REG. No. 24918 / 64
ISSN: 0022 - 1155
CODEN: JFSTAB

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

SSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (INDIA)

Vol. 32, No. 6 November-December 1995

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

Telephone : 521747; 28157; Telex 0846-241 FTRI IN, Cable : FOODSEARCH, MYSORE.
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- Advancement of all the aspects of Science and Technology relating to production, processing and distribution of food, with the ultimate objective to serve humanity through better food.
- Promotion of research, development and training in the science, Technology and Engineering of Food.
- To provide a forum for exchange, discussion and dissemination of knowledge and current developments, especially among Food Scientists and Technologists as well as the Public and Society at large.

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Volume 32 Number 6 November-December 1995

C O N T E N T S

Journal of Food Science and Technology, devoted to original R & D contributions in all branches of Science, Technology and Engineering of Foods and Food Products, is a bimonthly publication of the Association of Food Scientists and Technologists (India), Mysore. Review papers are also published to a limited extent.

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Dr. (Mrs) RUGMINI SANKARAN,

PRESIDENT, AFST (I)

FOR 1995-96

Helms of AFST(I) in the Gentle and Persuasive Hands of the Dynamic, Dedicated and Distinguished Food Microbiologist and Technologist

Dr. (Mrs) Rugmini Sankaran, Director, Defence Food Research Laboratory, Mysore has taken over the position of the President of the Association of Food Scientists and Technologists (India) on September 9, 1995 at the function organised on the concluding day of ICFoST '95 at the IFTTC Auditorium of the Central Food Technological Research Institute, Mysore.

Soon after assuming the charge, Dr. Rugmini Sankaran, in her presidential address, paid rich tributes to the outgoing Executive Committee of AFST(I) for the excellent work and also placed on record her appreciation for the wonderful cooperation extended to AFST(I) by the Premier Governmental R&D organisations, like the Central Food Technological Research Institute (CFTRI), Mysore and the Defence Food Research Laboratory (DFRL), Mysore. Further, she stressed that AFST(I) should play a pivotal role in the formulation of food policy and regulations by the Government, a need arisen out of the changing economic scenario and globalisation efforts of the food industries in the country.

In the absence of Dr. B.K. Lonsane, Editor, Journal of Food Science and Technology, who is on a foreign assignment in France, I, as Officiating Editor and being also the outgoing President of the Association, take the privilege to introduce our new President, Dr. Rugmini Sankaran to members of AFST(I) as well as readers of JFST. Incidentally, Dr. Rugmini Sankaran is the first lady to head this prestigious organisation.

Dr. Rugmini, born on September 10, 1935, at Trichur in Kerala, had her early education in her native place and later obtained her doctoral degree in Ecology from the prestigious Banaras Hindu University, Varanasi, U.P., in 1960. Her interest for research made her join the Defence Research Development Organisation, relinquishing her first assignment as a lecturer at the Banaras Hindu University. Dr. Rugmini had Advanced Training in Food Microbiology in U.K., at the University of Surrey.

Dr. Rugmini Sankaran's major contributions include, development of a number of analytical techniques and formulation of microbiological standards for foods, technologies for convenience foods, preservation and packaging of traditional foods and the development of an ecological method for the extension of shelf-life of meat. She has also designed and conducted several training courses in Food Science and Technology. Dr. Rugmini has played a crucial role in the successful transfer of technologies for a number of convenience foods to private entrepreneurs. She has achieved significant success in popularising science, specifically food science, through a number of seminars, workshops, expositions, radio talks and TV programmes, within India and abroad.

Dr. Rugmini has published about 170 research papers and technical reports, in addition to contributing chapters to two books. A very principled, committed and hard working person that she is, Dr. Rugmini's several contributions in food science are reflected in the various awards and honours she has received, including the outstanding woman professional award by Federation of Indian Chambers of Commerce and Industry (FICCI-FLO). She led an Indian delegation to Commonwealth Defence Scientists Organisation (CDSO) conference held in Kuala Lumpur, Malaysia in 1988.

Dr. Rugmini has also been an examiner for M.Sc. and Ph.D. candidates at the Universities of Mysore, Nagpur, Bombay, Mangalore and the University of Agricultural Sciences, Bangalore. She has been a member of various Committees of Bureau of Indian Standards (BIS) and Central Committee for Food Standards (CCFS) (Directorate General of Health Services). She has also been a member of Task Force on Agro-food Processing, Technology Information and Forecasting Assessment Council (TIFAC), Chairperson, Vegetable Panel and member, Department of Biotechnology (DBT) Task Force on Food Biotechnology.

Dr. Rugmini had a distinguished tenure as the Director of DFRL for about six and half years, before attaining superannuation on 30th September 1995. Her selection as Director of DFRL can be seen as a befitting recognition by the Defence Services of her achievements over the tenure, starting from a junior position. Like in case of AFST(I), Dr. Rugmini is the first lady to hold the directorship of not only DFRL but also in the entire Defence Research and Development Organisation. She is also an able administrator. She is not new to the AFST(I). She has been its member since 1969, and had the privilege of serving as its Vice-President (Hq), during 1982-83. She was President, Association of Microbiologists (India) during 1993-94. Under her able stewardship, the AMI conducted an International Conference 'Micon-94', at the DFRL in Mysore, which was attended by over 850 delegates from India and abroad. The "Proceedings" of this conference has been published in September 1995 as a book entitled 'Microbes for Better Living'.

