were 74.1 ± 0.2 and 85.7 ± 0.01, respectively, when treated with pepsin alone for 2 h and pepsin + trypsin mixture for 24 h. In case of casein, the corresponding values were 88.0 ± 0.7 and 98.0 ± 0.33, respectively. The comparatively lower digestibility of *Puntius* protein, as compared to that of casein, may be due to the use of the whole of the sun-dried *Puntius* body in the digestibility experiments, which were performed in six replicates. As has been pointed out earlier, the sun-dried *Puntius* has less flesh and lots of scales as well as bones. The *in vitro* digestibility

TABLE 1. DATA ON NUTRITIONAL CHARACTERISTICS OF THE SUN-DRIED *PUNTIUS SOPHORE* IN COMPARISON WITH CASEIN.

Attribute	Value	
	Casein	Sun-dried *Puntius sophore*
Alpha-amino nitrogen release, %	ND	3.26
Pepsin alone for 2 h	88.00	74.13
Pepsin + trypsin for 24 h	98.03	85.74
Digestibility data		
Total nitrogen in diet, g	7.67	8.76
Nitrogen in excreta, g	0.72	2.25
Apparent digestibility, %	90.61	74.32
True digestibility, %	92.69	82.08
Food intake, g	416.10	322.17
Nitrogen balance	ND	ND
Nitrogen retained, g	6.95	6.51
Nitrogen absorbed, g	7.16	6.72
In vivo protein digestion, %	92.69	82.08
Food conversion ratio, g	3.73	3.10
Biological value, %	97.06	96.75

will be much higher, if the flesh portion of the sun-dried *Puntius* is used in the experiment. There were some differences in the liberation of alpha-amino nitrogen during pepsin and pepsin + trypsin digestions. The release of alpha-amino nitrogen was greater in case of pepsin + trypsin digestion for 24 h, as compared to the digestion by pepsin alone for 2 h (Table 1). Similar results have also been reported in the Bombay fish by Valanju and Sohonie (1957).

The trends of the *in vivo* digestibility values of sun-dried *Puntius* were similar to those of the *in vitro* digestibility studies (Table 1). The lower *in vivo* digestibility of the fish sample may be attributed to inadequate contact of digestive enzymes in the gut of rats, due to the presence of scales and bones in the fish consumed.

Nutritional characteristics : The data presented in Table 1 revealed lower apparent and true digestibilities of sun-dried *Puntius*, as compared to those of casein. The total nitrogen in diet was lower, while the nitrogen excreted was higher in case of sun-dried *Puntius*. Similarly, the food intake, nitrogen retained, nitrogen absorbed were lower in case of sun-dried *Puntius*, as compared to those for casein diet (Table 1). Consequently, the food conversion ratio and biological value were also slightly lower in case of sun-dried *Puntius*. These differences are, probably, due to the fact that the relative proportion of dietary nitrogen, entering into growth and maintenance of the rats, depends upon the amount and nutritive quality of dietary proteins (Barnes et al. 1946).

The comparative weight gain and protein intake by rats fed on a casein, or on a sun-dried *Puntius* diet (Table 2) showed a more or less equal weight gain at 4-day intervals, in the case of the casein diet, but it was of irregular nature in the case of sun-dried *Puntius*. However, the total weight gain after 28 days feeding was slightly higher in case of sun-dried *Puntius*. Similar trends were also observed in the protein efficiency ratios. In contrast, the protein intake from fish-based diet was greater than that from casein diet, and resulted in a total protein intake of 58.6 ± 5.5 and 41.6 ± 0.99 for fish-based and casein diets, respectively.

The protein efficiency ratios after 28 days of feeding were 2.5 ± 0.5 and 2.0 ± 0.39 for casein and fish-based diets, respectively (Table 2). According to Indian Standards Specifications, protein-rich concentrated nutrient supplementary foods should have a protein efficiency ratio of 2.0 (ISI 1962). Thus, the sun-dried *Puntius* can be considered as a food, which meets this specification.

The growth rates of rats fed on casein and fish-based diets are represented in Fig. 1. It shows that the growth rate of the rats fed on fish-based diets

TABLE 2. PROTEIN INTAKE AND WEIGHT GAIN BY THE RATS DURING 28 DAYS OF EXPERIMENT AND PROTEIN EFFICIENCY RATIO

Days	Casein			Sun-dried *Puntius sophore*		
	Weight gain	Protein intake	PER	Weight gain	Protein intake	PER
1-4	14.0 ± 0.64	3.7 ± 0.37	3.9 ± 0.81	6.5 ± 2.12	5.5 ± 0.41	1.2 ± 0.47
5-8	13.3 ± 1.21	4.1 ± 0.67	3.2 ± 0.39	23.0 ± 4.24	6.8 ± 0.21	3.4 ± 0.52
9-12	16.3 ± 2.13	5.1 ± 0.09	3.2 ± 0.07	11.0 ± 7.07	7.7 ± 0.21	1.4 ± 0.88
13-16	12.7 ± 1.57	5.2 ± 0.06	2.0 ± 0.16	21.0 ± 4.24	8.7 ± 0.71	2.40 ± 0.29
17-20	16.0 ± 0.00	6.3 ± 0.35	2.6 ± 0.23	13.0 ± 4.24	9.6 ± 1.17	1.3 ± 0.28
21-24	18.0 ± 0.91	7.4 ± 0.91	2.4 ± 0.18	22.0 ± 16.97	9.6 ± 1.97	2.2 ± 1.32
25-28	21.3 ± 1.31	8.8 ± 1.04	2.4 ± 0.38	18.0 ± 2.82	10.78 ± 2.04	1.7 ± 0.05
1-28	111.7 ± 3.91	41.6 ± 0.99	2.5 ± 0.50	114.5 ± 32.2	58.63 ± 5.50	2.0 ± 0.39

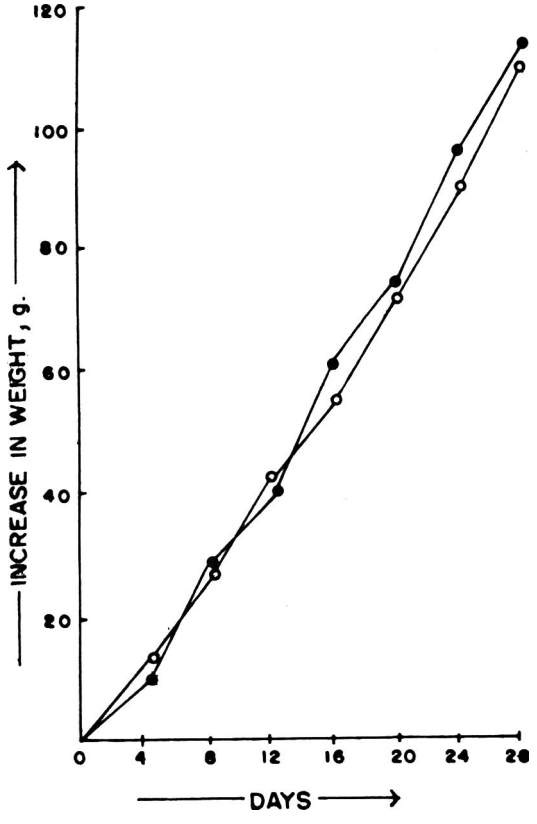

Fig. 1. Growth rate of male albino rats fed with casein and sun-dried *Puntius sophore* diets.
O : Casein, ● : *Puntius sophore*.

are slightly higher than that of rats on a casein diet. This is in spite of the comparatively lower protein efficiency ratio in case of fish-based diet, in comparison to casein diet (Table 2). These results show that the processed, small-sized *Puntius* has a nutritive value that is higher than, or comparable to, that of the casein.

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Biochemical Changes During Production of *Ogiri* - A Fermented Food Condiment from Castor Seeds *(Ricinus communis)*

GEORGINA N. ANICHE*[1] AND STELLA NWOKEDI[2]

[1]Department of Applied Microbiology and Brewing,
Enugu State University of Science and Technology, P.M.B. 01660, Enugu, Nigeria.
[2]Department of Applied Microbiology,
Nnamdi Azikiwe University, Awka, Nigeria.

The changes in the chemical constituents of castor seeds, as a result of cooking for 6 h, as well as during *ogiri* production by fermentation, were investigated. Most of the constituents of castor seeds such as crude proteins, free fatty acids, total acidity, and some mineral elements were unaffected by cooking. Cooking decreased alpha-amylase and protease activities. Alpha-amylase activity and the levels of reducing sugars decreased during primary fermentation, but increased during secondary fermentation. Protease activity increased with fermentation time, while lipase activity was minimal.

Keywords : *Ogiri*, Fermentation, Castor seeds, Food condiment, Food constituents, Biochemical changes.

Fermented foods are essential parts of diets in all parts of the world, especially South East Asia, the near East and parts of Africa and India (Barber et al. 1988; Matta et al. 1991). Such foods include dairy products, baked cereal products, alcoholic beverages, fermented oilseeds and beans (Barber et al. 1988; Soni and Sandhu 1990; Potty et al. 1978). In Nigeria, oilseeds, especially African locust bean seeds (*Parkia* sp), and African oil bean seed, *(Pentacletura macrophylla)*, are fermented to produce *iru*, a soup condiment, and *ugba*, which is consumed as a snack (Ogbadu and Okagbue 1988), besides *ogiri-okipi*, a fermented food from *Prosopis* seeds (Odibo et al. 1992). The microbiological and toxicological aspects of fermentation of castor seeds *(Ricinus communis)* for *ogiri* production have been studied (Odunfa 1985a; Barber et al. 1988).

Castor seeds contain approximately 50% oil, 18% proteins (Anosike and Egwuatu 1981). The oil from the castor bean is a glyceride, with a unique composition in comparison with the other common vegetable oils, as its fatty acids consist of about 90% ricinoleic acids (Hargreaves and Owen 1947). Some studies have also been done on the processed protein fractions, but these were found to be toxic even to rats (Saroj Dua et al. 1987). The toxicity of ricin is eliminated, when a water solution of the ricin is boiled (Spies et al. 1962). Given enough time, the toxicity diminishes with an increase in the extent of denaturation (Levy and Bengalia 1950). Although castor meal is classified as an inedible oilseed meal, because of its toxic constituents (Levy and Bengalia 1950), the seed product, *ogiri*, is used as seasoning agent in soups after being detoxified by boiling and fermentation (Odunfa 1985a).

In the present study, results on the biochemical changes in the food constituents and enzyme activities of castor seeds, during fermentation are reported.

Materials and Methods

Castor seeds (1 kg) were dehulled by lightly breaking the shells with a stone to expose the cotyledons. Unhealthy cotyledons were discarded, while selected good grades were washed in water. The cleaned seeds were boiled in water for 6 h, after which the seeds were wrapped in plantain leaves in small packs of 50 g each. The packs of boiled seeds were placed in calabashes (dried gourds), lined and covered with plantain leaves and left to ferment for 4 days at room temperature (19 ± 1°C). This step is termed primary fermentation.

At the end of primary fermentation, the fermented seeds were unwrapped and mashed into a paste, using a wooden pestle and mortar. Ash from palm frond (0.1% of weight of castor seed paste) was added, the mixture re-wrapped in fresh washed plantain leaves, and fermented further at room temperature for 3 days. This fermentation phase is referred to as secondary fermentation. Samples were removed at 24 h intervals during the primary and secondary fermentations and kept frozen until analysis.

Analyses : Ground cooked, uncooked,

* *Corresponding Author*

unfermented, primary fermented castor seeds, and secondary fermented pastes were analyzed. Ground sample (5 g), was weighed into a 100 ml conical flask and 50 ml ethanol-water mixture (50:50, v/v) was added (Odunfa 1985b). The suspension was then washed with 5 ml petroleum ether to extract the oil, centrifuged at 5000 rpm, and the clear supernatant used for analysis. Fat, crude proteins, moisture, pH, free fatty acids, total titrable acidity, total solids, organic matter, crude fibre, elemental composition, total ash, carbohydrates, iodine number, peroxide value, saponification value, acid value, and organic acid content of all the samples were determined by AOAC (1980) methods. Duplicate determinations were made for each sample.

The test for ricin toxicity, as outlined by Rhee (1987), was adopted for the determination of ricin content in the samples. Red blood corpuscles were prepared and fixed according to the method of Bing et al (1967). The washed, packed, red blood cells were chilled to 4°C in an ice bath. Glutaraldehyde (15%) was diluted to 1% (w/v) with a solution containing 1 volumes of 0.15 M sodium phosphate (pH 8.2), 9 volumes of 0.15 M sodium chloride, and 5 volumes of distilled water. The glutaraldehyde salt solution was chilled to 4°C and used to dilute the packed red blood cells to 1 - 2% (v/v). The mixture of cells and glutaraldehyde was incubated at 4°C for 3 min. with occasional gentle mixing. The fixed cells were collected by centrifugation at 3000 rpm at room temperature and washed five times with distilled water. The cells were suspended to a final concentration of 30% in distilled water. Methiolate was added to a final concentration of 1: 10,000 (w/v) and cells stored at 4°C. Agglutination test was carried out on the samples and the rate of agglutination of blood cells was noted (Rhee 1987).

The total free sugars were determined by the dinitrosalicylic reagent method (Summer and Howell 1935). The optical density was measured with Pye Unicam SP6 250 spectrophotometer at 600 nm. The total sugar concentration was determined from a standard maltose curve. For alpha-amylase assay, the enzyme was extracted from the samples by grinding a 3 g sample in 20 ml of 0.1% acetate buffer, containing 0.2% calcium chloride (Hargreaves and Owen 1947). The suspension was centrifuged at 5000 rpm for 5 min (Odunfa 1985b). Alpha-amylase assay procedure described by Bernfield (1955) was used. The optical density of the resultant solution was measured at 540 nm using an SP6 200 spectrophotometer. The blank was similarly treated, except that dinitrosalicylic acid was added before adding the starch solution. The amount of reducing sugars formed was calculated from a standard curve prepared with known concentrations of maltose.

For protease assay, the extracting buffer used was 0.1 M tris-hydrochloric acid buffer (pH 7.0) containing 5 mM calcium chloride. The assay method was that of Yong and Wood (1977). The optical density of the filtrate was measured at 275 nm. Enzyme activity was expressed in terms of an arbitrary unit called an Xs unit and defined as an enzyme, in the form of 0.15% solution, which produced a filtrate with an optical density of 0.500, and when measured in a 10 mm path length cell,

TABLE 1. CHEMICAL CHANGES OF UNCOOKED AND COOKED CASTOR SEEDS*

Parameter	Uncooked seeds	Cooked seeds**
Moisture, % wet weight	5.42	42.40
pH	5.68	6.30
Total acidity (as acetic acid), %	0.48	0.48
Free fatty acids (as oleic acid), %	1.08	0.56
Crude fibre, %	17.55	15.02
Crude proteins, %	23.10	23.45
Ash, %	3.41	3.02
Fat, %	61.87	65.61
Carbohydrates, %	11.62	7.92
Organic matter, %	96.59	96.98
Total solids, %	94.58	57.60
Iodine number	146.26	232.58
Peroxide value	2.15	1.11
Saponification number	7.01	336.06
Calorific value, K cal/g	6.85	7.12
Acid value	2.15	1.11
Alpha-amylase activity, unit/g	6.23	2.51
Protease activity, unit/g	225.08	122.70
Lipase activity (as oleic acid), mM/g	ND	0.16
Ricin, titre	1000.00	22.00
Total sugars, mg/g	52.50	51.88
Magnesium, µg/100 g	3.29	5.40
Calcium, µg/100 g	7.71	9.54
Zinc, µg/100 g	1.62	2.67
Lead, µg/100 g	ND	1.08
Copper, µg/100 g	10.83	10.80
Iron, µg/100 g	11.87	19.16
Potassium, mg/100 g	0.57	0.65
Sodium, mg/100 g	0.09	0.12
Phosphorus, mg/100 g	5.55	4.56
Oxalic acid	-	-
Lactic acid	-	tr

* Mean of duplicate samples. ** Castor seeds were cooked for 6 h. ND : Not detected, - : absent, tr: trace.