The achievements of Dr. Rugmini can be an excellent guiding factor to many. She comes from a large family of agriculturists and is one of the brilliant members among four brothers and four sisters, all of whom hold high ranking positions in the society. Dr. Rugmini is blessed with two equally brilliant and talented daughters. Chandrakanta, the elder one, is an electronics and communication engineer, who married a medical professional and teaches in a polytechnic in Kerala. Chandrakanta was recently in Canada under Indo-Canadian Exchange Programme. Sarita, the younger one, has high academic background, being the Gold Medallist in M.A. (Economics). After a short teaching job in Mysore University, Sarita was trained in journalism at the Bennett and Coleman School, appointed as Sub-editor-cum-Reporter of Economics Times in Bangalore and then given an independent charge of Reporter of Economic Times in Mysore.

Dr. Rugmini has many hobbies, which include reading, gardening, cooking and social service for upliftment of the under-previledged women. She was the University champion in hard-court tennis, had participated in dramas and also appeared on Doordarshan as an Outstanding Woman Professional.

On behalf of AFST(I) and readers of this journal and also on my own behalf, it is my privilege to welcome and felicitate Dr. Rugmini Sankaran and also the new Central Executive Committee members (as specified in this issue of the journal). I wish all success to this dedicated team in fostering further allround growth of AFST(I) and its activities.

P. NARASIMHAM
Officiating Editor
Journal of Food Science and Technology

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 441-452

Non-thermal Methods for Preservation of Fruits and Vegetables : A Critical Appraisal

D.S. KHURDIYA

Division of Fruits and Horticultural Technology,
Indian Agricultural Research Institute,
New Delhi - 110 012, India.

Fruits and vegetables are highly perishable. Hence, these must be saved from spoilage and deteriorative changes soon after the harvest. The avoidable losses can be reduced by post-harvest management. Proper storage of fresh produce and preservation can add to the value of the processed products and also made these available throughout the year. This review critically analyses the non-thermal methods for preservation of fruits and vegetables, with special reference to their advantages and limitations.

Keywords : Fruit and vegetable preservation, Non-thermal methods, Storage of fresh produce, Advantages and limitations.

Fruits and vegetables are highly perishable and get easily spoiled. The principal causes of spoilage in fruits and vegetables are the growth of spoilage microorganisms, the action of naturally occurring enzymes in these horticultural produces, chemical reactions, structural changes, and conditions of storage of the fruits and vegetables (Desrosier 1970). Moisture content, temperature, oxygen concentration, nutrients available, extent of contamination with spoilage organisms, and the presence of growth inhibitors are some of the factors, which control the type and extent of microbial food spoilage. Usually, the control of one or more of these factors prevents microbial spoilage. Fruit and vegetable preservation involves prevention of the above mentioned undesirable changes in fruits and vegetables, and their products. It encourages intensive fruit and vegetable production practices, while reducing the losses due to spoilage, and decay of produce (Desrosier 1970). Together, these increase frui and vegetable availability, and eventually lower their unit costs. Processing can change fruits and vegetables into new or more usable forms, and make them more convenient to prepare.

There are two basic principles in preventing microbial spoilage of fruits and vegetables. The first principle is to destroy the microorganisms in the fruits and vegetables, and prevent recontamination by microorganisms from outside (Cruess 1958; Lopez 1969). This is the basis of the canning technique, aseptic processing and packaging, while heat still is the most commonly used treatment to destroy microorganisms. The second principle is to alter the environment so as to prevent or retard the growth of undesirable organisms (Desrosier 1970; Girdharilal et al. 1986). The modern methods for preservation of fruits and vegetables, broadly classified by Girdharilal et al (1986), are presented in Table 1. The thermal processing, canning and aseptic processing are however, out of the scope

TABLE 1. CLASSIFICATION OF MODERN METHODS OF FRUIT AND VEGETABLE PRESERVATION

Physical Methods		
a)	Thermal processing	Pasteurization, Sterilization, Aseptic processing and packaging
b)	Storage at low temperatures	Refrigeration, Freezing, Dehydro-freezing
c)	Removal of water (evaporation or dehydration)	Sun-drying, Dehydration, Freeze-drying, Concentration
d)	Irradiation	Application of U.V. or ionizing radiation
e)	Other means	Carbonation, High pressure, etc
Chemical Methods		
a)	Addition of acid	Pickled vegetables
b)	Salting or brining	Vegetable/fruit pickles, lactic acid fermentation
c)	Addition of sugar and heatin	Fruit preserves, jams, jellies, marmalades, candies, etc.
d)	Addition of chemical preservatives	Sodium benzoate (benzoic acid), Potassium metabisulphite (Sulphur dioxide)
Fermentation		Alcoholic and acetous fermentation-fruit wines, apple cider, vinegar, etc.
Other Methods		A judicious combination of one or more than one methods mentioned above for synergistic preservation

Source : Girdharilal et al (1986)

* *Corresponding Author*

of this paper. In this review, an attempt has been made to consolidate all the available literature and to analyze critically the advantages and limitations of non-thermal methods for preservation of fruits and vegetables.

Refrigerated storage of fresh fruits and vegetables

Fruits and vegetables remain living entities after harvest, and their metabolism is a function of the temperature of the environment (Smock and Neubert 1950). Thus, low temperatures, near the freezing point of water, are effective in reducing the rate of respiration, and thus constitute a short-term preservation method of fruits and vegetables. Various pre-treatments, including washing, use of wax emulsions, pre-packaging, fungicides, and controlled as well as modified atmosphere coupled with refrigeration further improve the storage, and post -storage life of fruits and vegetables (Rao et al. 1982). The optimum low temperatures for storage of fresh fruits and vegetables have been specified by Wright et al (1954), Ryall and Lipton (1972), and Girdharilal et al (1986).

Fruits, and vegetables, which are to be stored, should be free of diseases as well as injury, and must be of proper maturity for best storage life (Desrosier 1970). The fresh produce, after harvest, is pre-cooled, either by application of cooled air blast, or by application of vacuum or more commonly by hydro-cooling, to a specified temperature, prior to storage at an appropriate refrigerated temperature. Fruits are susceptible to increased peel injury, decay as well as loss of quality, and vitality after hydro-cooling. For example, apples are pre-cooled in the storage rooms (Woodroof 1975a). The refrigerated storage can increase the shelf life of fruits and vegetables from a few days at ambient temperature to about 35 weeks at low temperature. Each commodity has a specified optimum refrigerated temperature, ranging from 0 to 20°C (Girdharilal et al.1986). The ready-to-eat fruit slices can be preserved for short time by refrigeration. The apple, peach slices, pineapple chunks, and citrus sections can be preserved at low temperatures, and the shelf life can be further improved by adding acidulants, antioxidants, microbial inhibitors, and sweeteners. The pH of fruit is reduced to 3.5 by the addition of ascorbic acid (0.05%), while sulphur dioxide prevents browning (Woodroof and Luh 1975).

Controlled atmosphere storage (CA)

It involves a system for holding fresh fruits, and vegetables in an atmosphere that differs substantially from normal air in respect of the proportion of nitrogen, oxygen and carbon dioxide (Lipton, 1975). The composition of the atmosphere may be altered by restricted venting of the storage room or the container, scrubbing the atmosphere of carbon dioxide, oxygen or by adding individual gases to the container, while reducing the proportion of others (Ryall and Lipton 1972). Internal atmospheres of fruits and vegetables also play a vital role in their storage under modified or controlled atmosphere (Williams and Patterson 1962; Peleg 1985). If the fruits are removed rapidly from the orchards to the storage room, cooled quickly, sealed, the oxygen level is reduced to an acceptable level in 2-3 days, and built up of carbon dioxide is limited, these can be processed as desired (Dewey 1965; Jewsen 1966). For fruits and vegetables, no one mixture of gases is suitable under all circumstances. For instance, cauliflower is injured during one week's storage at 5°C in 10% carbon dioxide, whereas broccoli remains in an excellent condition in that atmosphere. Many vegetables do well under a CA storage of 2 to 3% oxygen and 5% carbon dioxide (Ryall and Lipton 1972). Dewey (1965) recommended 0°C, 3% oxygen, and 2.5% carbon dioxide, as CA storage condition for "Delicious apple". CA storage does not prevent deterioration, but lengthens the storage life from a few days to as much as several months, depending upon the item involved (Ryall and Lipton 1972). Injury to fruit tissue may be caused by an abnormality in metabolism, induced by high carbon dioxide, and low oxygen concentrations. Some of these disorders are in the form of browning of the fleshy mesocarp, tissue breakdown, and the accumulation of certain organic acids (Smock, 1966; Stewart and Uota, 1971). Refrigerated storage, a short-term preservation of fresh fruits and vegetables, depends upon uninterrupted power supply, and is mostly situated near the consuming centres, rather than the growing areas. This is because of the prevalent system of marketing.

Low temperature storage, and transportation of tropical fruits under refrigerated conditions have not gained commercial popularity because of chilling injury or low temperature breakdown. Such fruits are characterized by poor colour, flavour, and taste due to improper ripening after transfer to high temperature (Rao et al. 1982). Krishnamurthy and Joshi (1989) observed poor development of carotenoid in mangoes, which did not show normal ripening after storage at low temperature (7°C). Some studies have also been carried out on storage of

fresh fruits and vegetables, based on principles of evaporative cooling, but these are at initial stage of research and development (Anon 1984; Maini et al. 1984; Roy and Khurdiya 1986; Habibunnisa, and Narasimham 1988; Kumar and Nath 1993). Therefore, it is imperative to accelerate this technology, to make it commercially viable.

Preservation by freezing

The basic principle of all rapid freezing methods is the speedy removal of heat from fruits and vegetables (Cruess, 1958; Desrosier 1970). These methods include freezing in cold air blast, direct immersion of produce in a cooling medium, contact with refrigerated plates in a freezing chamber, and by freezing with liquid air, nitrogen or carbon dioxide. Freezing in still air is the poorest method of all. By circulating cold air, the freezing rate is greatly accelerated. Quick freezing is a process where temperature of the food passes through the zone of maximum ice crystal formation (0 to -3.0°C) in 30 min or less (Desrosier 1970), which can be achieved in the following ways.

Freezing in air : There are two types of air systems for fruit and vegetable freezing (still air, and forced air). Still air freezing is accomplished by placing packaged or loose produce in suitable freezing rooms. Still air freezing is the cheapest and the slowest method, by which, products remain in the freezing chamber until frozen. The length of time required to freeze the food is dependent upon the temperature of the freezing chamber, the type of food being frozen, its temperature as it enters the freezer, the type, size and shape of the produce package, and the arrangement of the packages in the freezer (Bachtel and Kulp, 1960). The freezing time for a given package of food can be drastically reduced by installing fans in the freezing chamber. Very cold air blown at high speeds results in more rapid freezing. For quick freezing, cold air blast in an insulated tunnel is functional (Desrosier 1970).

Freezing by indirect contact with refrigerants : Fruits and vegetables may be frozen by placing these in contact with a metal surface, which is cooled by a refrigerant (Bechtel and Kulp, 1960). The produce may be packed in a can, and immersed in a refrigerant or packed in a paper board box, placed in contact with a refrigerated metal plate. The refrigerated metal plates may be moving in the form of a belt or may remain stationary. The refrigerated brine may be still or in turbulent motion. A 5 cm thick consumer package in a commercially contact freezer can be frozen in a hour and a half. The system can be batch type or continuous (Desrosier 1970). This method has the limitation of freezing regular size square or rectangular packs only.