TABLE.2. EFFECT OF FERMENTATION TIME ON THE CHEMICAL COMPOSITION OF CASTOR SEED AND PASTE DURING PRIMARY AND SECONDARY FERMENTATIONS*

Parameter	Primary fermentation ** time, days				Secondary fermentation *** time, days		
	1	2	3	4	5	6	7
Crude fibre, %	21.07	23.32	23.05	23.50	30.25	30.36	24.59
Crude proteins, %	21.17	20.21	20.04	20.91	19.08	17.85	17.15
Moisture, %	38.64	36.22	41.48	41.34	42.80	44.10	43.60
Ash, %	3.39	3.17	3.52	3.68	4.02	4.11	4.30
Carbohydrates, %	18.98	17.84	16.77	14.87	18.28	19.10	20.98
Fat, %	56.46	58.63	59.67	60.54	58.62	58.94	51.57
Organic matter, %	96.61	96.68	96.48	96.32	95.98	95.89	95.70
Total solids, %	61.36	63.78	58.52	58.66	57.20	55.90	50.40
Iodine number	208.10	189.10	154.50	125.00	151.66	162.15	179.58
Saponification number	294.10	350.60	308.60	245.40	105.19	100.05	98.18
Acid value	1.35	1.51	1.67	1.83	5.09	7.80	9.07
Magnesium, µg/100 g	5.10	4.58	5.35	5.30	5.45	5.56	5.52
Calcium, µg/100 g	2.23	1.43	1.78	1.97	12.87	13.17	13.10
Zinc µg/100 g	2.50	2.41	2.63	2.62	2.69	2.75	2.72
Lead, µg/100 g	6.24	4.03	3.50	3.39	7.69	8.28	7.27
Copper, µg/100 g	11.37	7.56	8.36	5.88	11.51	11.93	8.27
Iron µg/100 g	17.92	17.62	18.87	18.86	19.60	20.12	19.83
Potassium, mg/100 g	0.54	0.49	0.55	0.55	0.68	0.72	0.79
Sodium, mg/100 g	0.09	0.08	0.09	0.09	0.15	0.14	0.14
Total acidity (as acetic acid), %	0.60	0.66	0.66	0.54	0.72	1.54	0.72
pH	6.40	7.10	7.40	7.65	7.90	8.20	7.40
Free fatty acids (as oleic acid), %	0.68	0.76	0.84	0.92	2.56	3.92	4.56
Ricin, titre	22.00	22.00	22.00	11.00	11.00	11.00	-
Calorific value, K cal/g	6.53	7.00	7.17	7.95	7.47	7.04	7.25
Phosphorus, mg/g	4.77	3.99	4.18	4.16	5.44	4.56	4.99
Lactic acid	tr	tr	+	+	+	+	+
Oxalic acid	+	+	+	+	+	+	+

* Mean of two determinations. ** Primary fermentation is a period, when the boiled castor seeds were fermented. *** Secondary fermentation is a period, when the fermented seeds were ground to a paste and fermented further. - : Absent, + : Present, tr : trace.

had a strength of 36 x 5 units/g (Odunfa 1985b). For lipase assay, the extracting buffer was 0.1 M sodium acetate-acetic acid mixture (pH 5.5). The extract (5 ml) was added to a reaction mixture containing olive oil (1 ml), sodium taurocholate (0.4 g), calcium chloride (1 ml) and 0.1 M solution of acetate buffer (6 ml). The mixture was incubated at 35°C for 1 h. The reaction was terminated by adding 40 ml absolute alcohol. The mixture was then titrated with 0.02 M potassium hydroxide using phenolphthalein as an indicator. The blank was a mixture of the assay medium and 5 ml distilled water. The difference between the titres of the blank and that of the reaction mixture gave the amount of alkali required to neutralize the liberated fatty acids, and is expressed as oleic acid. The unit of the enzyme is that amount of enzyme which liberates 1.0 mg of oleic acid per min (Odunfa 1985b).

Results and Discussion

The chemical composition of uncooked and cooked castor seeds is shown in Table 1. The total acidity, crude proteins, ash and organic matter content of cooked and uncooked castor seeds were almost the same. Cooking decreased the free fatty acid content of castor seeds by about 50%. The alpha-amylase and protease activities decreased in the cooked samples, probably as a result of heat denaturation. The ricin content decreased from a titre of 1000 in the uncooked sample to a titre of 22, after cooking for 6 h. This result is in contrast to the reports of Spies et al (1962) and Jenkins (1962), which indicated that ricin and other allergenic proteins were destroyed by heating at 100°C for 1 h at pH 6.2.

The effect of fermentation on the composition of castor seeds and paste is shown in Table 2.

Crude protein level decreased as fermentation time increased. A possible reason for protein loss could be the high amount of ammonia given off during the fermentation (Hargreaves and Owen 1947). It could also be due to the hydrolysis of the protein by proteases produced by the fermenting microorganisms (Anosike and Egwuatu 1981; Oyenuga 1968). Even though oil (up to 50%) is the major component of castor seeds (Table 1), lipase activity in *ogiri* fermentation was minimal (Fig. 1). This was also observed in melon fermentation (Hargreaves and Owen 1947). The low lipase activity is desirable, since fatty acids produced might cause development of rancidity and consequent objectionable odour and taste. The presence of organic acids in the end product (*ogiri*) is desirable, since imparting of good flavours during fermentation has been attributed to the presence of these organic acids (Akinrele 1964; Banigo and Muller 1972). The increase in the level of calcium during fermentation might be due to the ash added at the end of primary fermentation. The reducing sugar levels showed a remarkable fluctuation with fermentation time (Fig. 1). The initial decrease in the sugar level might be due to the initial population of bacteria,

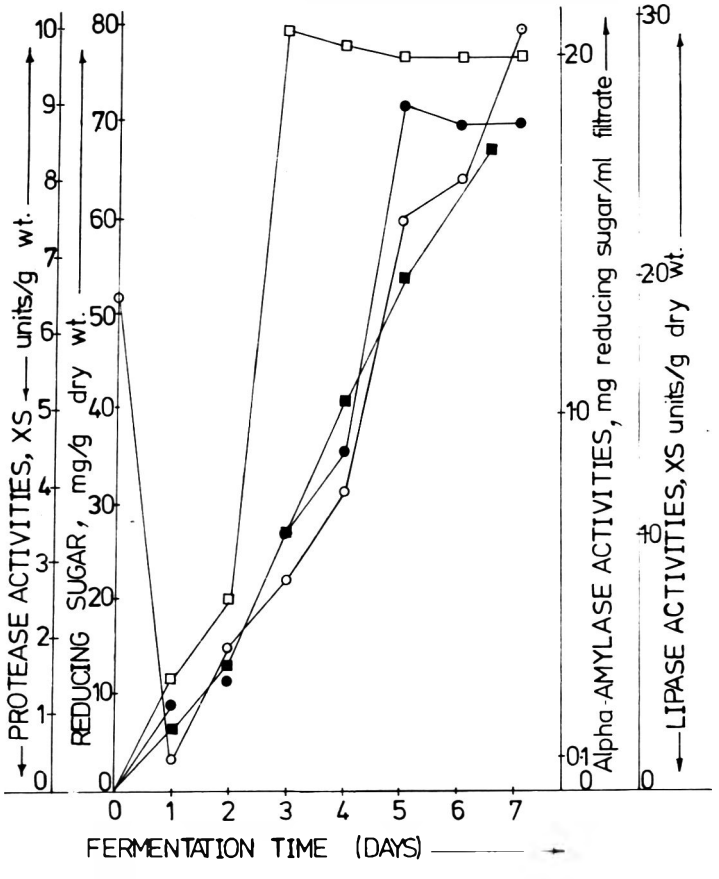

Fig. 1 Biochemical changes during *ogri* fermentation
O—O : Reducing sugar, ●—● : Alpha-amylase,
□—□ : Protease, ■—■ : Lipase.

which preferentially utilized the reducing sugars in the castor seed (Odunfa 1983). After exhaustion of the sugars, the initial population was probably succeeded by amylolytic microorganisms, which hydrolyzed starch in the castor seed, thereby increasing the sugar level. Alpha-amylase and protease activities increased with increase in fermentation time (Fig. 1), as a result of the probable presence of amylolytic and proteolytic microorganisms in the fermenting castor seeds and paste. Ricin titre was constant during the first 3 days of primary fermentation, but it decreased to half of the value, as fermentation progressed and disappeared completely on the final day of fermentation.

The increase in the level of the mineral elements of *ogiri* is nutritionally important, considering the level of these minerals in other traditional African foods (Oke and Umoh 1975). However, the actual amount of the mineral elements available to the consumer might be limited, because of the presence of oxalic acid in the fermenting castor seed, and the consequent formation of complexes with some of these mineral elements (Table 2). The pH of the fermenting castor seed became alkaline, probably due to the amines and ammonia (Table 1), normally produced during the hydrolysis of proteins (Odunfa 1981).

From the foregoing discussion, it can be concluded that castor seed fermentation resulted in the production of *ogiri* with high nutritional quality. It is important that fermentation should bring about a total elimination of ricin toxicity in the *ogiri* product.

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Semi-quantitative Purification and Assessment of Purity of Three Soybean Proteins - Glycinin, β-Conglycinin and α-Conglycinin - by SDS-PAGE Electrophoresis, Densitometry and Immunoblotting

D. DREAU*[1,2], C. LARRE[3] AND J.P. LALLES[1]

[1]INRA, Laboratoire du Jeune Ruminant, 65 Rue de St. Brieuc, 35042 Rennes Cedex, France.
[2]INRA, Laboratoire de Pathologie Infectieuse et Immunologie, 37380 Tours-Nouzilly, France.
[3]INRA, Laboratoire de Biochimie et Technologie des Proteines, 44026 Nantes, France.

Semi-quantitative purification of the three most antigenic proteins of soybean seeds, e.g., glycinin, β-conglycinin and α-conglycinin, was studied. In first step, fractions were pre-purified by successive precipitations of respective proteins at an appropriate isoelectric point and/or ionic strength. Then, glycinin, β-conglycinin and α-conglycinin fractions were isolated by gel filtration. The procedure was carried out on a semi-preparative scale, and used to purify about 2 g glycinin and 1 g each of β-conglycinin and α-conglycinin, from 1 kg of defatted soybean flour. Purity of glycinin, β-conglycinin and α-conglycinin fractions was checked by sodium dodecylsulphate polyacrylamide gel electrophoresis and immunoblots. Contaminants detected in both glycinin (9%) and α-conglycinin (4%) fractions, but the β-conglycinin obtained was pure.

Keywords : Soybean proteins, Purification, Successive precipitation, Gel filtration, SDS-PAGE, Immunoblotting.

Some of the proteins from soybean are involved in digestive intolerance (Barratt et al. 1978). Some studies have also been done on the electrophoretic and solubility characteristics of proteins from soybean varieties (Gupta et al. 1982). Furthermore, two major globulins, glycinin and β-conglycinin, have been shown to be antigenic in some animal species (Barratt et al. 1978; Sissons et al. 1982). α-conglycinin, also called Kunitz trypsin inhibitor (Catsimpoolas and Ekenstam 1969; Koshiyama et al. 1981a), could enhance T lymphocyte proliferation (Richard et al. 1989). These proteins differ in their physico-chemical properties. Glycinin is a polymeric globulin of 350-380 kDa with an isoelectric point (pI) of 6.4. It is formed by the association of 6 acidic (20-22 kDa) and 6 basic (35-40 kDa) polypeptides (Badley et al. 1975). Precipitating at a pI of 4.8, β-conglycinin has a polymeric structure. It contains two sub-units called α (72 kDa) and β (52 kDa) (Medeiros 1985). α-conglycinin is a monomeric protein of 20.1 kDa with a pI of 4.5 (Catsimpoolas and Ekenstam 1969; Koshiyama et al. 1981b).

Based on these physico-chemical differences, various processes for isolating proteins from soybean flour have been developed, and effect of urea on these proteins has also been documented (Wolf et al. 1962; Thanh and Shibasaki 1976; Winters et al. 1990; Sood et al. 1991). Small quantities of highly purified material were obtained by efficient techniques like electrophoresis, sedimentation gradient and HPLC (Wolf et al. 1992). Studying biological and immunological properties of soybean protein *in vitro* and *in vitro* requires large amounts of highly purified protein material. These have been generally produced, based on pI and ionic strength properties of glycinin (Thanh and Shibasaki 1976), β-conglycinin (Thanh and Shibasaki 1978; Winters et al. 1990) and α-conglycinin (Koshiyama et al. 1981b).

A quantitative process, using ion exchange chromatography (Gueguen et al. 1984), has been developed for the major proteins of pea viz., legumin and vicilin, which are considered homologous to soybean glycinin and conglycinin, respectively (Gueguen 1983). This method was adapted to produce immunologically pure glycinin, β-conglycinin and α-conglycinin at g levels. Since ion-exchange chromatography was unsatisfactory (D Dreau, unpublished observations; J Gueguen, personal communication), a simple and quantitative process, which associated ionic and ammonium sulphate precipitation as well as a gel filtration step, was used to prepare soybean proteins in the present study. Purity was assessed by SDS-PAGE electrophoresis, followed by scanning densitometry, or immunoblotting.

Materials and Methods

Pre-purification : An extract of defatted soy flour (Protein Technologies International, St. Louis, MO,

* *Corresponding Author.* [1]*Present address : Department of General Surgery Research Carolinces Medical Center, P.O. Box 39861 Charlotte, N.C. 98939-9861, USA*

USA) was prepared in citrate-phosphate buffer (0.16 M, pH 7.2). First, the three soybean protein fractions were obtained by successive precipitations at their pI values of 6.4, 4.8, and 4.5, respectively (Nielsen 1985). Pelleted β-conglycinin and α-conglycinin were resuspended in citrate-phosphate buffer and stored at 4°C. Glycinin (pI 6.4), which precipitates at low temperature, was stored at 12°C. Glycinin and β-conglycinin fractions were further purified, using ammonium sulphate between 55-65 and 85-95% saturation, respectively (Gueguen 1983). Pellets were resolubilised in citrate-phosphate buffer, and then desalted on a DSK150 unit (Pharmacia, Uppsala, Sweden). After freeze-drying, fractions were resuspended in citrate-phosphate buffer before the gel filtration step.

Gel filtration : These proteins were further purified by gel filtration on ACA 34 Ultrogel (100 x 10 cm, 101, Pharmacia). A solution of the proteins (300 ml, 600 mg) was loaded onto the column and the elution was performed at a flow rate of 300 ml/h. Protein elution was monitored by UV absorbance at 280 nm. Fractions of 150 ml were collected and those corresponding to purified proteins were pooled, desalted on trisacryl GF 05 (40 x 10 cm, Pharmacia) and then freeze-dried.

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) : Protein fractions were analyzed using SDS-PAGE (4% stack, 12% slabs) under reducing conditions (Dithiotreitol 0.1 M; Sigma, St Louis, MO, USA) in a mini-Protean II cell (Bio-Rad Laboratories, Richmond, CA, USA). Five µg protein of each fraction was applied on the gel, and electrophoresis was carried out in a tris-glycin buffer (0.1 M, pH 8) Laemmli 1970). After migration, protein fractions were stained by a mixture of acetic acid, methanol, water (1:3:6) solution, containing 60 µg/ml brillant blue G 250 (Serva, Heidelberg, Germany). The gels were destained for 2 h in a solution of acetic acid (5%). Protein bands were also stained using silver as described elsewhere (Rabilloud 1992).

Densitometric analysis : Densitometric analysis was conducted on the Bio-image system (Millipore Corporation, Bedford, MA, USA), following manufacturer's recommendations. The molecular weight (MW) of each band was estimated by comparison with standard proteins (Sigma) and quantitated by measuring its optical density. Fraction purity was determined by evaluating the proportion of contaminants in the mixture.

Immunoblotting: Antisera against purified proteins were raised in rabbit, following the immunization protocol of Kilshaw and Sissons (1979). SDS-PAGE electrophoresis of the purified materials was achieved as described above. After migration, gels were equilibrated and blotted onto a nitrocellulose membrane (N+Hybond; Amersham International, Buckinghamshire, England) (Towbin et al. 1979), using semi-dry technique (Pharmacia). Then, membranes were saturated with milk (5% w/v) in Tris-HCl 10 mM, NaCl 150 mM, pH 8 and incubated with a mixture of specific rabbit antibodies, prepared against whole soybean protein extracts as well as purified fractions of glycinin, α-conglycinin and β-conglycinin, prepared at analytical level (Tukur et al. 1993). Indeed, detection of some purified proteins like α-conglycinin was impossible by using polyclonal antisera raised against unpurified soybean extracts. This may indicate its low immunogenicity, when injected as a protein mixture. Specific bands were detected by including the membranes in a solution of anti-rabbit immunoglobulin peroxidase conjugate (Sigma) in tris-milk buffer. Revelation was done using diaminobenzidine (Sigma) as peroxidase substrate.

Results and Discussion

Though successfully used for pea globulins separation (Gueguen et al. 1984), ion-exchange chromatography was unsatisfactory for soybean protein purification (D Dreau, unpublished data; J Gueguen, personal communication). Indeed, no clear peak was obtained on chromatographic profiles, when tested at various conditions of pH, using anionic Q-sepharose (pH range 6-8) or cationic S-sepharose (pH range 2-4) columns (data not shown). Aggregates of glycinin and β-conglycinin as well as uncontrolled adsorption of β-conglycinin sugar residues on the column beads may constitute the major reasons for non-separation of the soybean proteins in ion exchange chromatography (Gueguen 1983). Techniques like chromatography, based on lectin affinity or reverse phase HPLC (Wolf et al. 1992), seem to be able to produce only partially purified glycinin or β-conglycinin. The ammonium sulphate precipitation preceded by a precipitation with Mg^{2+} has been used for isolation of partially purified 11S and 7S fractions, and of pure 2S fraction of soybean (Rao and Rao 1977). Moreover, ammonium sulphate precipitation, associated with a gel filtration step, allowed the separation of pure β-conglycinin (Winters et al. 1990).