Direct immersion freezing : It is a very rapid method of freezing. In this method, the prepared fruits and vegetables are directly immersed in a liquid refrigerant such as sugar solution, and sodium chloride solution, respectively. Liquids are good heat conductors, as compared to air or gases. The produce can be frozen quickly, and the contact is intimate between the food, and refrigerant. High heat exchange rates can be obtained, using turbulent flow techniques. Fruit and vegetable pieces or packages can be frozen in liquid bath, sprays, and fog system. Individual fruits and vegetables can be frozen in a matter of minutes, using suitable solution, and brine, and at suitable temperatures (Desrosier 1970).

Cryogenic freezing : Cryogenic media, viz., liquid nitrogen, liquid carbon dioxide, and halogenated hydrocarbon can also be used for direct immersion freezing. Solid carbon dioxide can be used by mixing with vegetable pieces, and during transportation. The thermal shock due to vigorous boiling occurs, when fruit is directly dipped into the liquid nitrogen (Holdsworth 1983). A cyclic dip of full mango slices in liquid nitrogen (-196°C), for 8-23 min till complete frozen, was developed by Vilaschandran et al (1985). The quality of frozen slices was as good as the fresh slices. Generally, freezing is the better method to preserve the taste, texture, and nutritional value of fruits and vegetables as compared to other preservation methods. Consequently, ever-increasing quantities of food are being frozen throughout the world. In order to obtain high quality frozen foods, high quality raw materials are necessary, and processing, distribution, and storage must be carefully controlled. Quality can not be gained, but it certainly can be lost (George 1993).

Freezing stops microbial activity. Enzyme activity is only retarded at freezing temperatures. Enzymes control is the easiest by destroying them by blanching prior to freezing, and storage (Mundt et al. 1960; Splittstoesser et al. 1961). To obtain the best performance of frozen fruits and vegetables, storage temperatures should be maintained constant, and not higher than -17.8°C. Repeated freezing, and thawing are detrimental to the quality of frozen products. Fruits and vegetables are better preserved at -23.3°C and best at -34.4°C. The produce is significantly altered, if stored at higher temperatures

(Farley 1958). Different fruits have different storage lives at -18°C (Girdharilal et al. 1986). Constant temperatures help to minimize recrystallization, a major cause of quality loss during the storage of frozen products. A major cause of product degradation is the amount of unfrozen water present in the frozen matrix (George 1993). The unfrozen water is known to be reactive, particularly during storage, rendering the product susceptible to deteriorative, and enzymatic reactions, thereby limiting its frozen shelf life (Reid 1983). The more by the stable state, the better is the retention of quality in the frozen food (Desrosier 1970).

Dehydration (sublimation) is a critical factor to be considered, when freezing unpackaged foods by either still or blast air method. Packaging foods, prior to freezing, has obvious advantages in controlling this dehydration, which irreversibly alters the colour, texture, flavour, and nutritive value of frozen fruits and vegetables. However, it has the disadvantage of insulating the produce. Packaging protects the frozen fruits and vegetables from oxidation and destruction of many nutrients, including vitamins. However, in vegetables such as peas, lima beans, corns, diced carrots, the freezing precedes packaging (Desrosier 1970). George (1993) has critically reviewed the freezing process, which also includes "Superchill System" and the cryo-mechanical systems used in food industries. The latter process offers the advantages of both cryogenic, and mechanical systems, i.e., the flexibility, and the lower unit-cost factors, respectively. The ultra-rapid freezing is only possible with the use of cryogenic freezers (Holdsworth 1983). However, the economic considerations have limited the use of cryogenic techniques as the sole mechanism of freezing. Mechanical techniques (air blast or contact freezing) are, therefore, often widely used (George 1993).

Preservation by drying and dehydration

The main objective of drying is removal of free water (lowering of water activity below 0.7) from fruits and vegetables to the extent, where microorganisms do not survive, and reproduce (Desrosier 1970; Somogyi and Luh 1975). Simultaneously, the total solids, viz., sugars, organic acids, etc., are concentrated, exerting osmotic pressure to inhibit the microorganisms. Thus, reduction in water content, and packaging, controls the biological, and chemical forces, respectively, which act upon fruits and vegetables, facilitating preservation of these perishables. (Desrosier 1970). Fruits and vegetables after selection are given anyone or many pre-drying treatments, viz., sorting and grading, washing, peeling, cutting into halves, wedges, slices, cubes, nuggets, etc., making pulp, juices or concentrates, blanching, sulphuring or sulphitation (Somogyi and Luh 1975).

Blanching in boiling water or under steam will stop all life processes, and destroy microorganisms. It will also inactivate enzymes that would cause discolouration, and changes in flavour, and aroma; fix green colour, and remove certain harsh flavour common in vegetables (Girdharilal et al. 1986). Blanching times vary. In general, 1 to 3 min are adequate for leafy vegetables; 2 to 8 min for peas, beans, and corns; and 3 to 6 min for potatoes, carrots, and similar vegetables (Desrosier 1970). Sulphur dioxide is used for its antioxidant and preservative characters. In addition to preventing enzymatic browning, sulphur dioxide treatment reduces destruction of carotene, and ascorbic acid (Somogyi and Luh 1975). Loading per square feet of tray may range less than a half kg to 1.5 kg, and dried either in the sun or in a dehydrator (Cruess 1958). Fruits are dried to 15 to 25%, and most vegetables to 4%, while most of their powders to 2 to 3% moisture content (Desrosier 1970).

Sun-drying : In sun-drying, the sound semi-ripe fruits, after pre-treatment, are loaded on trays, transferred to the sun-drying yard, and allowed to dry, until the fruits are about two thirds dry. Then the trays are stacked in the shade to allow the later stages of drying, which proceeds slowly. Apricots, nectarines, peaches, pears, and grapes are some fruits that are sun-dried (Salunkhe et al. 1976). Among vegetables, cucumbers, potatoes, *kair*, *sangri*, etc., are also sun-dried in rural areas. Information on details of sun-drying of various fruits is available (Mrak and Phaff 1949; Cruess 1958). Lower capital investment is required to apply this simple method. Since sun-drying depends on uncontrollable factors, production of uniform, and high quality product is not expected. Some over-drying, contamination by dust as well as dirt, and insect infestation of the finished product are usually tolerated. The most obvious disadvantage of this technique is its complete dependence upon the sun light. It is a slow process, unsuitable for producing high quality products. Generally, it will not allow the fruit products to dry below 15-20% moisture level. The resulting product will have a limited shelf life (Somogyi and Luh 1975).

Incorporating some improvement in the sun-drying technique, a hot box process, which improved

the product quality by reducing contamination by dust, insect infestation, and animal or human interference was developed in Syria (Szulmayer 1971a,b). It reduced the drying time to half the usual time, and the finished product had more appealing appearance and better flavour than that dried in the conventional way. Szulmayer (1971a,b) has also described an indirect solar drying process, in which the product is exposed to heated air rather than to the direct sun. Use of hot air reduces relative humidity of the air, thereby leading to drying at a reasonable rate. Solar drier with *chimni* effect has also been claimed to improve the quality of the finished products (Pawar et al. 1988; Khurdiya and Roy 1986).

Dehydration : It involves application of artificial heat to vapourize water, and the system of removing water vapour after its separation from fruit and vegetable tissues (Somogyi and Luh 1975). Dehydration of fruits and vegetables can be performed in various ways (Table 2), as described by Cruess (1958), Desrosier (1970), Anon (1971), Van Arsdal et al (1973), and Salunkhe et al (1976). Post-drying treatments include sweating, screening, inspection, 'flaking' process, and packaging (Roberts and Faulkner 1965). In sweating process, the dehydrated fruits are held in bins or boxes for equalization of moisture (Somogyi and Luh 1975).

Merits and demerits of sun-drying, and dehydration of fruits and vegetables have been described in detail by Cruess (1958) and Desrosier (1970). Dried and dehydrated fruits and vegetables are more concentrated than those obtained by any other preserved form. They are less costly to produce, involve a minimum of labour requirement, processing equipment is limited, dried product storage requirements are at a minimum, and distribution costs are reduced (Woodroof and Luh 1975; Von Loesecke, 1955).

TABLE 2. DEHYDRATION PROCEDURE

Cross flow drying	
i) Cabinet drying	Fruits and vegetables
ii) Tunnel drying	Fruits and vegetables
iii) Pneumatic drying	Cooked potatoes
iv) Continuous conveyer drying	Fruits and vegetables
Through flow drying	
i) Kiln drying	Apples
ii) Tower drying	Fruits and vegetables
iii) Belt trough drying	Vegetables
iv) Bin drying	Vegetables
v) Fluidized bed drying	Vegetables
Drum drying	Fruit and vegetable *purees*, paste, sludge
Spray drying	Fruit and vegetable juices
Puff drying	
i) Explosive puff drying	Fruits and vegetables
ii) Vacuum puff drying	Fruits and vegetable juices
Freeze drying	Fruits and vegetables
Accelerated freeze drying	Fruits and vegetables
Foam mat drying	Fruit juices
Microwave drying	Fruits and vegetables
Dehydro freezing	Fruits and vegetables
Osmotic drying	Fruits and vegetables

Sources : Cruess (1958); Desrosier (1970); Anon (1971); Van Arsdel et al (1973); Salunkhe et al (1976).

Preservation by ionizing radiation

The utilization of ionizing radiation for stabilization of foods offers a method of cold sterilization, wherein foods are preserved without marked change in their natural character (Desrosier 1970). It has about six distinct areas of application for radiation processing of foods, the important being the preservation without refrigeration, prolonging storage life of fresh fruits and vegetables, and disinfestation of packaged foods. Food irradiation consists of exposing the food to ionizing radiation, emanating from radioactive isotopes ^{60}Co and ^{137}Cs or from electrical machines, generating electrons or X-rays. This exposure results in preserving effects due to the applied irradiation dose (Grunewald 1984). The limitations of the applicability of this technique are on grounds of microbial safety, wholesomeness, physical properties, and economy.

According to Grunewald (1984), a high dose of above 10 kGy is employed in commercial sterilization, which lends to a particular food with shelf stability. A medium dose is considered to be between 1 and 10 kGy, and is generally used for the reduction of microbial load, and elimination of pathogenic microorganisms or for the extended shelf life. Of course, these two effects are obtained by one irradiation of the product. A low dose is considered to be below 1 kGy. Insect disinfestation, delayed senescence and ripening, and sprout inhibition are the applications that fall into the low dose category. In this dose range, irradiation requires less energy than other treatments. Irradiation at 2.5 kGy reduced the losses to 1%, as compared with 15% for non-irradiated strawberries, when kept at 5°C for 12 days (Kiss

1982). Mango, dipped in water at 55°C for 5 min, and irradiated with the dose of 7.5 kGy, had an extended shelf life of more than 4 weeks at 10°C. This treatment also controlled the mango weevil (*Sternochetus magniferae*), but the flavour got reduced (Grunewald 1984).