Protein purification : As shown by gel filtration chromatograms (Figs. 1a, b, c), neither isoelectric pH nor ionic strength precipitations were able to

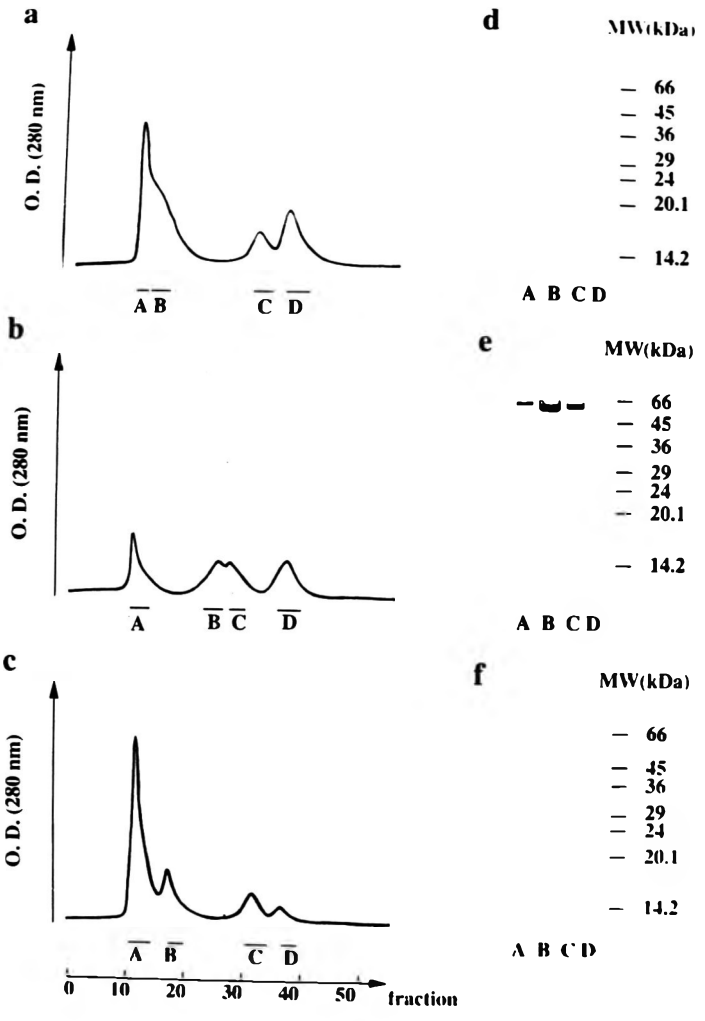

Fig.1. Gel filtration and electrophoretic patterns of glycinin (1a, 1d), β-conglycinin (1b, 1e) and α-conglycinin (1c, 1f) fractions. Gel filtration was carried out on ACA34 gel (100 x 10 cm) eluted with citrate-phosphate buffer (0.1 M, pH 7.2) at the flow rate of 300 ml/h. Protein absorbance was monitored at 280 nm (1a, 1b, 1c). Fractions of 150 ml were collected. Peak fractions were pooled and analyzed under reducing conditions on Coomassie blue stained SDS-PAGE gels (1d, 1e, 1f).

provide purified glycinin, β-conglycinin or α-conglycinin, since the main peak was always surrounded by several minor peaks. Data on the SDS-PAGE patterns, obtained for each fraction (Figs. 1d, e, f), indicated that the exclusion volume (A) contained mainly glycinin (Fig. 1d), β-conglycinin (Fig. 1e) and α-conglycinin (Fig. 1f). Peak D (Figs. 1d, e, f) resulted from the strong absorbance of residual ammonium salts at 280 nm, and contained no protein of molecular weight higher than 10 kDa.

In Fig. 1a, the glycinin fraction, which was the shoulder of the excluded peak (peak B), was contaminated by β-conglycinin (Fig. 1d). The gel filtration profile of the β-conglycinin fraction (Fig. 1b) illustrated the heterogeneity of β-conglycinin monomer association. This fraction seemed to

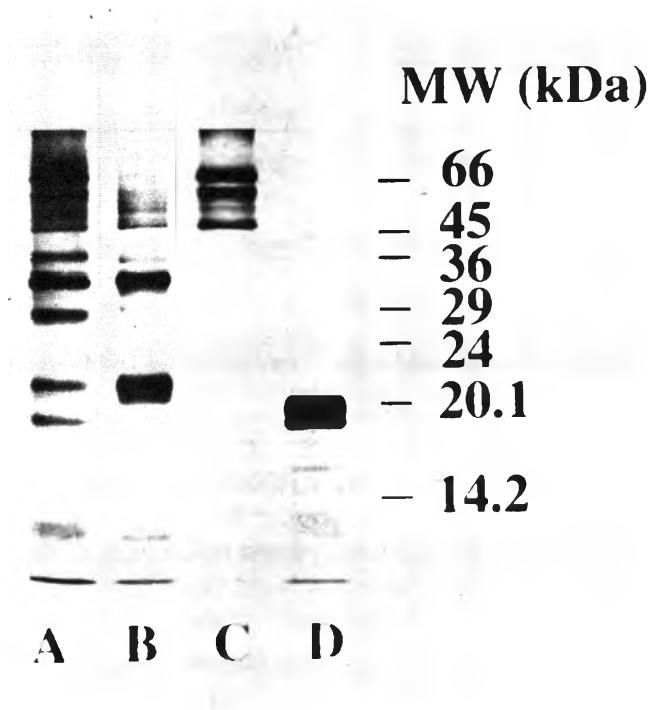

Fig.2. Silver staining of the gel after SDS-PAGE of defatted soybean flour extract (lane A), glycinin (lane B), β-conglycinin (lane C) and α-conglycinin (lane D) fractions under reducing conditions.

contain two different proteins. However, similar subunits (α: 72 kDa, α: 69 kDa, β: 52 kDa) were detected in both parts of this peak (Peaks B-C, Fig.1e). The α-conglycinin peak (C, Fig. 1c) was eluted just before ammonium salts and buffer. This peak contained only one constituent of 20.1 kDa (Fig. 1f), corresponding to α-conglycinin monomer (Koshiyama et al. 1981a,b).

Purity of glycinin, β-conglycinin and α-conglycinin fractions : Purity of these three proteins is usually checked by electrophoretical analysis (Winters et al. 1990; Arrese et al., 1991) and immunological techniques (Moreira et al. 1981; Iwabuchi and Yamauchi 1987). These techniques have become more accurate and sensitive over the last twenty years (Stott 1989; Rabilloud 1992), so as to enable the notion of protein purity to evolve. Here the purity of glycinin, β-conglycinin and α-conglycinin was further analyzed by silver staining of gels (Fig. 2). The β- and α-conglycinin fractions (lanes C,D) appeared free of glycinin contaminants. In the glycinin fraction (lane B), the main contaminant had a molecular weight corresponding to the β subunit of β-conglycinin (52 kDa). Other minor bands were present in glycinin and α-conglycinin fractions in which at least two bands were detected (MW<15kDa). A densitometric analysis was performed on this SDS-PAGE gel to estimate

TABLE 1. ESTIMATED CONTRIBUTION (%) OF CONTAMINANTS IN PURIFIED FRACTIONS GLYCININ, β-CONGLYCININ AND α-CONGLYCININ

Contaminant	Fractions		
	Glycinin	β-Conglycinin	α-Conglycinin
Glycinin	-	0	0
β-conglycinin	4	-	0
α-conglycinin	2	0	-
Other bands	3	0	4
Estimated purity, %	91	100	96

After SDS-PAGE under reducing conditions, protein bands were revealed using silver staining. Percentage of contaminants in each purified fraction was determined using optical densitometry

contaminating bands (Table 1.). Taking into account the stained bands corresponding to the MW of the sub-units of glycinin, β-conglycinin or α-conglycinin, the purity of these fractions was evaluated to be 91, 100 and 96%, respectively (Table 1).

Basic polypeptides of glycinin were not recognized by the antibody prepared against whole and purified soybean proteins in the purified fraction by immunoblot, thereby suggesting epitope modifications during the process used (lane 2, Fig. 3). Otherwise, results obtained by immunoblotting confirmed SDS-PAGE analysis (Fig. 3). This indicates that the process, and especially precipitation steps, may modify some antigenic sites, as previously shown (Gueguen 1983). However, β-conglycinin, α-conglycinin and acidic glycinin sub-units were recognized by antibodies produced against the respective native protein, suggesting that their immunoreactive epitopes were not influenced by the purification procedure. No cross reaction of the β-conglycinin fraction was found here by immunoblotting, in agreement with Winters et al (1990).

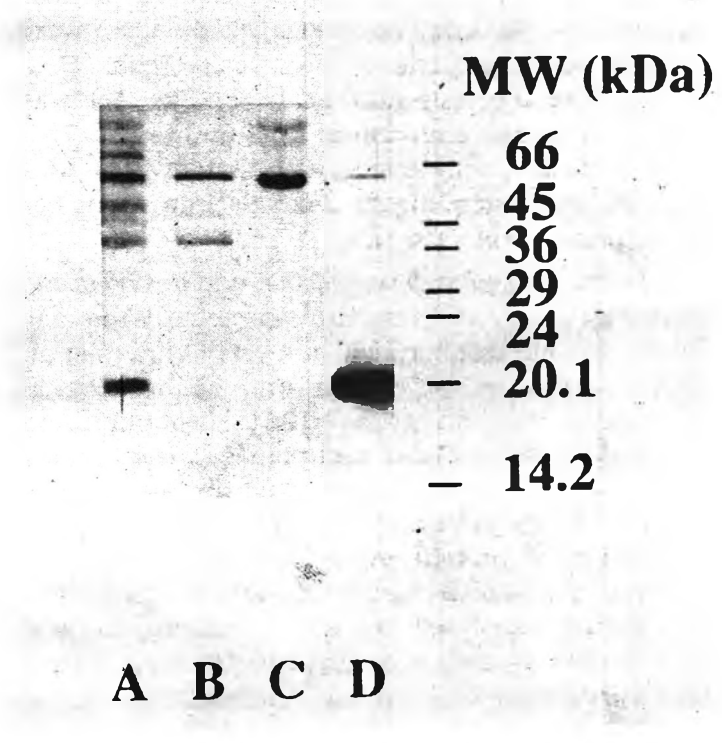

Fig.3 Western blot of a gel after SDS-PAGE migration of defatted soybean flour extract (lane A), glycinin (lane B), β-conglycinin (lane C) and α-conglycinin (lane D) fractions under reducing conditions. Separated proteins were immunodetected, using a mixture of polyclonal antibodies prepared against whole soybean extracts, glycinin, β-conglycinin and α-conglycinin.

Production of pure glycinin, β-conglycinin and α-conglycinin : A high yield of immunologically pure β-conglycinin was achieved by the process described here, whilst the other soybean fractions (glycinin and α-conglycinin) were contaminated at least by the 52 kDa sub-unit of β-conglycinin. This constitutes the main difficulty to be overcome, before one can arrive at a fully-automated separation procedure. Nevertheless, the process was found to be the most efficient for purification of glycinin, β-conglycinin and α-conglycinin from cultivars, or soybean mixtures, in large amounts, as already mentioned (Thanh and Shibasaki 1976; Winters et al. 1990). These amounts were low, compared to the purification of pea globulins, partly because of protein aggregation. This latter phenomenon is a complex one, and depends on ionic strength, pH, protein concentration and temperature (Gueguen 1983).

Now-a-days, electrophoretic processes (Righetti et al. 1989), used for purifying animal proteins including human growth hormone (Ettori et al. 1992), do not seem to be useful for plant protein separation, partly because of their higher molecular weight, and because of the complex interactions among them. We are now intending to improve the purity of glycinin and α-conglycinin by removing β-conglycinin, using a ConA affinity chromatography (Kitamura et al. 1974) or specific antibodies.

Conclusion

Using the process described here, it was possible to prepare about 2 g of purified glycinin (91%) and 1 g each of β-conglycinin (100%) and α-conglycinin (96%) from 1 kg of defatted soybean flour. Only β-conglycinin seemed to be highly pure, as assessed by SDS-PAGE and immunoblotting. It is felt that the precipitation method, associated with affinity chromatography, will provide a rapid and convenient way to isolate soybean protein

quantitatively, without significant contamination, and will lead to significant advances in the study of the biological properties of these three soybean proteins, especially in the gastrointestinal tract.

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Storage Studies on *Suji* in Different Packages

R.K. UPADHYAY, M. THANGARAJ* AND P.K. JAISWAL

Central Agmark Laboratory,
North Ambazari Road, Nagpur- 440 010, India.

Effect of storage of *suji*, packed in low density polyethylene, high density polyethylene, high molecular-high density polyethylene, polypropylene, cloth and jute bags, under accelerated environment (38°C and 90% RH) on moisture, total ash, acid insoluble ash, gluten and alcoholic acidity has been studied. Moisture and alcoholic acidity increased, while gluten decreased on storage. The changes in total ash and acid insoluble ash were not significant during the storage period. The rates of increase in moisture and alcoholic acidity as well as rates of decrease of gluten were high in *suji* stored in jute/cloth bags, as compared to those in other packages. Among the plastic packages, low density polyethylene gave the least protection, while polypropylene gave the maximum protection against spoilage of *suji* during storage. Microscopic observations showed mould growth after 15 days storage of *suji* in cloth and jute packages, but only after 30 days of storage in other packages. The onset of insect infestation in *suji* was observed after 45 days in cloth and jute packages, whereas there was no indication of infestation even after 60 days in other packages. The organoleptic acceptability remained unchanged for 40 days in case of *suji* stored in plastic packages, and for 20 days in case of jute/cloth packages.

Keywords : *Suji*, Packing materials, Storage, Chemical changes, Mould growth, Insect infestation.

Suji, or semolina, is a wheat product which is widely consumed in a variety of preparations such as savoury, *uppuma*, sweet dishes, etc. *Suji* is highly prone to infestation. The infested samples may show defects such as cake formation, development of musty flavour, discoloration and infestation (Peleg and Bagley 1983; Kent-Jones and Amos 1967). These ultimately affect the organoleptic and baking qualities of *suji* (Arya et al. 1971). Factors such as the type of insect infestation, mould attack, absorption of moisture, heat and dynamic stress, need to be taken care of, while making selection of packaging material (Newatia 1986). Storage studies on *suji* using high density polyethylene (HDPE) as packing material have been reported by Kumar and Anandaswamy (1977), but no work has been done on comparative suitability of various packaging materials for packaging and storing of *suji*, and the changes in chemical characteristics on storage, under simulated field conditions, although some studies have been reported on the packaging and storage of *atta* (Premavalli, et al. 1972; Kameswara Rao and Malathi 1969). Hence, the present study was undertaken.

Fresh and clean samples of *suji* were obtained from a local flour mill. Preformed packages made of 100 μ low-density polyethylene (LDPE), 100 μ high-density polyethylene (HDPE), 100 μ high-molecular, high-density polyethylene (HMHDPE), 100 μ polypropylene (PP), cotton cloth, and jute cloth were obtained from the local market in the form of pouches/bags of 12 x 16 cms to accommodate 400 g sample. Jute and cotton bags were thread-stitched, while other packages were heat-sealed, after filling. Immediately after filling, 18 replicates of packages were stored at 38±1°C and 90 ± 2% RH in a controlled humidity cabinet for 2 months. Initially, and after every week, the samples were analyzed for moisture, total ash, acid insoluble ash, gluten and alcoholic acidity (ISI 1968). The samples were also microscopically examined, (Harrigan and McCance 1976) at regular intervals, for mould growth and insect infestation (ISI 1971a). The stored *suji* samples, as such in raw form, were evaluated for organoleptic acceptability (ISI 1971b) at an interval of 7 days by a panel of judges and the data obtained were subjected to statistical analysis (ISI 1971c).

The fresh samples, at the time of packaging, showed (%) moisture 12, total ash 0.5, acid insoluble ash 0.029, gluten 8.13 and alcoholic acidity 0.069. The samples were free from mould growth and insect infestation. The changes in total ash and acid insoluble ash on storage of *suji* (data not shown), were not ($P>0.9$) significant in all the packaging materials.

The increase in moisture content of *suji*, in jute and cloth packages was comparatively higher (Fig. 1) and could be attributed to their poor barrier property. In jute and cotton packages, the moisture content of *suji* was 13.6 and 13.7%, respectively on 22nd day, and crossed the limit of 13.5% set under the standards (Agmark 1987). The main drawback of the jute and cotton bags is the easy access to moisture at periphery, thereby leading to excessive accumulation of moisture in *suji* near the

* Corresponding Author

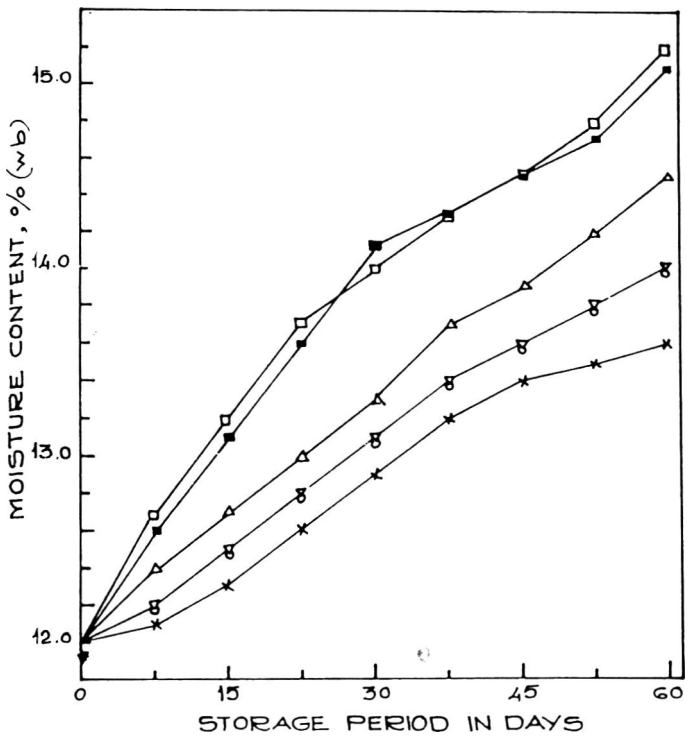

Fig. 1. Changes in moisture content of *suji* in different packing materials, and stored at 38 ±1°C and 90±2% RH Δ : LDPE, ∇ : HDPE O : HMHDPE, X : PP, □ : Cloth, ■ : Jute.

peripheral portion. In other packages, this moisture limit was crossed after 38 days, except in case of LDPE packages. The variation in moisture of *suji* stored in LDPE, HDPE, HMHDPE, and PP packages, on any particular day (Fig.1), is attributed to variations in the moisture vapour permeability rates of the packing materials (Kumar and Balasubrahmanyam 1988), as the gauges of all of these packing materials were the same. Among the plastic packing materials, the rate of moisture absorption by *suji* was minimum in PP bags and maximum in LDPE bags.