Bacterial spores are more resistant to the germicidal action of ionizing radiation than are vegetative cells. Enzymes are more resistant to the effects of ionizing radiation in natural substrates than in pure solutions. The radiation sterilization can bring about a rapid deterioration of food substances, unless enzymes are controlled. Constant oxygen tension, temperature above freezing point, and upto about 20°C have little effect on irradiation (Desrosier 1970). Fruits and vegetables, after heat inactivation of enzymes, and proper packaging had storage life of 2 to 5 years at 0°C (Desrosier and Rosenstock 1960). Fruits and vegetables must be cleaned, well cured, and free from diseases and bruises, before being considered for irradiation (Grunewald 1984). Fruits, which are slightly less than fully ripened need be processed, as the radiation treatment will only temporarily thwart normal ripening processes (Burns and Desrosier 1956).

Weight loss due to dehydration can not be prevented by irradiation, but only by packaging, which also prevents contamination by air-borne microorganisms or accidental contamination (Tripp and Crowley, 1957). Cans, glass containers, and polyethylene pouches, which are resistant to irradiation upto 10 kGy, can be used for food packaging (Grunewald 1984). Maxie et al (1971) have reported infeasibility of irradiating fresh fruits and vegetables because of many undesirable side effects caused by high dose of irradiation required for certain purposes. However, the first commercial food irradiation facility was established in Florida (USA) in 1992. It is being successfully used for treating strawberries, grape fruits, tomatoes, onions, and oranges for super markets in Florida, and Illinois (Ahmed and Loaharanu 1994).

Consumer acceptance of irradiated foods will probably depend on thoughtful consideration of at least three important areas : safety for consumption, sensory qualities and value including costs. Ionizing radiation is a need-based technology, and it can not be generalized to all types of foods with one dose. It requires high capital cost. Its successful application depends upon the proper packaging and handling (Tripp and Crowley, 1957). There is no detection method to distinguish irradiated food from the unirradiated one. It is, therefore, imperative to have proper regulation in its application, especially under the Government control, and inspection. The great advantage of this technology is in preventing the food-borne pathogens, provided recontamination is checked by proper packaging, and control. It is also very useful as a supplement, and complement with other methods of preservation (Personal communication with Dr. Paul Thomas, BARC, Bombay).

Preservation with carbon dioxide

Carbon dioxide has been found to have preservative properties at higher pressure than normally encountered in the atmosphere (Desrosier 1970). Carbon dioxide is currently being used in controlling the maturation, and storage quality of fresh fruits (Desrosier 1970, Ryall and Lipton 1972), as discussed earlier. Aerobic moulds, and yeasts require oxygen for their growth, and become inactive in the presence of high levels of carbon dioxide. The preservative action increases in line with the number of volumes of carbonation used (Cruess 1958). Carbonation is the process of dissolving carbon dioxide in a beverage so that it gives off the gas in fine bubbles, when served, and has the pungent taste characteristic to the carbonated beverage.

Juices can be carbonated directly or they can be stored as such or in the form of concentrates for carbonation, whenever necessary. In Europe, apple juice is preserved in bulk in glasslined tanks by first germproof filtration, and then impregnation with carbon dioxide at about 100 to 110 psi pressure (Cruess 1958). Carbonated beverages keep well for about a week without the addition of any preservative. It is, however, necessary to add 0.005% sodium benzoate to the finished product, if the beverages are to be kept for longer periods (Girdharilal et al. 1986).

In using the syrup, about 45 ml of syrup is added to each 200 ml soda water bottle. Carbonated water at 30 to 40 psi pressure is added to fill the bottle. The bottles are sealed with crown caps at once, placed in a pasteurizer, and heated to 66°C for 30 min (Cruess 1958). At present, synthetic carbonated beverages that have been marketed in India have practically very little or no nutritive value (Girdharilal et al. 1986). If fruit juices are carbonated, the nutritive value of these beverages could be increased considerably (Khurdiya 1990). This method has the limitation of using only the clear fruit juices (Unpublished data).

Preservation by pressure

It has been reported by Cruess (1958) that grape juice in active fermentation could be sterilized by subjecting it to a pressure of 75,000 psi for 30 min, and by a pressure of 30,000 psi applied for a somewhat longer time. Apple juice was sterilized by subjecting it to 60,000 to 80,000 psi in 30 min, and actively fermenting sugar solution was sterilized by 60,000 psi in 30 min. The fruit juices preserved by this method were comparable to the fresh fruit in flavour and general quality.

In these experiments, a small collapsible tin tube was filled with fruit juice or other liquid, and the tube was sealed. The tube was then placed in a lead cylinder, which in turn, was placed in a heavy-walled steel cylinder into which water or oil was forced by hydraulic pressure. The method of preservation by pressure is generally being used at laboratory level only for the fruit juices, and has not been utilized commercially so far. It may have an added advantage that it will avoid the need of high temperature, and chemicals, but its effects on nutritional aspect have to be studied in detail.

Preservation by pickling

The preservation of fruits, and vegetables in common salt or in vinegar is called pickling. Spices and edible oil may be added to the product. Raw mango, lime, turnip, cabbage, cauliflower, *kair*, etc., are preserved in the form of pickles, which have become popular in several countries. Pickling is done (i) by curing or fermentation with dry salting or fermentation in brine, or salting without fermentation; and (ii) by finishing, and packing (Girdharilal et al. 1986). In dry salting, for every 100 kg prepared vegetables, 3 kg salt is used in alternate layers in the keg or barrel. After 3/4th filling, the mass is covered with wooden board under some weight. Brine is formed in about 24 h. Fermentation is usually completed in 8 to 10 days at 27-32°C, but it may take 2-4 weeks in cold weather. Vegetables such as cucumber, which do not contain sufficient juice to form brine with dry salt, are fermented in brine (Girdharilal et al. 1986).