The gluten content of *suji*, did not change to a significant extent (P>0.05) in all types of packages, when the moisture content of *suji* was less than 13% (Fig.2) After 15 days, the rate of reduction in gluten on storage was greater in *suji* samples stored in cloth and jute bags, whereas the rate of reduction increased after 30 days of storage in case of LDPE, HDPE and HMHDPE packages. The decrease in gluten content of *suji* was minimum in PP bags, which can be correlated to minimum ingress of moisture through PP bags, as compared to other packaging materials. The reduction in gluten of *suji* has been attributed to excessive absorption of moisture and insect infestation (Arya et al. 1971). On the 30th and 60th days, the gluten contents of *suji* were 7.97 and 7.69%, respectively, when stored in LDPE bags, but these were 8.05 and 7.86%, respectively in case of PP bags (Fig. 2). This is because of poor barrier properties of LDPE, as compared to those of PP for ingress of moisture.

In general, the alcoholic acidity of *suji* increased with time, the rate of increase being higher in jute/cloth packages, followed by LDPE packages (Fig. 3). There was no significant variation (P>0.2) among HDPE, HMHDPE and PP packages, with respect to increase in alcoholic acidity of *suji* samples. On storage, the rate of increase in alcoholic acidity of *suji* was higher, after the moisture content of *suji* reached 13%, which corresponded to 30 and 15 days of storage of *suji* in plastic and cloth/jute packages, respectively (Fig.3). Thus, alcoholic acidity of *suji* could be correlated with its moisture content. In case of higher ingress of moisture by *suji*, the increase in its alcoholic acidity will also be higher upon storage (Leelavathi et al. 1984). This is evidenced by comparatively low alcoholic acidity in *suji* stored in PP, which has the lowest water vapour permeability rate amongst all packaging materials used in this study.

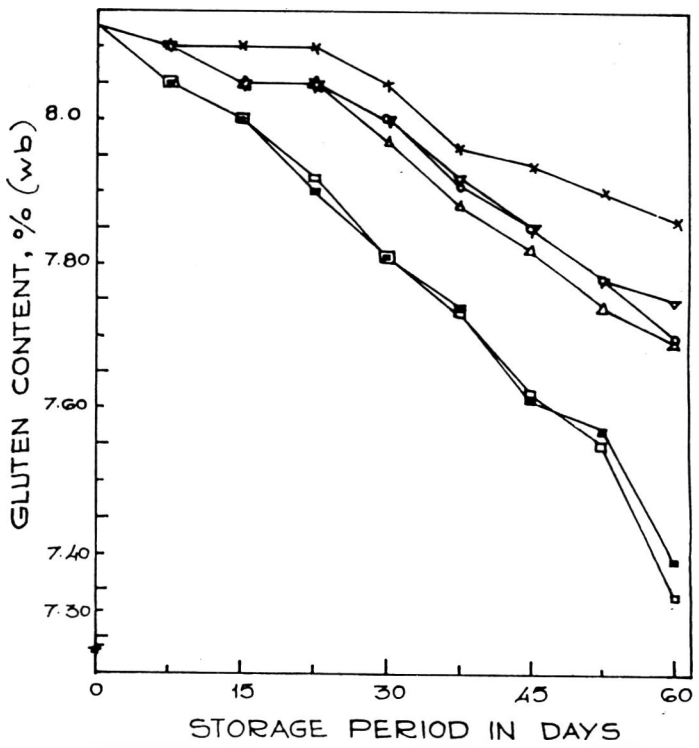

Fig. 2. Changes in gluten content of *suji* packed in different packing materials, and stored at 38 ±1°C and 90±2% RH Δ : LDPE, ∇ : HDPE O : HMHDPE, X : PP, □ : Cloth, ■ : Jute.

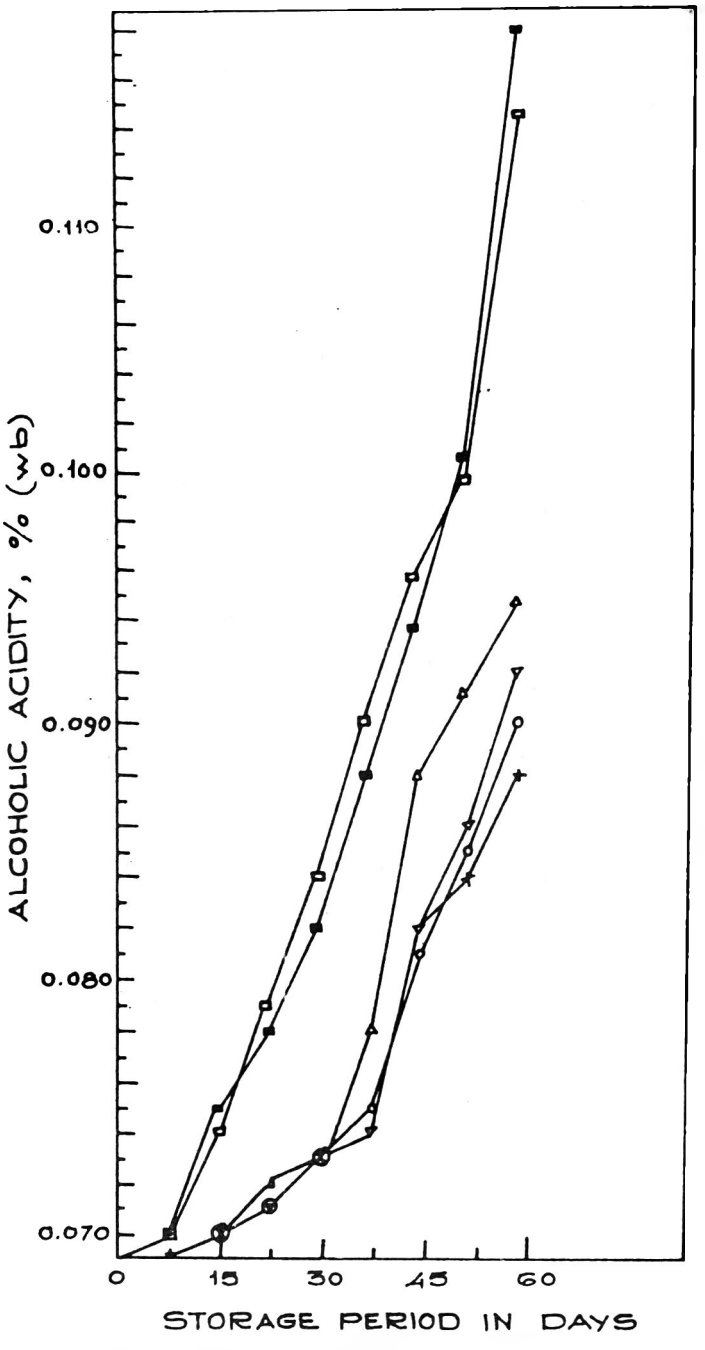

Fig. 3. Changes in alcoholic acidity of *suji* packed in different packing materials, and stored at 38 ±1°C and 90±2% RH Δ : LDPE, ∇ : HDPE O : HMHDPE, X : PP, □ : Cloth, ■ : Jute.

The microscopic observations of *suji* revealed mould growth, after storage of about 15 days in cloth and jute packages. In LDPE, HDPE, HMHDPE and PP packages, the mould growth was observed in the *suji* samples stored for 45 days. Early onset of mould growth in *suji* stored in cloth and jute packages is attributed to excessive ingress of moisture in *suji* and poor protection afforded by the packaging materials against mould attack. The onset of insect infestation in *suji* was observed after 45 days of storage in cloth and jute bags. In case of plastic packages, there was no sign of insect infestation even after 60 days of storage. The colour of *suji* in LDPE, HDPE, HMHDPE and PP packages remained unchanged during the entire period of storage. However, there was a slight change from its normal colour at the periphery, after storage period of 30 days in cloth/jute packages. Data on the organoleptic qualities showed that the quality of *suji* remained unchanged for 40 days in plastic packages. In contrast, the quality of *suji* in cloth and jute packages started deteriorating after 20 days of storage, as determined by the changes in taste and development of musty flavour.

It is concluded that the *suji* stored in jute bags offers the least resistance to spoilage, and the jute bags provide about 15 days of shelf-life. In contrast, plastic packages, of the aforesaid specifications, usually, give a shelf-life of about one month to stored *suji*. Among the plastic packages, LDPE gave the least protection and PP gave the maximum protection, against the spoilage of *suji*.

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Antifertility and Cannibalistic Properties of Some Mycotoxins in Albino Rats

D.N. CHOUDHARY*, G.R. SAHAY AND J.N. SINGH

Bioscience Research Centre
Tej Narayan Banaili College, Bhagalpur-812 007, India.

Effect of oral administration of aflatoxin B_1 (AF-B_1), sterigmatocystin and kojic acid at 10, 10, and 50 µg dose per animal/day, respectively, on reproductive performance of male albino rats was studied. None of these toxins interfered with spermatogenesis, but anti-implantational effect was observed in females, mated by the males treated with sterigmatocystin and kojic acid. A significant loss of viability among the litters and cannibalism in mothers, 2-3 days after littering, was observed.

Keywords : Aflatoxin B_1, Sterigmatocystin, Kojic acid, Spermatogenesis, Anti-implantation, Cannibalism.

The mycotoxins are a group of toxins that possess multiple biological properties (Krishna et al. 1992). Domestic and farm animals are generally exposed to the risk of mycotoxins through consumption of mouldy, or mycotoxin-contaminated, feed (Krishna et al. 1992). Several workers have studied the effects of aflatoxins on growth and reproduction in laboratory animals. After feeding of aflatoxin, progressive degeneration of germinal cells in rat (Jayaraj et al. 1968), intra-uterine resorption in pregnant rats (Panda et al. 1970, 1975) and cannibalistic behaviour in mother albino rats (Shankaramurti et al. 1972) have been reported. Sterigmatocystin has also been found to possess toxic properties (Shreemannarayan et al. 1989). Recently, anti-implantational/abortifacient effects of some mycotoxins in albino rats have also been reported (Chaudhary et al. 1992). The present communication reports the effects of oral administration of aflatoxin B_1, sterigmatocystin and kojic acid on reproductive organs and fertility of male albino rats.

Mycotoxins (aflatoxin, sterigmatocystin and kojic acid) were procured from Sigma Chemical Company, St. Louis, U.S.A. Male ('Sprague Dawley' strain) rats (150-200 g body weight) of proven fertility were orally administered with a suspension of mycotoxin in propylene glycol at a dose of 10 µg/rat/day (aflatoxin B_1 and sterigmatocystin) and 50 µg/rat/day (kojic acid) for 21 days, individually in each group. A separate group of rats, taken as control, received the solution of propylene glycol alone for the same period. Fertility performance of the individual rat was studied from day 16 to day 21 of treatment. Each male was caged separately with two coeval females of proven fertility. Presence of sperms in the vaginal smear indicated that the females had mated to the particular male and day of mating was taken to be day 1 of pregnancy. Laparotomy was done on day 8 of pregnancy, to examine and record corpora lutea and implantation sites. Litter was examined and litter size recorded at term. Teratogenic effects, or death of young ones, if any, and the behaviour of mothers were also recorded.

Male rats were sacrificed on day 22 and tissues were collected and weighed on a torsion balance. Serial sections of testis were prepared for microscopic examination. Fructose content in coagulating gland (CG) and acid phosphatase activity in ventral prostate (VP) were estimated by the method adopted by Mann (1964) and Sigma (1963), respectively. Spermatozoa collected from caput, corpus and cauda epididymis, and vas were examined under a microscope, and their number, morphology and mortality were recorded (Singh et al. 1969). The data were analyzed statistically using Student's t-test (Raghavarao 1983).

Body and organ weights : These were significantly decreased in case of oral administration of aflatoxin B_1 and kojic acid, which may be due to suppression of growth hormone (Table 1). Decline in weight of the pituitary gland in males treated with aflatoxin B_1 further supports such a relationship. Aflatoxin B_1 also decreased the weight of epididymis. Sterigmatocystin caused significant decrease in the weight of the testis and the seminal vesicle, whereas kojic acid significantly reduced the weight of testis and epididymis. The decrease in organ weight could be attributed to low gonadotrophic activity (Choudhary et al. 1990).

Metabolic changes : A significant reduction in the fructose content of coagulating gland and acid

* Corresponding Author

TABLE 1. CHANGES IN GENITAL ORGANS AND PITUITARIES OF MALE ALBINO RATS AFTER ORAL ADMINISTRATION OF SOME MYCOTOXINS.

Treatment, (No. of animals)	Change in body weight, g	Weight of organs, mg/100 g body weight				Fructose, µg/100 mg	Acid phosphatase unit/h/mg
		Testis	Epididymis	Seminal vesicle	Pituitary	CG	VP
Control, (7)	14.45 ±1.36	1374.13 ±12.32	553.56 ±16.23	286.45 ±14.34	3.49 ±0.21	25.47 ±1.96	155.52 ±12.50
Aflatoxin B_1 10 µg/rat/ day, (7)	8.42^b ±1.39	1385.68^{NS} ±16.62	472.70^b ±17.04	256.92^{NS} ±12.41	2.74^a ±0.19	13.27^c ±2.33	83.45^b ±16.53
Sterigmatocystin 10 µg/rat/day, (8)	16.50^{NS} ±1.50	1313.32^a ±22.10	515.91^{NS} ±35.13	148.59^c ±16.88	3.46^{NS} ±0.25	10.79^c ±2.68	115.39^a ±13.45
Kojic acid 50 µg/rat/day, (8)	8.87^b ±1.17	1334.40^a ±13.37	482.79^b ±15.52	349.82^{NS} ±43.52	3.45^{NS} ±0.19	23.55^{NS} ±1.34	172.46^{NS} ±07.68

NS = Not significant; a (P<0.05); b (P<0.01) and c (P<0.001). CG = Coagulating gland, VP = Ventral prostate.

phosphatase activity in ventral prostate occurred after oral administration of aflatoxin B_1 and sterigmatocystin in the males. Decline in acid phosphatase activity in ventral prostate, and in the fructose content in coagulating gland of male rats treated with aflatoxin B_1 and sterigmatocystin, might be due to a decline in endogenous androgen production (Choudhary et al. 1991), which, in turn, is induced by anti-gonadotrophic effect of the toxins.

Spermatogenesis : All the spermatogenic elements, including sperms, were present in normal fashion, thereby indicating no adverse effect by any of the mycotoxins. Similarly, no adverse effect on density, morphology and mortality of spermatozoa, collected from caput, corpus and cauda epididymis and vas of the extract-fed rats, were observed.

Fertility performance test : Female rats, mated by males treated with sterigmatocystin and kojic acid, showed significant reduction in implantation sites and litter size. Loss of viability among the litter on second or third day of post-delivery and cannibalistic behaviour of the mothers were also noticed. This may be due to either inhibition of implantation or increased resorption of foetuses (Sharma et al. 1986), or due to the presence of some toxic components (Choudhary et al. 1992). It is evident from Table 2, that decline in litter size is primarily due to decline in implantation sites, which may be due to the direct effect of toxin reaching the female genital tract with the semen (Choudhary et al. 1990). Late delivery by the females treated with sterigmatocystin further supports the fact. Loss of viability among the litter indicated the toxic effect of aflatoxin B_1, sterigmatocystin and kojic acid.

Cannibalism : The female rats, which were mated by males treated with aflatoxin B_1 and kojic acid, started eating their litter, 2 days after delivery (Table 2). This cannibalistic behaviour may be due to disturbance in the chemical interaction of the mothers with their litter (Shankaramurti et al. 1972). Since the mothers in the control group did not show cannibalism (Table 2), the possibility of any nutritional deficiency is ruled out.

Authors are grateful to the Principal, T.N.B.

TABLE 2 MATING PERFORMANCE TEST OF MALE RATS TREATED WITH MYCOTOXINS

Treatment	No. of breeders	No. of successful males	No. of mated females	Corpora lutea sites	Implantation sites	Litter size			Other trends
						Total	Dead	Viable	
Glycol, control	7	6	8	10.26 ± 2.82	7.87 ± 0.95	5.94 ± 0.84	-	5.94 ± 0.84	
Aflatoxin B_1	7	6	9	9.22^{NS}± 0.68	6.12^{NS}± 0.88	4.95^{NS}± 0.75	1.77^b ± 0.54	3.14^c ± 0.34	Cannibalism in mothers, and death of litters
Sterigmatocystin	8	5	7	6.28^{NS}± 0.72	4.71^b ± 0.43	3.14^b ± 0.47	0.85^{NS}± 0.56	2.89^b ± 0.47	Late delivery death of litters
Kojic acid	8	6	8	8.25^{NS}± 0.81	4.62^a ± 0.74	3.62^a ± 0.45	1.85^b ± 0.46	1.79^b ± 0.79	Cannibalism in mothers, death of litters

NS = Not significant; a (P<0.05); b (P<0.01); and c (P<0.001)

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Changes in Soluble Sugars and Other Constituents of Bamboo Shoots in *Soibum* Fermentation

S.S. GIRI[1]* AND L.S. JANMEJAY[2]

[1]Department of Biochemistry,
Manipur College, Imphal-795 001, Manipur, India.
[2]Department of Life Sciences, Manipur University, Canchipur-795 003, Manipur, India.

Fermentation of two species of edible bamboo shoots for the production of *Soibum* was followed for the change in individual soluble sugars, pH, lactic acid, titratable acid and activities of amylase as well as invertase. Among the soluble sugars, only glucose was found to be the most preferentially utilized. Except for xylose, none of soluble sugars was completely utilized during fermentation of *Bambusa tulda*. The levels of amylase and invertase were found to fluctuate widely. Formation of lactic and titratable acids during fermentation caused a drop in the pH of the fermenting mash.

Keywords : Traditional fermentation, Edible bamboo shoot fermentation, *Soibum*, Changes during fermentation.

Traditional processing of fresh edible bamboo shoots by natural fermentation for producing an indigenous food, *soibum*, is popular in Manipur. (Pravabati Devi and Singh 1986). The microorganisms involved in this fermentation were identified to be lactic acid bacteria and yeasts, under the existing anaerobic condition (Giri and Janmejay 1987). The improved traditional fermentation time is as long as 30 days. The fermentation time of *soibum* could be reduced considerably by efficient conversion of bamboo constituents into sugars. The changes in the sugars have been studied during lactic acid fermentation of other plant materials (Odunfa 1983; Kotzekidou and Roukas 1987). The present study reports the data on the changes in individual soluble sugars, lactic and titratable acids, pH, activities of amylase and invertase during the *soibum* fermentation.

The edible succulent bamboo shoots (about 20 cm in diam and 15 cm height) of the species *Bambusa tulda* and *Dendrocalamus giganteus* were collected during the rainy season. The outer hard layers were removed manually and discarded. The bamboo shoots were cut into thin slices, mixed thoroughly, and fermented individually, so as to imitate a traditional methodology, by packing the slices compactly in sterile polythene bags. The bags were closed to prevent entry of air, placed in 500 ml capacity beakers for fermentation at room temperature (26±4°C). A sufficient number of bags was run for each species. Samples were removed in triplicate at the end of 0, 2, 5, 15 and 30 days for immediate determination of pH, titratable acid (Szarvas and Stiaszny 1977), and lactic acid (Barker 1957). Assays of amylase and invertase in these mashes were conducted by following the methods described by Mahadevan and Sridhar (1982). Identification and determination of individual soluble sugars (Lewis and Smith 1969) were conducted on 0, 2, 15 and 30 days old samples. The activities of amylase and invertase were estimated at pH 3.5 and 6. Each of the results is presented as a mean of three replicate mashes.

Data in Table 1 show that the fermentation rate was higher during the first 15 days, as evidenced by the changes in various parameters. The rate, however, slowed down in the further fermentation period. The levels of lactic and titratable acids increased gradually during the entire period (30 days) of fermentation. At 30 days, the pH was lowest and lactic acid concentration was highest. Thus, it may be inferred that the preparation of *soibum* involves lactic acid fermentation. Earlier work (Giri 1988) confirms this inference as *Leuconostoc mesenteroides* was found to be dominant in the initial period of fermentation; thereafter, it was replaced by *Lactobacillus corneformis*. It was further noted that lactic acid bacteria, such as *Lactobacillus brevis* and *Streptococcus lactis*, continued to dominate in the fermentation medium, during the subsequent period, in spite of the appearance of yeasts belonging to the genera *Candida*, *Debaromyces*, *Hanseniaspora*, *Hensenula*, *Pichia*, *Saccharomyces* and *Torulopsis* (Giri 1988).

Data indicated that a variety of soluble sugars were formed during the course of fermentation (Table 1). These included sucrose, galactose, glucose and either mannose or arabinose, or both. All of these soluble sugars were utilized to different degrees by the cultures during the course of

* *Corresponding Author*

TABLE 1. CHANGES IN INDIVIDUAL SOLUBLE SUGARS, pH, LACTIC ACID, TITRATABLE ACID AND ACTIVITIES OF AMYLASES AND INVERTASES DURING THE FERMENTATION OF *SOIBUM*

Fermentation time, days	Soluble sugars, mg/100 g								pH	LA %	TA %	Amylase activity at pH			Invertase activity at pH		
	Ra	Su	Ga	Gl	Ma	Ar	Ri	Xy				3	5	6	3	5	6
***B.tulda* fermentation**																	
0	33	152	143	323	295		218	105	5.1	0	0	5.9	6.9	7.6	3.6	8.0	4.6
2	32	51	56	107	86		67	22	4.1	0.1	0.4	0.3	1.2	1.9	1.2	0.4	0.7
15	8	15	6	41	6		11	3	3.7	0.6	0.9	0.4	1.0	0.9	7.4	0.7	0.3
30	6	16	8	54	9		5	1	3.7	0.7	1.0	0.9	1.5	3.4	6.3	1.2	0.7
***D.giganteus* fermentation**																	
0	170	151	128	383	288		235	139	4.9	0	0	2.1	5.9	7.3	2.6	4.1	2.7
2	109	115	86	100	129		115	129	4.7	0.1	0.3	0.1	1.2	1.2	2.2	0.6	0.7
15	52	44	75	108	72		46	52	3.7	0.7	1.2	0.3	0.6	0.8	1.8	1.3	0.9
30	15	13	18	23	20		18	16	3.6	0.9	1.2	0.3	1.2	3.0	2.6	1.1	0.7

Ra : Raffinose, Su : Sucrose, Ga : Galactose, Gl : Glucose, Ma : Mannose, Ar : Arabinose, Ri : Ribose, Xy : Xylose, LA : Lactic acid, TA : Titratable acid (expressed as lactic acid equivalent). Activities of amylases and invertases are expressed as mg glucose liberted/100 g mash/24 h. All the values are means of triplicate determinations.

fermentation. However, none of these soluble sugars was utilized completely during the 30 days of *soibum* fermentation. It is interesting to note that the utilization of soluble sugars was higher in case of fermentation of *B. tulda*, as compared to that of *D. giganteus*. The residual soluble sugars in case of *B. tulda* fermentation were less than 100 mg/100 g fermenting mash. Among the soluble sugars, fructose could not be detected in the present studies, probably because it was utilized as soon as it was formed due to action of invertase on sucrose.

Comparatively faster degradation of all the soluble sugars studied in case of *B. tulda* fermentation, as compared to that of *D. giganteus*, indicates that the microorganisms involved in the former have greater abilities to produce the enzymes for degradation of these sugars. Bamboo shoots are known to contain starch, xylan, arabinogalactan and galactan (Maekawa 1975; Maekawa and Kitao 1973). The growth of *Leuconostoc mesenteroides* in the earlier phase of *soibum* fermentation has also been observed to be associated with biosynthesis of dextran (Giri 1988). Low phenolic content of *B. tulda*, as compared to that in *D. giganteus* (Giri 1988), might also be responsible for the higher utilization of the soluble sugars in the fermentation of the former.

Data on the activities of amylases at different pH values showed that the optimum condition for the enzyme present in plant material at 0 h is at pH 6. Similarly, the amylases, elaborated by the cultures during the course of fermentation, have their optima at pH 5 to 6 at 15 days, in case *B. tulda* and *D. giganteus* fermentations. In one case, amylases, with pH optima at 3, became conspicuous in both fermentations. On the contrary, the optima for invertases were at pH 5 at 0 h fermentation of both the bamboo shoots. However, the optima for invertases elaborated by the cultures were at pH 3 from second day onwards. The involvement of amylases and invertases in the biochemical changes caused during the fermentation of *soibum* can be inferred from various facts such as a) activities of these enzymes were greater in *B. tulda* mashes, whose respective sugars were rapidly utilized as compared to those in *D. giganteus* fermentation; b) activities of these enzymes were greater in 30-day mashes, which contained lesser soluble sugars, as compared to mashes fermented for shorter periods; c) amylases with optima at pH 3 appeared during the course of fermentation; and d) extracellular production of these enzymes by the microbial cultures involved in the fermentation has been reported by other workers (Buchanan and Gibbons 1974; Sills and Stewart 1982).

High concentrations of most of the residual soluble sugars even at the end of 30 days fermentation of *soibum* indicate the possibility of reducing fermentation time by adding respective enzymes to the mash at 0 h or during the course of fermentation. The presence of higher residual sugars also indicates the need to subject the fermented *soibum* to drying, or else spoilage microorganisms may grow in the moist-fermented product during storage.

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Efficiency of Four Methods for Isolation of Proteins from Defatted Rapeseed Meal and Use of the Residue for Yeast Growth

S. SHARMA* AND S.K. GARG[1]

Department of Bio-Sciences,
Himachal Pradesh University, Shimla-171 005, India.

The extraction of proteins from the defatted rapeseed meal (RSM) was carried out using water, salt, alkali and alcohol. Alkali was the most effective in the extraction of proteins, whereas the protein content of the residues left after extraction ranged from 9.3 to 28.0 g/100 g meal, and was found to be maximum, when extraction was done with alcohol. The residues were analyzed for amino acid composition and evaluated for nitrogen supplement value, using *Candida curvata*. The variability in yeast biomass production on substrates containing RSM digests, might be due to differences in the protein content.

Keywords : Defatted rapeseed meal, Protein isolation, Amino acid composition, Efficiency of methods, *Candida curvata*, Biomass production.

Rapeseed meal (RSM), obtained in the process of oil extraction of the seeds, is considered to be a good quality feed due to its high protein content (Bell and William 1953). However, its use as potential animal feed has been hindered by the problems associated with palatability, digestibility and toxicity (Sosulski 1981; Garg et al. 1982). Trials have been carried out to improve the rapeseed protein by fermenting it with microorganisms (Phillipchuk and Jackson 1979; Garg et al. 1983, 1985). The studies, carried out with RSM as the protein source, established that the meal was deficient in certain amino acids, which interfered with normal metabolism (Leslie and Summers 1975). The present study deals with the comparative assessment of the efficiency of different solvents for protein isolation, and the use of residual meal to support the growth of *Candida curvata*.

The seeds of *Brassica campestris* (var. 'Toria') were pulverized into a fine powder and defatted by Soxhlet extraction (AOAC 1975) using petroleum ether (BP 60-80°C). The analyses of defatted RSM for moisture, crude proteins, crude fibre and ash contents were carried out by standard methods (AOAC 1975). Protein isolates were prepared by individually stirring 50 g RSM in 700 ml of distilled water, salt (10% NaCl), alkali (0.1 N NaOH) and alcohol (70%) for 1 h at 25±1°C. The suspensions were centrifuged at 16,000 rpm (4°C) for 20 min and filtered through Whatman No. 1 filter paper. The residues left over were designated as RWE, RSE, RAE and RAlcE, respectively. Nitrogen was determined by micro-Kjeldahl method (AOAC 1975) and the values were converted to crude proteins.

Rapeseed meal and the residues were hydrolyzed by 6 N HCl and autoclaved at 0.752 kg/sq. cm for 30 min (Phillipchuk and Jackson 1979). The acid hydrolysates were ice-cooled, filtered, and their pH was adjusted to 5.5 with 20% NaOH. The acid digests of RSM (RSM-HA) and the residues (20 ml/flask) were autoclaved at 1.054 kg/sq. cm for 30 min. The flasks containing different digests were inoculated uniformly with *C. curvata* cell suspensions ($15x10^6$ cells/ml), in triplicates, and incubated at 25±1°C on rotary shaker (220 osc/min). The growth was recorded by measuring the absorbance at 670 nm at 24 h interval.

The RSM digests containing proteins (15 mg) and 6 N HCl (10 ml) were autoclaved at 1.054 kg/sq. cm for 30 min. After cooling and filtration, the contents were dried, and subsequently dissolved in 0.2 N citrate buffer (pH 2.2), for the determination of amino acid composition (Spackman et al. 1958) on an automatic amino acid analyzer (AAA-881).

Rapeseed meal used in the present study had the following composition (%): crude proteins, 31.99; crude fibre (cellulose), 14.89; ash (minerals), 3.85; and moisture, 4.85. The protein contents of water, salt, alkali and alcohol isolates were 14.63, 19.25, 23.48 and 4.23%, respectively, and of the respective residues were 16.75, 13.03, 9.30 and 28.00%. Proteins were extracted employing polar and non-polar solvents. It was observed that the protein extractability was in the order of alkali > salt > water > alcohol. High extraction in the polar solvents could be accounted for by the predominance of hydrophilic amino acids. Sixteen different amino acids were identified in the hydrolysates of RSM

* *Corresponding Author.* [1]*Present Address : Department of Molecular Biology and Biochemistry, Guru Nanak Dev University, Amritsar-143 005, India.*

TABLE 1. AMINO ACID (mg/100 mg protein) COMPOSITION OF ACID DIGESTS OF RSM RESIDUES LEFT OVER AFTER PROTEIN ISOLATION

Amino acid (mg/100 mg protein)	RSM-HA	RWE	RSE	RAE	RAlcE
Lysine	3.77	4.43	2.01	9.84	12.16
Histidine	13.42	-	18.26	2.60	6.33
Arginine	5.05	4.10	1.22	6.58	0.26
Aspartic acid	12.19	9.59	4.20	2.73	0.55
Threonine	0.57	0.22	0.18	11.49	0.56
Serine	16.37	18.18	5.73	8.33	9.25
Glutamic acid	11.08	10.87	5.57	4.52	7.80
Proline	0.10	10.38	5.09	6.40	9.98
Glycine	6.85	10.28	15.29	8.34	1.50
Alanine	6.44	8.33	12.31	19.18	6.17
Cysteine	-	0.23	-	-	-
Valine	0.08	2.15	0.58	2.51	2.16
Methionine	0.11	1.33	0.70	4.22	0.75
Isoleucine	1.14	1.94	0.41	2.45	0.05
Leucine	13.22	4.62	3.24	2.68	7.95
Tyrosine	3.61	2.96	1.11	4.14	0.66
Phenylalanine	4.26	2.03	5.86	9.96	0.14

Values are based on actual recoveries and are average of duplicate estimations. RSM-HA: Hydrochloric acid digest; RWE: Residue water extract; RSE: Residue salt extract; RAE: Residue alkali extract; RAlcE : Residue alcohol extract.

and in the residues (Table 1). Cysteine was present only in RWE. The amino acid composition of RSM is comparable to that reported by Sharma and Ingalls (1974).

Candida curvata was grown on digests of RSM and the residues to assess their ability to support the growth. The RSM-HA showed maximum cell growth after 24 h incubation, while yeast biomass production on RAlcE was comparable to that of RSM-HA after 48 h incubation (Fig. 1). The growth ceased after 24 h incubation on all digests. Maximum growth on RSM-HA might be due to sufficient levels of certain growth promoting amino acids, such as, histidine, serine, isoleucine, tyrosine, aspartic acid and glutamic acid. The levels of isoleucine and tyrosine were extremely low, while lysine, histidine and serine were more in RAlcE in comparison to RSM-HA digest. The methionine content was low in all RSM digests except for RAE. The poor growth on different residues of RSM might be due to low or inadequate levels of growth promoting amino acids. Phillipchuk and Jackson (1979) reported that some amino acids, when absent in the medium, were found to be limiting for yeast growth. The present data indicate the variability in yeast biomass production due to differences in the protein contents of RSM digests.

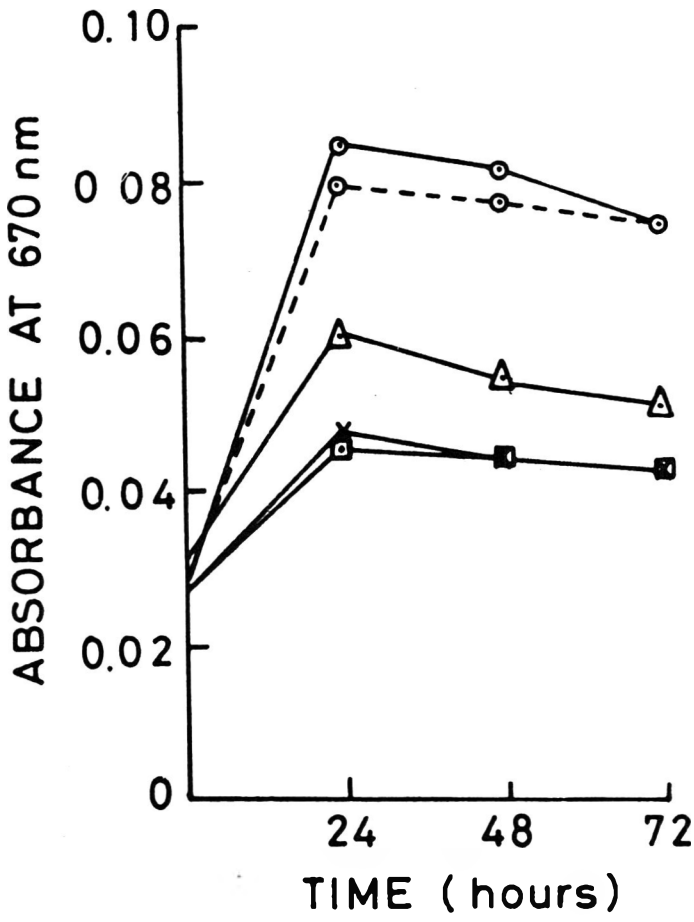

Fig. 1. Growth of *Candida curvata* on acid digests of rapeseed meal (RSM) and the residues left over after protein isolation. O——O : RSM-HA; Δ——Δ : RSM-RWE; x——x : RSM-RSE; □——□ : RSM-RAE; O- - - O : RSM-RAlcE.

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Effect of Vital Gluten and Disodium Phosphate on the Quality of Flat Bread and Noodles from Sprouted Wheat Flour

K.S. SEKHON[1], H.P.S. NAGI*[1], NARPINDER SINGH[2] AND SAVITA SHARMA[1]

[1]Department of Food Science and Technology,
Punjab Agricultural University, Ludhiana-141 004, India.

[2]Department of Applied Chemistry,
Guru Nanak Dev University, Amritsar-143 005, India.

Addition of 4% vital gluten and 0.15% disodium phosphate was found to be beneficial for getting good quality flat breads from sprouted wheats. Good quality noodles could also be prepared from moderately sprouted wheats with the addition of 2% vital gluten and 0.15% disodium phosphate. However, highly sprouted wheats, require extra addition of gluten to the flour to produce quality noodles.