Lactic acid fermentation : The addition of salt permits the naturally present lactic acid bacteria to grow, thereby rapidly producing sufficient acid to supplement the action of salt (Desrosier 1970). In this process, the fermentable carbohydrate reserve is converted into acid, whose level in cucumber ranges from 0.8 to 1.5%, expressed as lactic acid. In the commercial production of fermented salted cucumbers, the salt concentration is maintained at 8 to 10% during the first week, and thereafter until 16% salt concentration is obtained in solution. The fermentation is completed within 4 to 6 weeks, as evidenced by the change in the tissue characteristics. The salt stock is freshened twice in warm water (43-45°C) for 10 to 14 h. The freshened salt stock is packed in consumer units with weak vinegar (2.5% acidity) to prepare sour pickle. Packing is done in sweet, spiced vinegar for sweet pickle and in dill herb for dill pickle. Spices are added to the acidified brine (Desrosier 1970).

Sauerkraut is another lactic acid fermented product made from cabbage (Cruess 1958; Desrosier 1970; Girdharilal et al. 1986; Pederson and Albury 1954). The shredded cabbage is mixed with salt and on an average 1.5 to 2.0% lactic acid is produced, as fermentation may be complete in a little over a week. About one-half as much acid is produced, as there is sugar present in the cabbage. In India, oil pickles are highly popular. Cauliflower, lime, mango, turnip, bamboo, jackfruit, *kair*, *karonda*, etc., are used to make oil pickles. Raw material (whole or cut into desired pieces) is washed, then mixed with spices such as chilli powder, turmeric powder, cumin, cardamom, cinnamon, clove, black pepper, fenugreek, nigella, fennel, ginger, onion, mustard seed, methi, etc. Mustard or gingelly oil is generally used (Khurdiya and Verma 1969 a,b; Haware and Rao 1979; Girdharilal et al. 1986).

Pickles are good appetizers. They add to the palatability of a meal, and aid in digestion by stimulating the flow of gastric juice. Pickles with lactic fermentation, when consumed, impart intestinal tone and reduce putrefactive agents/ processes in the colon. These foods have a laxative effect on many human subjects. Very little scientific data are, however, available regarding their nutritive value. Different kinds of pickles contain varying amounts of nutrients, depending upon the raw materials used, and the method of preparation followed (Girdharilal et al. 1986). The stabilized pickles contain other nutrients in adequate amounts, when compared with original perishable tissue (Desrosier 1970). In some instances, nutrient levels are increased due to the presence of yeasts. Freshened salt stock of cucumbers retained 100% calcium and iron (Fellers 1960).

The main problem in pickles is the spoilage by either yeasts or moulds, since both can grow in the presence of acid. Thus, an anaerobic environment can control these microorganisms, which can be provided by putting extra layer of

oil over the fruits and vegetables in oil pickle, and brine or vinegar layer in pickles packed with brine or vinegar. As acidity is not favourable for the growth of putrefactive bacteria, fruits, and vegetables are not generally spoiled by them. The principles of making the conditions most unfavourable for the growth and multiplication of microorganisms have been relied upon in pickling. Thus, the lactic acid fermentation is dependent on production of pickled fruits and vegetables, to suppress undesirable microbial activity, and create a favourable environment for the desired fermentation (Girdharilal et al. 1986). Hence, under properly controlled conditions, the salted fermented cucumber, so called salt stock, may be held for several years, as the salt protects the fruit pickles against microbial spoilage (Kanekar et al. 1989).

Preservation by sugar and acid

The fruits are preserved in the form of jams, jellies, preserves, marmalades, and candies by relying upon the high solids-high acid principle (Desrosier 1970). It is an important utilization avenue of fruits, which, though otherwise of excellent qualities, do not have an appeal to the eye. Such fruits do not enter usual fresh market channels. In addition to the pleasing taste of such preserved fruits, they possess substantial nutritive value. Jams, jellies, preserves, marmalades, and fruit butters are products from fruits with added sugar, after concentrating by evaporation to a point, where microbial spoilage can not occur. Mould growth on the surface of fruit preserves is controlled by exclusion of oxygen, i.e., covering with paraffin. Modern practice replaces the paraffin with vacuum-sealed containers, which bring the moisture losses, mould growth, and oxidation under control (Desrosier 1970).

Jellies and jams are semi-solid masses made from not less than 45 parts by weight of fruit juice/ pulp ingredient to 55 parts by weight of sugar. The substrate is concentrated to not less than 65 or 68% soluble solids in jellies and jams, respectively (Desrosier 1970; Girdharilal et al. 1986). If the small containers are cleaned prior to use, the heat of the boiling jelly is usually sufficient to eliminate spoilage microorganisms (Cruess 1958). Narrow limits of operation for successful jelly manufacture, as described by Desrosier (1970), include optimum concentration of pectin 1.0%, sugar 67.5% and pH value of 3.2. Marmalade, a jelly-like product, is made from citrus juice, and peel, along with sugar. It is concentrated to achieve the gel structure.

A jam has similar definition as a jelly, with the exception that it includes fruit ingredient, rather than the fruit juice or extract. Fruit preserves (*murabba*), candied, and glazed fruits are prepared similarly. The whole or pricked pieces are blanched, and slowly impregnated with syrups of progressively increasing sugar concentrations, until the sugar concentration in the tissue is high enough to prevent the growth of spoilage microorganisms. Fruit is removed from syrup, washed, dried, packaged and marketed as candied fruit. Such candied fruits are dipped into syrup and again dried, to obtain glazed fruit. The details of methods of their preparation are described by Cruess (1958), Desrosier (1970), and Girdharilal et al (1986).