Keywords : Sprouted wheat flour, Vital gluten, Disodium phosphate, Flat bread, Noodles.

Untimely rains during the harvesting and threshing period lead to field sprouting of wheat grains and bring about extensive chemical changes and increased hydrolytic enzymes. For example, the water absorption capacity of sprouted flour decreases and it becomes ill-suited for baking (Waltl 1977; Kulp et al. 1983; Sharma et al. 1988). The amylolytic enzymes degrade the starch to the extent that dextrins are produced, which impart stickiness to the dough. In addition to alpha-amylase, proteases and solubilized carbohydrates are also responsible for deteriorated quality of products made from sprouted wheat (Bean et al. 1974a). Sodium chloride and trisodium phosphate, generally used in various noodle products, are known to restore the quality of noodles prepared from sprouted wheat (Bean et al. 1974b). Therefore, to make up the loss of gluten in sprouted flour, experiments were carried out to prepare flat bread and noodles, with added gluten and disodium phosphate at different concentrations.

A commercial variety of wheat, 'WL-1562', was obtained from the Department of Plant Breeding of the University. Samples of wheat (5 kg) were cleaned manually to remove impurities and given the sprouting treatment for 36 h, by first soaking in sufficient water (10 l) for 10 h in a germination chamber at 30°C and 80% R.H. The sprouted wheat grains were dried in forced air circulation drier at 30°C for 24 h, to about 14% moisture. The sound and sprouted samples were conditioned at the appropriate moisture levels before milling in a Quadrumat Junior Mill (Brabender, OHG, Germany). Flour proteins, fat, ash, total sugars and minerals were determined by standard AACC (1990) methods.

Recipe for preparing flat bread consists of (g) flour 100, compressed yeast 1, common salt 1, vital gluten 2 or 4, anhydrous disodium phosphate 0.15 and water 65 ml. Mixing, fermentation and proofing times were 3-4, 45 and 15 min, respectively. Sheeting and cutting were done to obtain 3 mm thick and 15 cm round diam pieces, for baking at 300°C for 3-4 min. For noodles, a stiff dough was made using flour, gluten, water and disodium phosphate, in the above mentioned proportions. It was then extruded through a manually operated noodle making machine (No. 5, Smart and Co. Aligarh), fitted with a sieve having round holes (diam 1 mm). The dough strands were then fried in refined cottonseed oil at 180°C for 5 sec, in the automatic deep-fat-frier (thermostatically controlled fryer, ITT, Deluxe-2, USA).

During preparation, the flat bread doughs were evaluated for dough handling and puffing (Austin and Ram 1971). The colour of the freshly baked flat bread was also noted visually. In case of noodles, the optimum cooking time was noted by cooking 10 g noodles in 200 ml distilled water, till the disappearance of white core, as judged by squeezing between glass plate. Gruel solid losses were determined by evaporating an aliquot of the water in which noodles were cooked to optimum time. The pH of the noodles was estimated by taking 10 g in 100 ml water. A 9 point hedonic scale was used to organoleptically evaluate the flat bread and cooked noodles (Larmond 1970). The data from duplicate experiments were statistically analyzed using the factors in randomized block

* Corresponding Author

TABLE 1. EFFECT OF VITAL GLUTEN AND DISODIUM PHOSPHATE ON WATER ABSORPTION, DOUGH HANDLING AND ORGANOLEPTIC PROPERTIES OF FLAT BREAD.

Flour samples	Characteristics	Gluten, % 0 Sound wheat	0 With di-sodium phosphate, 0.15%	2 Sound wheat	2 With di-sodium phosphate, 0.15%	4 Sound wheat	4 With di-sodium phosphate, 0.15%
Sound wheat (Falling number 483)	Water absorption, %	65.0	65.0	67.0	67.5	69.0	69.5
	Dough handling	NS	NS	NS	NS	NS	NS
	Puffing	F	F	F	F	F	F
	Colour, visual	CW	CW	CW	CW	CW	CW
	Organoleptic quality						
	Colour	8.0	7.8	7.8	8.3	7.8	7.3
	Texture	7.5	7.0	7.8	8.3	6.5	7.0
	Flavour	7.8	7.0	7.5	8.3	7.0	7.3
	Taste	7.8	6.8	7.5	8.0	6.8	7.3
	Overall acceptability	7.8	7.1	7.1	8.2	7.0	7.3
Sprouted wheat (Falling number 109)	Water absorption, %	58.0	58.0	59.0	59.5	59.5	60.0
	Dough handling	S	SS	SS	NS	NS	NS
	Puffing	P	F	F	F	F	F
	Colour, visual	LB	LB	LB	CW	CW	CW
	Organoleptic quality						
	Colour	6.3	6.3	6.3	7.0	7.0	7.3
	Texture	5.3	5.3	5.3	6.8	6.5	7.0
	Flavour	5.0	5.5	5.8	6.5	6.5	6.8
	Taste	5.5	5.3	5.5	6.5	6.5	6.8
	Overall acceptability	5.8	5.6	6.7	6.7	6.6	6.9

S : Sticky, SS : Slightly sticky, NS : Non-sticky, F : Full puffing, P : Partial puffing, CW : Creamish white, LB : Light brown, LSD : (0.05), Water absorption: 1.3, Colour : 0.7, Texture : 0.7, Flavour : 0.4, Taste : 0.3, Overall acceptability : 0.3

design, as described by Steel and Torrie (1960).

Water absorption increased significantly with the addition of gluten (Table 1). With the addition of 4% gluten, the water absorption increased by 5 and 2% in case of flours from sound and sprouted grains. There was no difference in the dough handling of flours from sound grains, with or without added gluten. All the samples were non-sticky and puffed full. The breads were creamish white in colour, with pleasing flavour. In the case of flour from sprouted grains, the sample without any added gluten was sticky to handle. The stickiness problem was overcome with the addition of disodium phosphate and 2% gluten individually. The combined effect of 2% or higher gluten, along with disodium phosphate, proved extremely useful and the dough behaved satisfactorily. Bread, made from flour of sprouted grains and without any addition, showed partial puffing, whereas other breads puffed full. The colour of the flat bread was light brown, up tc 2% gluten addition, and it improved with further addition of gluten, along with disodium phosphate (Table 1).

Organoleptically, all the flat breads from sound wheat were highly acceptable to consumers. However, flat breads with added 2% gluten and 0.15% disodium phosphate rated superior to the others. Further addition of gluten was in no way helpful in increasing the sensory scores. In case of bread from flour of sprouted grains, the addition of 4% gluten, along with 0.15% disodium phosphate, scored maximum, and these breads were liked by the panelists and were rated as normal (Table 1).

Noodles, prepared from the flour of sprouted grains, had lower water absorption, cooking time and total scores, as against the higher loss of solids in cooking water (Table 2). The noodles, prepared from flours of the grains sprouted for 36 h, were very sticky and soggy, after cooking. The pH of the noodles was not significantly affected with the use of flour from sprouted grains. However, addition of disodium phosphate increased the pH from 5.4 to 6.3 of the noodles made from sound grains and from 5.2 to 6.4 of those made from flours of sprouted wheat grains. Addition of vital gluten significantly improved all the attributes, which otherwise get deteriorated due to the use of flour from sprouted grains. These deteriorated attributes were found to show improvement upon addition of disodium phosphate. Good quality noodles,

TABLE 2. EFFECT OF VITAL GLUTEN ON COOKING AND ORGANOLEPTIC SCORE OF NOODLES.

Flour samples	Characteristics	Gluten, % 0 Normal	0 With di-sodium phosphate	2 Normal	2 With di-sodium phosphate	4 Normal	4 With di-sodium phosphate
Sound wheat (Falling number 483)	Water absorption, %	47.0	47.5	49.5	50.5	50.0	52.0
	Cooking time, min	2.55	2.6	3.4	4.2	4.6	5.4
	Gruel solid loss, %	11.2	10.5	10.0	9.8	9.2	9.0
	pH	5.4	6.3	5.5	6.4	5.4	6.6
	Total scores (9.0)	7.5	7.6	8.0	8.2	8.1	8.3
Sprouted wheat (Falling number 109)	Water absorption, %	38.3	41.0	40.0	41.5	41.1	42.3
	Cooking time, min	2.1	3.4	2.9	3.7	4.2	4.7
	Gruel solid loss, %	12.0	11.4	10.8	10.6	9.9	6.6
	pH	5.2	6.4	5.2	6.1	4.3	6.4
	Total scores (9.0)	5.5	6.2	6.7	7.1	7.4	7.7

LSD : (0.05), Water absorption : 0.5, Cooking time : 0.4, Gruel solid loss : 0.3, Total scores : 0.04, respectively; Disodium phosphate used was 0.15% in all the cases.

comparable to those made from sound grains, could be produced from a moderately sprouted (36 h) wheat, with the addition of 2% gluten and 0.15% disodium phosphate.

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Enzymes for Improved Extraction and Stabilization of Colour and Flavour of Orange Juice

A. SOLEHAH, V.T. BALAUMANI, K. DAS* AND M.A. AMIZA

Division of Food Technology, School of Industrial Technology, Universiti Sains Malaysia, Minden, 11800 Penang, Malaysia.

Treatment of orange pulp with pectinase at 45°C and a pulp to water ratio of 1:1, resulted in about 1.4-fold increase in °Brix, as compared to that without addition of enzyme. Cellulase alone did not show any improvement, whereas, the combined effect of cellulase and pectinase improved the °Brix to 1.8-fold with synergistic effect, as compared to that without the use of enzyme. Glucose oxidase-peroxidase system was found to be effective for the chemical stability of the stored juice. Stabilization of flavour and colour indicated that both pH and total soluble solids were higher in the enzyme-treated juice stored at 5°C, as compared to those in controls, and these remained constant throughout the storage period. A higher loss of about 59% glucose in treated juice, compared to that of 18% in controls, led to less browning in enzyme-treated samples. Recoverable oil %, and thus the estimated d-limolene, decreased during storage, with corresponding reduction in the oxidation of d-limolene. The loss of d-limolene in control juice was higher than that in enzyme-treated samples.

Keywords : Orange juice, Enzymic extraction, Stabilization of colour and flavour, Pectinase, Cellulase, Browning, Glucose oxidase-peroxidase system, Storage.

Extraction of citrus juice, specially orange juice, needs a specific extractor, which can extract the pulp (Braddock and Kesterson 1979) and at the same time separate the peel and the albedo. It is not economical to extract the whole juice from the pulp, as this will need the use of a high pressure screw press (Albrigo and Carter 1977). Moreover, the use of high pressure screw press produces a low quality juice (Braddock and Kesterson 1979). It has been reported that pectin, present in citrus juice, creates difficulties in the processing of soluble solids (McKinnis et al. 1964; Doesburg 1965). Soft fruit pulp is quite difficult to press (Braddock and Kesterson 1976) and extraction is better done with the use of a pectinolytic enzyme (Braddock and Kesterson 1976, 1979; Sreekantiah et al. 1968, 1971). In fruit processing, pectinase is usually used to rupture the fruit structure for maximising juice yield, decreasing juice viscosity and facilitating pressing and filtration (Braddock and Kesterson 1976, 1979). Similar technology has been in use for blackcurrant, strawberry and raspberry extraction (Kulp 1975; Kertesz 1951).

Browning in orange juice is essentially caused by Maillard reaction or ascorbic acid (Eskin et al. 1981). The effective way of minimising browning reaction, apart from using sulphur dioxide, is by removing the reactants involved. As it is not practicable to remove the ascorbic acid and the amino acids, removal of glucose by glucose oxidase-peroxidase system is a more promising process (Reynolds 1965; Woods and Swinton 1991). The most important category of flavour ingredients in citrus fruits is the essential oil consisting of aldehydes, ketones, esters, alcohols and acids (Kirchner and Miller 1957; Veldhuis et al. 1972). Many of these are isoprenoids and the cyclic terpene d-limolene (4-isopropanyl-1-methyl cyclohexane) is one of the most abundant constituents (90-95%) in citrus fruit (Eskin et al. 1981). Types of containers can also effect shelf-life of citrus juices. Vitamin and possibly flavour stabilities are poorer in containers that are permeable to atmospheric oxygen (Tatum et al. 1975). With a view to improve the juice recovery and to reduce discoloration due to browning, as well as off-flavour on account of oxidation of d-limolene, the present work on evaluation of potentials of enzymes was undertaken.

Enzymic treatments : Pectinase-ultrazyme (activity of 5.7 IU/mg protein with 17 mg protein/ml), cellulase-cellulast (activity of 1,500 IU/g protein with 1.2 g protein/ml) and *A. niger* glucose oxidase-Novozyme 358 (activity of 2,000 IU/ml, plus added peroxidase) were obtained from Novo Industries, Holland. Seedless oranges (*Citrus sinensis* Osbeck) were thoroughly washed, cut, and the flavedo as well as the albedo were discarded. The pulp from the extractor (auto-juicer) was diluted with water in the ratios of 1:1 and 1:2 (w/w). Each dilution was treated individually for 1 h with pectinase (0.32 IU/30 g pulp), cellulase (3 IU/30 g pulp) and with a mixture of both at different temperatures. In subsequent experiment, 1:1 diluted juice was treated at 45°C with a mixture of pectinase + cellulase for 1 h. The enzymatically recovered juice was divided

* Corresponding Author

into two parts, and diluted with water in the ratio 1:500. One half was treated with glucose oxidase-peroxidase system, while the other half was used as an untreated control. Both samples were packed in air- sealed plastic bottles, and stored at 5°C. During the storage period, regular tests of samples were conducted.

Analytical methods : Total soluble solids (TSS) were determined by a refractometer and expressed as °Brix. The °Brix differentials were the differences between the initial and final (after 1 h) reading. For determination of glucose concentration (free of fructose), an auto glucose and L-lactate analyzer (Yellow Spring Instrument Co., Ohio, model 2000) was used. An improved method, developed by Klim and Nagy (1988) was used for determining browning (UK 16A model, Shimadzu, Japan). Bromate titration method (Scott et al. 1965), using distillation with isopropanol, was used for determination of recoverable oil and d-limolene. Sensory analysis was conducted by a simple preference test (Michael O'Mahony, 1986). Most of the assays were carried out in duplicates.

Use of enzymes in the extraction of juice from the pulp

Effect of pectinase and cellulase individually: The increase in total soluble solids (°Brix differentials) of aqueous extract of pulp juice at dilutions 1:1 and 1:2, due to action of pectinase at 35, 40, 45 and 50°C, is shown in Fig.1. Data indicate an increase in °Brix differentials with increase in temperature up to 45°C for all the enzyme treatments. But after 45°C, the °Brix differentials decreased until it became zero, i.e., no changes at 50°C. This shows that the rate of thermal deactivation of the enzymes exceeds the catalytic rate after 45°C. Further, it is found that the juice dilution at the ratio of 1:1 has resulted in a higher increase in soluble solids, as compared to that of 1:2 in case of all the enzyme treatments. At 1:1 dilution of juice, the increase in °Brix is about 1.4-fold due to action of pectinase at 45°C, as compared to that of control (without enzyme). The increase may be explained by the fact that pectinase solubilises pectic materials and wall membranes of the juice cells, thereby resulting in softening of tissues in juice pulp (Braddock and Kesterson, 1976, 1979). The results indicate that cellulase has no effect, when it is used alone (Fig.1).

Effect of combined use of pectinase and cellulase: Fig. 1 shows the effect of treatment of aqueous extract of pulp juice (at 1:1 and 1:2

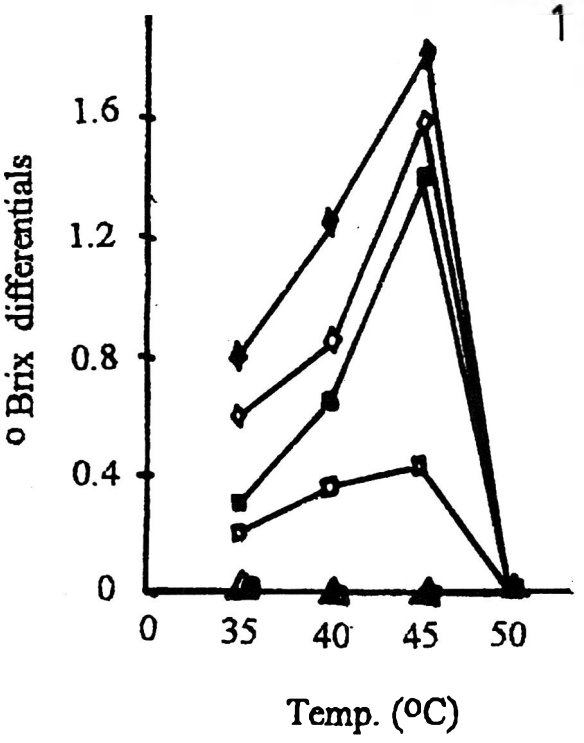

Fig. 1 The effect of individual and combined enzymes on °Brix differentials of the different dilutions on the juice. Solid symbols : 1:1 ratio of juice dilution with water, Hollow symbols: 1:2 ratio of juice dilution with water, ■ and □ : Pectinase alone; ▲ and △ : Cellulase alone, ◆ and ◇ : Mixture of pectinase and cellulase, O: Control, no enzyme.

dilutions) with a mixture of pectinase and cellulase (0.32 and 3 IU/30 g pulp, respectively) at different temperatures. It is found that the combined use of the enzymes has further improved the °Brix differentials, and showed as high as about 1.8-fold increase in °Brix, as compared to that without the addition of enzyme. Such synergistic effect of these two enzymes was also reported by Wolnak (1972) in the extraction of apple juice.

Use of enzymes in stabilization of colour and flavour

Both pH and °Brix values were observed to remain constant throughout the storage period of the juice, whether treated with glucose oxidase/ peroxidase system or not.

Effect of browning : Results show that the browning, measured as absorbance at 420 nm, is linear with storage time. Notable decrease in browning is observed in the enzyme-treated juice, as compared to that in the control. It is known that glucose takes part in the Maillard reaction (Eskin et al. 1981). When the mixture of glucose oxidase-peroxidase was added to the juice, it catalyzed both oxidation of glucose to gluconic acid

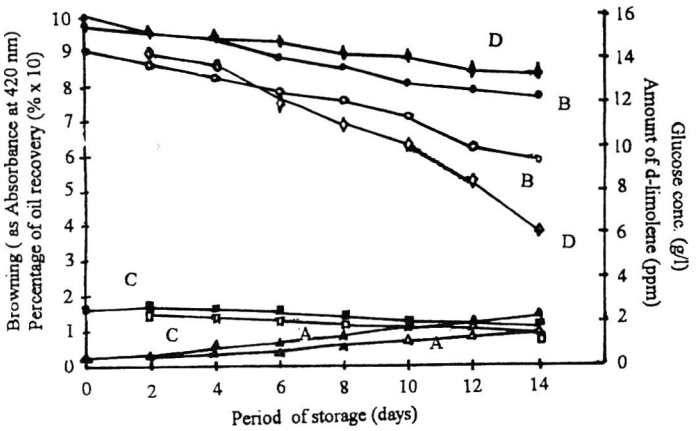

Fig. 2. Effect of treatment of the juice with glucose oxidase/peroxidase system on browning (A), recovery of oil (B), d-limolene (C), and glucose concentrations (D) during storage, Solid symbols; Control experiments, no enzyme, Hollow symbols : Enzyme treatment of juice with glucose oxidase/peroxidase system. ▲ and Δ : Browning, ● and O : Recovery of oil, ■ and ❑ : d-limolene, ♦ and ◊ : Glucose concentration.

and removal of oxygen, thereby slowing down the Maillard reaction, which facilitates browning.

Effect on glucose content : Concentrations of glucose in the juice at different storage times are presented in Fig.2. The control sample stored at 5°C exhibited an 18% loss of glucose, whereas, the loss in glucose oxidase/peroxidase-treated juice was about 59%, after two weeks of storage. Such greater loss of glucose, due to its oxidation by glucose oxidase-peroxidase in the enzyme-treated samples, is responsible for lower browning.

Effect on essential oils and d-limolene : Results show a decrease in the amount of essential oils recorded in both enzyme-treated and control juice (Fig.2). Recovery of d-limolene was 38 and 32% for treated and untreated juice, respectively. The corresponding loss of d-limolene in both sample is due to the fact that, under acidic conditions, it is oxidized to α-terpineol (Matthews and Braddock, 1987) which is a non-volatile terpene that causes off-flavour (Lundahl et al. 1989). The study indicates that glucose oxidase was effective in reducing the oxidation of d-limolene, and to stabilize the flavour of orange juice to a certain extent, due to removal of oxygen from juice.

Sensory evaluation : Results drawn from a simple preference test indicate that the enzyme-treated juice was more attractive and preferred by all panelists because it tasted better. This shows that enzyme treatment is effective in preserving the flavour and colour of orange juice.

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A New Method for Isolation of Proteases from Cocoa Beans by Ammonium Sulphate Precipitation

T.K. SEOW[1], S. JINAP*[2], K.H. LEE[1], P.M. LEE[3]

[1]Department of Biotechnology, [2]Department of Food Science,
Faculty of Food Science and Biotechnology,
Universiti Pertanian Malaysia,
43400 UPM Serdang, Selangor, Malaysia.

[3]Department of Chemistry, Indiana University,
Shah Alam Campus in Malaysia, ITM/MUCIA, Section 16,
40000 Shah Alam, Selangor, Malaysia.

Protease was successfully isolated from the cotyledons of ripe cocoa beans *(Theobroma cacao* Linneaus) by a precipitation method, using 20 mM sodium phosphate buffer (pH 6.5) containing 1.5% polyvinylpyrrolidone and ammonium sulphate. Four fractions, that were proteolytically active against casein were isolated, using 0 - 20%, 20 - 40%, 40 - 60% and 60 - 80% saturations of ammonium sulphate. Addition of Triton X-100 and sodium dodecyl sulphate did not enhance the efficiency of the isolation process.

Keywords : Cocoa beans, Protease isolation, Ammonium sulphate precipitation, Detergents, Polyvinylpyrrolidone.

Although the role of proteases in the production of cocoa flavour and flavour precursors has long been recognised (Ziegleder and Biehl et al. 1991), and the presence of this group of enzymes in cocoa beans has been detected since 1915 (Forsyth and Quesnel 1963), a detailed investigation on these enzymes in cocoa beans has never been carried out (Lehrian and Patterson 1983). Actually, the acids and flavour produced during fermentation determine the overall quality of cocoa beans (Samah et al. 1992). In fact, there were even reports of failure to detect proteases in cocoa beans (Holden 1959), even though there were earlier reports of success (Forsyth et al. 1958). Isolation of protease from cocoa bean cotyledons *(Theobroma cacao* Linneaus) is necessary for conducting studies on the properties of the enzyme. Previous workers have used an acetone precipitation method for isolating proteases from cocoa beans (Forsyth et al. 1958; Biehl et al. 1991). This study reports an isolation of protease from cocoa beans by ammonium sulphate precipitation method, along with data on determination of sulphate ammonium sulphate saturation ranges, and effect of certain reagents on the efficiency of the isolation procedure.

Ripe cocoa pods, 'PBC 128' variety, were harvested from the Sime Darby Estate in Malacca, Malaysia, transported in dry carbon dioxide to the University, and stored immediately in the freezer at -20°C. For extraction of crude enzyme, the frozen cocoa pods were thawed, split open with a knife, the beans were taken out and their mucilage and testa were removed carefully using a knife. The beans were homogenized in chilled 20 mM sodium phosphate buffer (pH 6.5; 1:2 w/v), using a National MX-291N blender. The solution was sieved through a cloth strainer and the resulting filtrate was centrifuged in a Beckman J2-21M/C refrigerated centrifuge (4°C; 22,100 x **g**; 30 min). The resulting crude extract was fractional precipitated by ammonium sulphate (Green and Hughes 1955). The precipitate was separated by centrifugation, as above, and resuspended in a minimal volume of 20 mM sodium phosphate buffer (pH 6.5), before dialysis against 20 mM sodium phosphate buffer (pH 6.5; 4°C). The dialysing medium was changed twice every 24 h. The crude extract, dialysed and non-dialysed fractions were assayed.

The casein digestion method (Kunitz 1947) was used to assay protease activity. For the assay, 0.5 ml enzyme solution was mixed with 1 ml casein solution (0.5%), and incubated at 37°C for 1 h. One ml trichloroacetic acid (10%) was added and centrifuged (1,700 x **g**; 15 min; 25°C) in Clements GS150 bench top centrifuge. The supernatant was removed carefully by a Pasteur pipette and its absorbance at 280 nm was determined in a Hitachi U-1100 spectrophotometer. The control solution was prepared in the same way, but for the addition of 1 ml trichloroacetic acid (10%) before incubation. Protease activity was arbitrarily considered as the difference between absorbance readings per ml of fraction, after and before incubation. Protein

* *Corresponding Author*

concentration was determined by the spectrophotometric method of Lowry et al (1951), using bovine serum albumin (BSA) as a standard.

Polyvinylpyrrolidone (PVP), at 1.5% (w/w, based on cocoa beans) was added into the chilled 20 mM sodium phosphate buffer (pH 6.5), before the mixture was homogenized (National MX-291N blender) for 30 sec. To determine the effect of Triton X-100 (a non-ionic commercial detergent) and sodium dodecyl sulphate (SDS) (an anionic detergent), each was added to the fraction to a final concentration of 0.1% (v/v or w/v). As Triton X-100 contains a phenyl group, which interferes with the spectrophotometric measurement at 280 nm in the casein digestion method, 10% trichloroacetic acid was added to 0.1% (v/v) Triton X-100, for use as a control.

Three fractions were precipitated by 0 - 50%, 50 - 70%, and 70 - 90% saturations of ammonium sulphate, but none of them was proteolytically active, probably due to interference by the ammonium sulphate, which is present in all the fractions. Ammonium sulphate was found to exhibit a reading at 280 nm. However, none of the three fractions was proteolytically active even after dialysis.

Effect of PVP : All the fractions treated by PVP were proteolytically inactive. However, these showed proteolytic activity after dialysis (Table 1). PVP, a phenolic arrester (Suelter 1985), removes phenolic compounds, normally present in large amounts in plant tissues (Ziegleder and Biehl 1988). If not removed, these compounds or their oxidation products, could deactivate the proteases through the formation of insoluble protein complexes (Suelter 1985; Whitaker 1972). The interference caused by ammonium sulphate was eliminated after dialysis and the protease activity of the fractions could be detected. Data in Table 1 also confirmed the suitability of the casein digestion method to detect proteolytic activity in the fractions. The control, containing a casein solution, showed no reading, thereby demonstrating that this method could be used with confidence.

TABLE 1. PROTEASE ASSAY OF AMMONIUM SULPHATE PRECIPITATED FRACTIONS, WITH OR WITHOUT FRACTIONATION

Sample	Protease activity, abs/ml
Ammonium sulphate fractionation, %	
0 - 50	0.402 ± 0.006
50 - 70	0.134 ± 0. 002
70 - 90	0.012 ± 0.002
Control	0
Treated 0-50% fraction	
with Triton X-100	0.018 ± 0.002
with SDS	0.240 ± 0.004

Effect of detergents : Both the detergents reduced proteolytic activity of the fraction obtained by 0 - 50% ammonium sulphate precipitation (Table 1). Triton X-100 showed a more pronounced effect, than did SDS. Triton X-100 and SDS are used as solubilizing agents of proteins, and are sometimes employed in improving isolation of enzymes (Suelter 1985).

The inhibitory effect of Triton X-100 may be due the presence of contaminants that oxidize sulphydryl groups (Suelter 1985). The results also indicate that the proteolytic property of the 0 - 50% fraction may be due to a protease that is classified as cysteine protease, which is dependent on the sulphydryl groups for its activity (Lowe 1976). The reduction (60%) of protease activity by SDS, might be due to its ability to denature proteins (Suelter 1985).

Fractionation of crude extract : The crude extract was fractionated with 0-20%, 20-40%, 40-60% and 60-80% ammonium sulphate concentrations. All the fractions were proteolytically active (Table 2). The 0-20% fraction had the highest activity, while the lowest activity was found in 60-80% fraction. However, 60-80% fraction exhibited the highest specific activity, while the specific activity of the 0-20% fraction was only about half. The difference is due to the relatively higher concentration of protein in 0-20% fraction, which is about 4.5 times of that in 60-80% fraction.

TABLE 2. PROTEASE ACTIVITY OF VARIOUS AMMONIUM SULPHATE PRECIPITATED FRACTIONS

Ammonium sulphate fractionation, %	Sample volume, ml	Protease activity, abs/ml	Protein concentration, mg/ml	Specific activity
0 - 20	660	0.065 ± 0.002	2.267 ± 0.05	0.028 ± 0.001
20 - 40	460	0.056 ± 0.003	1.885 ± 0.04	0.029 ± 0.002
40 - 60	330	0.028 ± 0.001	1.470 ± 0.04	0.019 ± 0.001
60 - 80	192	0.027 ± 0.001	0.493 ± 0.06	0.054 ± 0.001

The results of this study are in agreement with those reported by Forsyth et al (1958) and Biehl et al (1991). The fact that all the ammonium sulphate precipitated fractions are proteolytically active could imply the existence of more than one type of protease in cocoa beans. Similar implication could also be derived from the results of the work by Biehl et al (1991). However, Holden (1959) reported unsuccessful attempts to detect protease in cocoa beans. The results of this study provide positive confirmation for the existence of proteases in cocoa beans.

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Foliar Application of Maleic Hydrazide for Improving Storability of Potatoes Under High Temperature Storage Conditions

H.N. KAUL* AND ASHIV MEHTA

Division of Crop Physiology and Biochemistry,
Central Potato Research Station, Jalandhar-144 003, India.

Efficacy of 0.3% maleic hydrazide as a foliar spray, applied 3 weeks before haulm cutting, was studied in four cultivars of potatoes in two crop seasons. The harvested potatoes were stored in an evaporatively cooled potato store (15-29°C, RH 68-90%) and at room temperature (20-36°C, RH 30-72%) from March to May. Maleic hydrazide treatment significantly reduced the mean number of sprouted tubers by 27% upto 10 weeks of storage; sprout weight was also reduced by 76% after 12 weeks of storage. Interaction between maleic hydrazide treatment and storage environment was significant.

Keywords : Potato, Maleic hydrazide, Foliar spray, Evaporatively cooled potato storage, Ambient temperature storage, Sprouting, Weight loss.

Storage of potatoes under high temperature conditions is still prevalent in the plains of India, because of inadequate capacity of refrigerated stores. Consequently, there are excessive sprouting, weight loss and rottage of potatoes (Booth and Proctor 1972; Verma et al. 1974; Kaul et al. 1976; Burton and Wilson 1978). To overcome these problems, vapour heat treatment (Rama and Narasimham 1985) and the use of insulated, evaporatively cooled potato stores (ECPS) have been suggested (Mehta and Kaul 1987, 1988; Kaul and Mehta 1988). Evaporative cooling storage of potatoes, in small naturally ventilated chambers, and zero-energy cool chambers, has also been found to be beneficial (Roy and Khurdiya 1983; Rama et al. 1990; Rama and Narasimham 1991), side by side with chemical sprout inhibitor treatments (Rama and Narasimham 1987, 1989). Folication of maleic hydrazide (MH) on the standing crop has been reported to suppress sprouting in potatoes stored at high temperatures (Singh et al. 1959; Vliet and Schrilmer 1963; Ahmed et al. 1981; Badshah 1984); but no information is available on its efficacy, when the produce is stored in ECPS. In the present study, efficacy of foliar application of MH on four potato cultivars has been evaluated, when the produce was stored in ECPS and at room temperature (RT).

Treatment : Field trials were conducted in sandy loam soils at the Institute Farm. The experiments involved MH spray at two levels (0 and 25 kg/ha) on four cultivars viz., 'Kufri Chandramukhi', 'Kufri Sindhuri', 'Kufri Badshah' and 'Kufri Jyoti', with four replications in plots of size of 16.5m^2. N, P and K were applied at 150, 60 and 120 kg ha^{-1}, respectively. Diethanolamine salt of MH (Desprout, Micro Chemicals, India, 30% MH) in a 0.3% solution was sprayed on the crop, three weeks before haulm cutting, at the rate 25 kg MH ha^{-1}, an effective concentration, which leaves MH residues in tuber at the permissible limits (Sukumaran et al. 1979). The plants were irrigated 10 times during growth, with such a schedule that there was no irrigation for 10 days before haulm cutting. The harvested tubers were heaped in the field for 15-20 days for curing, and then the undamaged tubers within the weight range of 40-60 g were selected. Composite samples of potato were stored, treatment-wise, in an insulated 20-tonne capacity ECPS, and at RT, in hessian cloth bags of 20 kg for 12 weeks, starting from the first week of March.

Analytical aspects : Temperature and relative humidity were recorded daily, while physiological weight loss, sprouting, rotting, and sprout weight were determined every two weeks. Loss in weight was recorded from 20 marked tubers, and sprouting and rotting from bulk stored tubers. Tubers having at least one sprout longer than 0.5 cm were counted as sprouted. Tubers were desprouted and sprout weight (g kg^{-1} tuber weight) was recorded in undisturbed separate lots, at each observation. Total losses were calculated as sum of weight loss, sprout weight and rottage. Since the high order interaction MH x Storage x Cultivars was generally not significant, the same has been omitted. The data recorded during the period of storage were statistically analyzed in 2 x 2 x 4 factorial completely

* *Corresponding Author*

TABLE 1. EFFECT OF FOLIAR APPLICATION OF MH AND STORAGE ENVIRONMENT ON STORAGE ATTRIBUTES

Attribute	Storage period, weeks									
	4		6		8		10		12	
	RT	ECPS	RT	ECPS	RT	ECPS	RT	ECPS	RT	ECPS
					Sprouting, %					
Without MH spray	39.30	36.80	81.30	47.40	94.60	92.90	98.10	100.00	100.00	100.00
With MH spray	23.90	17.80	45.90	34.60	74.70	60.30	91.10	80.40	100.00	100.00
					Sprout weight, g kg^{-1} tuber weight					
Without MH spray	0.62	0.27	1.64	2.87	8.72	7.23	11.43	13.01	12.26	22.21
With MH spray	0.21	0.20	0.61	0.42	2.10	1.91	2.94	2.38	3.28	4.83
					Rottage, %					
Without MH spray	ND	ND	ND	ND	ND	ND	2.20	0.60	4.10	0.60
With MH spray	ND	ND	ND	ND	ND	ND	2.00	0.30	14.10	1.10
					Weight loss, %					
Without MH spray	3.47	1.07	6.17	2.69	7.78	5.43	12.18	6.15	16.21	11.02
With MH spray	3.21	1.06	5.74	2.67	7.28	4.56	11.97	5.39	15.86	9.21
					Total loss, %					
Without MH spray	3.53	1.09	6.33	2.97	8.65	6.15	15.52	8.05	21.44	13.84
With MH spray	3.23	1.08	5.80	2.71	7.49	4.75	14.26	5.92	30.28	10.79

ND : Nil

randomized design (Gomez and Gomez 1984).

Storage environment : The daily maximum temperature inside the ECPS was 6-9°C lower than the RT. The latter increased from 23.7 to 38.2°C from March to May. The relative humidity in ECPS was initially high (81-85%) and decreased only a little as the outside temperature increased. RH in ECPS was in the range of 81.5-93.7%, as compared to a wide variation of 34.6-85.5% at RT.

Effect of MH application : Maleic hydrazide foliar spraying considerably decreased the mean percentage of sprouted tubers and the differences were significant at each sampling (Tables 1 and 2). Reduction in sprouting of tubers at high temperatures, due to the application of MH, has also been reported earlier (Matlob 1979; Ahmed et al. 1981; Badshah 1984). After 10 weeks, sprouting was 100% in both the MH treatments (data not given). Sprouting in ECPS was low by 36 and 4.6% at 6 and 10 weeks storage. Similar was the trend of sprout weight (Tables 1 and 2) and these results are in conformity with the results of Weis et al (1980). Mean sprout weight after 12 weeks was higher by 74% in the ECPS due to the effect of high relative humidity, which is known to be favourable for sprout growth (Kaul and Mehta 1988).

There was no rottage upto 8 weeks under both the storage conditions. MH treatment, however, increased the mean rottage of tubers; this trend was similar to that reported by Singh et al (1959) and Ezekiel et al (1984). Mean rottage of potatoes in the ECPS was, however, significantly less than at RT (Tables 1 and 2). Weight loss of potatoes remained lower in MH treatment, but the differences were statistically non-significant. MH spray is reported to be effective in reducing weight loss, primarily by restricting the sprout growth

TABLE 2. SUMMARY OF STATISTICAL DATA (CD AT 0.05)

	Storage period, weeks				
	4	6	8	10	12
			Sprouting		
MH	0.34	0.43	0.42	0.48	-
Stores	0.34	0.42	0.42	0.48	-
MH x Stores	0.49	0.59	0.60	0.68	-
			Sprout weight, g kg^{-1} tuber weight		
MH	0.03	0.20	0.42	0.55	0.53
Stores	0.03	0.20	0.42	ND	0.53
MH x Stores	0.05	0.29	0.59	0.77	1.07
			Rottage, %		
MH	ND	ND	ND	0.14	0.27
Storage	ND	ND	ND	0.14	0.27
MH x Stores	ND	ND	ND	0.20	0.39
			Weight loss, %		
MH	NS	NS	NS	NS	NS
Stores	0.25	0.33	0.37	0.52	0.79
MH x Stores	0.35	0.47	0.53	0.73	1.12
			Total loss, %		
MH	NS	NS	NS	NS	NS
Stores	0.27	0.36	0.38	0.63	1.09
MH x Stores	0.40	0.51	0.55	0.89	1.53

ND : Nil, NS : Non-significant

(Matlob 1979). Weight loss of potatoes in the ECPS was significantly less at each sampling. Mean total losses due to the combined effect of rottage, weight loss and sprout weight were not affected by the MH treatment. However, these were significantly reduced in the ECPS (Tables 1 and 2), probably due to lower temperature and high humidity.

The studies indicated that foliar application of maleic hydrazide, in combination with improvised storage structures like ECPS, can effectively reduce the sprouting and weight loss of tubers, thereby resulting in reduced total losses under high temperature storage conditions in the tropics.

The authors are thankful to Mr. Jaspal Singh and Mr. Raj Kumar for the assistance rendered in the field and data analysis. Thanks are also due to Dr. J.P. Singh for criticism and suggestions.

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Food Hydrocolloids - Structure, Properties and Functions : edited by Katsuyoshi Nishinari and Etsushiro Doi, published by Plenum Press, 233, Spring Street, New York, N.Y.10013, 1993; pp 510, Price US $ 125/-

"Food Hydrocolloids" is a pot-pourri of papers presented at the International Conference at Tsukuba and organised by the Japanese Society of Food Hydrocolloids. The editors have presented 77 papers in this significant volume of very useful information on a wide range of hydrocolloids and their functional properties and food applications.

The book begins with a block of 30 papers dealing with the structure, properties and functions of polysaccharide hydrocolloids derived from plants, seaweed and microbial sources.

The main objective of the editors is to bring together technical and industrial considerations with discussion of the underlying functionality of food hydrocolloids. Structure-property relationships are repeatedly emphasized for each class of biopolymers.

Functional properties are described in a separate section of 19 papers, which include discussions on application of hydrocolloids to dairy products and *surimi* preparations and effect of pressure on gel formation.

Protein hydrocolloids are described in a series of 11 papers, where sol-gel formation with egg proteins, gelatin, collagen, rice and sesame seed proteins are dealt with and the factors affecting the stability of these gels and emulsions have been thoroughly discussed.

The emulsifying properties of proteins and stability of emulsions are dealt with effectively in a series of 6 papers. Interactions between different types of biopolymers and mixed polysaccharides on gel formation are described in the next 6 papers.

The last block of 5 papers deals with the physiology and nutritive value of food hydrocolloids, dealing with dietary fibre and associated changes in metabolism of experimental animals.

This book is sure to have a wide appeal to food scientists concerned with food hydrocolloids. The scientist specializing in a narrow field will also not be disappointed, because of the breadth of information contained within the covers of this book.

The book attempts to bring about an intermarriage between molecular conformation, interactions, and functional properties of the main food hydrocolloids. Using this information, one can develop safe and convenient foods of better quality with desired texture. The wealth of information provided shows how biopolymers - such as starch and other polysaccharides; gelatin and other proteins; and microbial polymers; can be used to provide different textures to thicken, gel, stabilize, emulsify, retain moisture, prevent crystallization, form foams, etc.

The book will open new vistas for the researchers in this field and also serve as a standard text for all working in the enchanting field of food hydrocolloids. The documentation provided in the book at the end of each chapter would help to widen the horizons of knowledge in this field.

P.J. DUBASH
UNIVERSITY DEPARTMENT OF CHEMICAL TECHNOLOGY, BOMBAY - 400 019

AFST (I) ANNOUNCEMENTS

ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA)

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

Invites Nominations for Fellows of AFST (I) for the year 1994

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

General

1. The awardee will be called as Fellow of Association of Food Scientists and Technologists (India) and in an abbreviated form will be termed as FAFST.
2. The total number of Fellow of the Association will not exceed 5% of the total membership, including regular and life members of the Association, in any given year or 100, whichever is lower.

   The title of Fellows has so far been awarded to 32 AFST(I) members and 6 non-members who have contributed to the progress of Food Science and Technology.

Eligibility

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

Nominations

1. The nomination for Fellow should be proposed by five AFST(I) members of good standing for a minimum of 5 years or by 2 Fellows of the Association. This is applicable to AFST(I) members as well as non-members.
2. Any regular or life member of AFST(I), who has been continuously a member of the Association can sponsor the nomination for only one Fellow in a particular year.
3. The nomination shall be accompanied by acceptance of the person proposed.
4. The nomination shall be in the format given overleaf. A brief bio-data of the nominee, highlighting the Scientific or Technological achievements in the area of Food Science and Technology, supported by a list of publications not exceeding 10 important research papers or other supporting documents **not exceeding 20 pages**, must accompany the nominations.
5. Central Executive Committee Members of AFST(I) are not eligible to be nominated as Fellows.
6. The nomination duly proposed and accepted by the nominee shall be sent to the Hon. Executive Secretary, AFST(I) by **31st March 1995.**

Selection of Fellows

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

Privileges of a Fellow

The Fellow shall be entitled to the following rights :

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

Cessation of Fellow

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

Conferring as Fellows

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

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

Please forward your nominations duly filled as per the format given overleaf and mail it by Registered post to the Hon. Executive Secretary, AFST(I), CFTRI Campus, Mysore-570 013, before **31st March 1995.**

The envelope containing the nomination along with the bio-data and contributions (5 copies) should be superscribed 'Nomination for Fellow AFST(I)'.

K. UDAYA SANKAR
HON. EXECUTIVE SECRETARY

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

Nomination Form For Fellows

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

Full name and academic distinction

FULL NAME

DATE OF BIRTH

AREAS OF SPECIALIZATION

ACADEMIC QUALIFICATIONS

---

for election as the Fellow of AFST(I).

We append below the statement of his/her claims for election as Fellow and certify that in our opinion he/she is fully qualified for that distinction.

We also certify that he/she has been informed of the obligations attached to the fellowships of the AFST(I) and is agreeable to abide by them, if elected.

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

.....

Proposer's name & signature
Date :
Station :

Seconder's name & signature
Date :
Station :

(Signatures of supporters from their personal/general knowledge)

(1)

(2)

(3)

I agree for the above nomination

(Name & Signature)

.....

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

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

(3) Last date for receipt of nomination at the AFST-office is **31st March 1995.**

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

INVITES

NOMINATIONS FOR AFST (I) AWARDS FOR 1994

Nominations for the following awards of the AFST(I) for the year 1994 are invited. All nominations would be sent by Registered Post, so as to reach Honorary Executive Secretary, Association of Food Scientists and Technologists (India), CFTRI Campus, Mysore - 570 013, **before 31st March 1995.**

PROF. V. SUBRAHMANYAN INDUSTRIAL ACHIEVEMENT AWARD

The guidelines for the award are :

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

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

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

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

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

The envelope containing the nominations, along with bio-data and contributions (five copies) should be superscribed "**Nomination for Prof. V. Subrahmanyan Industrial Achievement Award - 1994.**

LALJEE GODHOO SMARAK NIDHI AWARD

The guidelines for the award are :

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

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

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

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

The envelope containing the nominations along with bio-data and contributions (five copies) should be superscribed "**Nomination for Laljee Godhoo Smarak Nidhi Award 1994".**

BEST STUDENT AWARD

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

The guidelines for the award are :

(i) The applicant must be an Indian national

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

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

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

(iii) He/She should not have completed 25 years of age on 31st December 1994.

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

The envelope containing the nomination should be superscribed **"Nomination for Best Student Award 1994".**

YOUNG SCIENTIST AWARD

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

The guidelines for the award are :

(1) The candidate should be an Indian national, below the age of 35 years on 31st December 1994, working in the area of Food Science and Technology.

(i) The candidate should furnish evidence of either;

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

OR

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

The application along with details of contributions and bio-data (five copies) may be sent by registered post with the envelope superscribed **"Nomination for Young Scientist Award 1994".**

BEST PAPER AWARD

This award is to be given by the AFST(I) Educational and Publication Trust to the author(s), who have contributed the best paper to the *Journal of Food Science and Technology* published in 1994. A panel of experts, constituted by the Central Executive Committee, will scrutinize the issues and select the best paper for the award.

K. UDAYA SANKAR
HON. EXECUTIVE SECRETARY

STRATEGIES FOR PACKAGING AND STORAGE OF FRESH AND PROCESSED FRUITS AND VEGETABLES IN 21ST CENTURY

SEMINAR HELD AT NEW DELHI ON SEPTEMBER 2 AND 3, 1994

SUMMARY AND RECOMMENDATIONS

The National Seminar on "Strategies for packaging and storage of fresh and processed fruits and vegetables in 21st century" was organized by the Delhi Chapter of the Association of Food Scientists and Technologists (India) at New Delhi during 2nd and 3rd Sept. 1994 with the following objectives :

(i) To examine the environment friendly and energy saving technologies in the area of packaging and storage

(ii) To disseminate the results of R&D in the field of packaging and storage

(iii) To highlight technological advancement and attention required for future research and

(v) To provide impetus to domestic and international trade through upgradation of technology.

The seminar was inaugurated by Shri R.P. Ratawal, Hon. Minister of Tourism and Social Welfare, Govt. of Delhi, on 2nd September at the India International Centre. The inaugural session was presided by Shri Gokul Patnaik, Chairman, APEDA, Dr. K.L. Chadha, DDG (Hort.), ICAR, delivered the keynote address and Shri C.K. Basu, Jt. Secretary, Ministry of Food Processing Industries gave a special address. Dr. Susanta K. Roy, National President of AFST (I) briefed the audience about the seminar. Prof. B.S. Bhatia, President, AFST (I) Delhi Chapter proposed the vote of thanks.

On the 2nd September, after the inauguration, two technical sessions were held. The first technical session on packaging of fresh and processed fruits and vegetables was chaired by Dr. D.K. Uppal, Executive Director, NHB. Eight invited papers were presented. This was followed by the session on Storage of fresh and processed fruits and vegetables under the chairmanship of Dr. G.L. Kaul, Horticulture Commissioner, Government of India, in which six invited speakers delivered their talk.

The 2nd day seminar was held at the NRL, Auditorium of the IARI., New Delhi on 3rd September, 1994 with the poster presentation under the chairmanship of Dr. M.K. Roy, Principal Scientist, NRL, IARI. There were 63 posters during the session. After this, the 3rd session on Export marketing of fresh and processed fruits and vegetables was held under the chairmanship of Shri Gokul Patnaik, Chairman, APEDA. Dr. V. Prakash, Director, CFTRI presented the lead paper. Three invited speakers also spoke in this session.

The Plenary session was held at IARI., New Delhi, on the same day in the after noon under the chairmanship of Shri C.K. Basu, Joint Secretary, Ministry of Food Processing Industries, Govt. of India. Shri Basu in his opening remarks expressed his happiness that a National Seminar on this very important topic was being organised at this juncture, when the Govt. Of India is laying great emphasis. He congratulated the AFST (I) for making such a novel effort, which was attended by more than 200 scientists, technologists, processing and marketing experts. Shri Basu emphasized that it was high time that scientists should focus their attention on packaging and storage of these fresh perishable commodities as well as their processed products, which are lacking at present, so that their efforts could make an impact in the world market. Mr. Basu pointed out that in different fora, it is stated that 30-40% of our horticulture produce perishes because of lack of post-harvest infrastructure and processing facilities. Mr. Basu, however, did not agree with these assertions, as these are not supported by exact data. He, therefore, urged the scientists to state only those which are backed up by data. He, however, admitted that there are losses due to imperfect post-harvest infrastructure for these perishable horticultural commodities and urged the AFST on their own to study these exact losses and the requirements of post-harvest infrastructure facilities for the country. He further stressed the need of development of infrastructure facilities for better handling of these commodities. Shri Basu pointed out that AFST (I) should act as a think tank so that the Govt. of India can refer the problems to the Association and in return, the association can put forward suggestions for solving them. While praising CFTRI as a leading institute for providing research and training in different fields of Food Science and Technology, Shri Basu

felt it is high time that other institutes dealing in horticultural commodities should come up for standardizing the packaging and storage requirements of these perishable commodities. In this regard, he mentioned that IARI has done enough ground work to make a good base for higher research work. This centre can be upgraded to become an advanced centre for imparting training in research and development. After his brief speech, Shri Basu invited the chairmen of different sessions to present the recommendations of their sessions. Immediately after his speech, a lively and fruitful discussion took place and the following recommendations emerged for consideration.

1. A scientific and systematic study should be made for assessing the exact post-harvest losses at different stages of post-harvest delivery system of horticultural produce, including processing, with suggestions for suitable economically viable infrastructure facilities to overcome the problems.
2. Controlled and modified atmosphere techniques may be adopted for storage and packaging of fresh fruits and vegetables, both for internal and export markets.
3. The R&D efforts on the development of edible eco-friendly coating materials or films may be intensified to cope up with the developments in the advanced countries.
4. Alternate packaging materials like weight heat sterilizable glass containers and flexible packaging materials like retortable pouches may be introduced for packing of processed products.
5. Modern packaging system like aseptic packaging using suitable packaging materials may be adopted for RTS beverages and bulk packaging of fruit pulps/concentrates, without using any preservatives and refrigeration for economic benefit and better consumer health.
6. All out efforts should be made to set up packing station and see that all fruits and vegetables after harvest should pass through the packing station before marketing, as followed in the advanced world.
7. Stress on quality maintenance for fresh produce as well as processed products to meet the stringent standard of importing country.
8. Environmentally pure and pesticide-free fruits and vegetables are being sought after not only in western countries but also in India. However, their production and producing level are poor. It is suggested that Govt. of India and State Governments should ban the use of biologically non-degradable pesticides like DDT, BHC, etc.
9. Due to higher application of pesticides during production of various crops, it is essential to estimate them in various food products and suggest methods to bring down the levels of pesticides within the safe permitted limits.
10. In view of the Montreal protocol on phasing out the use of chemical fumigants for the quarantine treatment of fruits and vegetables, APEDA should explore the possibility of using irradiation as an alternative.
11. All efforts should be made for the commercialization of irradiation-processing of onions and potatoes for reducing post-harvest losses.
12. The evaporative cool storage technology is recommended for adoption by farmers and traders for short and medium term storage of the horticultural produce at different handling levels. viz. farms, mandis, halting stations, etc.
13. Realizing the importance of post-harvest technology of horticultural crops, it was suggested that an advanced centre on the lines of CFTRI on the specific field of horticultural crops may be created. It was recommended that IARI with its available infrastructure and multi-disciplinary facilities should have an independent Centre/Division of Post-harvest Technology of Horticultural Crops, to meet the requirements of the country in the area of research and training in this field.
14. It was emphasized that there should be close linkages with various organisations, so that more collaborative work can be taken up avoiding individualistic approach. In this regard AFST (I) should take the leading role.

S.K. ROY
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
NEW DELHI-110 012

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ACKNOWLEDGEMENTS

The Editor expresses his gratitude to the following referees for their valuable help in the evaluation of papers published in the JOURNAL during 1994. The figures in bracket indicate the number of papers evaluated.

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Alur, M.D. (1)
Appaiah, K.M. (1)
Appu Rao, A.G. (1)
Arumughan, C. (1)
Arun Sharma (2)
Arya, S.S. (6)
Ashok Pandey (1)
Azeemoddin, G. (1)

Balasubrahmanyam, N. (1)
Basappa, S.C. (1)
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Bhat, S.G. (2)
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