Preservation by chemical additives

There are several chemical additives, which are defined as non-nutritive substances that are intentionally added in small quantities to food, to improve its appearance, flavour, texture or storage properties. The additives can contribute substantially in the preservation of fruits and vegetables. The preservatives are also used to supplement the effectiveness of traditional methods of food preservation. Desrosier (1970) has described the legitimate and undesirable uses of chemical additives. These are summarized below :

(a) The chemical preservatives are advantageous in maintaining the nutritional quality, enhancing the keeping quality, making fruits and vegetables attractive, and helping in their processing. (b) The use of a chemical preservative is not in the best interest of the consumer. It disguises the faulty processing or handling techniques, causes a substantial reduction of the nutritive value, and deceives the consumer. Desired preservation effect with chemicals can be obtained by good economically feasible, manufacturing practices. (c) Certain fruit and vegetable products, viz., squashes, cordials, syrups, ketchup, sauces, need chemical preservatives, for a fairly long period after opening the seal of the bottle. Pasteurization may cause cooked flavour in these products and (d) The preservative used should not be injurious to health. It should be non-irritant, and easy to detect as well as estimate.

The preservative is any substance, which is capable of inhibiting, retarding or arresting the process of fermentation, acidification or other decomposition of food. It does not include common salt, sugar, acetic acid or vinegar, alcohol, spices, essential oil, etc. Since the preservation power of

chemical preservatives is governed by acidity of the product, these are only recommended for preserving acid products, whose pH is 4.5 or below (Woodroof 1975a). They should not be added in powder or crystal form. The best method is to dissolve the preservative in a small quantity of water, and then mix with the product. Safety in using an additive is an all important consideration. The amount of an authorized additive used in a fruit or vegetable should be minimum, necessary to produce the desired effect (Girdharilal et al. 1986). Two chemical preservatives, permitted for use in fruit and vegetable products by Fruit Products Order (1955), are briefly described below :

Sodium benzoate : It is a salt of benzoic acid, which is practically tasteless, and odourless. Benzoic acid, the effective agent, is sparingly soluble in water (Girdharilal et al. 1986). Sodium benzoate is 170 times more soluble than benzoic acid and hence preferred. Sodium benzoate is added promptly, as the juice or pulp comes out from the press. Even a few hours delay may permit fermentation to begin. An amount of 0.1% sodium benzoate is usually sufficient to preserve a product, which has been properly prepared, and adjusted to pH 4.5 or below (Smith et al. 1962; Woodroof 1975b). It is commonly used in preserving apple cider, fountain syrup, pickles, relishes, other acid foods and especially in the products, which contain water soluble plant pigments such as anthocyanin (Desrosier 1970; Girdharilal et al. 1986). Benzoic acid is more effective against yeasts than moulds. However, a benzoate-resistant yeast was isolated from spoiled mango pulp (Ethiraj and Suresh 1988). It does not stop lactic and vinegar fermentation. A combination treatment with mild heating or carbon dioxide is complementary (Desrosier 1970). Benzoates in fruit juice at a concentration of 0.1% may be noticeable, and can impart a disagreeable peppery or burning taste.

Sulphur dioxide : It is available in gas, liquid, and solid state. Generally, potassium metabisulphite, a dry chemical, is used. It is, however, decomposed by weak acids like citric, tartaric, malic and carbolic acids, to form potassium salt, and sulphur dioxide, which is liberated from potassium sulphurous acid with water, when added to the fruit juice or squash. Free sulphurous acid is more effective (120 times) than combined sulphurous acid. The undissociated sulphurous acid molecule prevents the multiplication of yeasts, while the sulphurous acid ion inhibits the growth of bacteria. Pure sucrose does not combine with sulphurous acid molecule. Glucose, aldehydes, ketones, pectin and breakdown products of pectin, etc., which are found in fruit juices, have the properties of combining with sulphur dioxide with the result that the effectiveness of sulphur dioxide is reduced (Girdharilal et al. 1986).

Being more effective against moulds than yeasts, sulphur dioxide has found wide use in the fermentation industries. Sulphur dioxide in dried fruits and vegetables not only protects certain nutrients, and controls discoloration, but also is equally effective in controlling microbial and insect activities (Ough et al. 1960). Sulphur dioxide is largely eliminated by boiling or heating, when the sulphured food is reconstituted. It can not be used in the case of some of the naturally coloured juices like *phalsa, jamun*, pomegranate, strawberry pulp, etc., on account of its bleaching action on anthocyanin. It can not also be used in products, which are to be packed in tin containers, because it not only acts on tin container causing pinholes, but also forms hydrogen sulphide, which has an unpleasant smell and also forms a black compound with the iron of the base plate of the tin container (Girdharilal et al. 1986).

Quattrucci and Masci (1992) have reported the nutritional aspects of food preservatives. Sulphur dioxide, usually classified as an antimicrobial agent, is also used in controlling enzymatic and non-enzymatic browning, preventing oxidation, and modifying protein texture. Its use has also been proposed for the destruction of aflatoxin (Doyle and Marty 1978; Yagen et al. 1989). Because of these versatile properties and cheapness, sulphur dioxide, sulphite and bisulphites are largely used by the food industry, in the production of wine, beer, dehydrated fruits and vegetables, fruit jams as well as juices (Quattrucci and Masci 1992). Fatal cases of congenital sulphite oxidase deficiency have been reported by Irreverre et al (1967). The resultant compounds, hydroxysulphonate of reaction with reducing sugars, produce gastrointestinal effects similar to those produced by the sulphites themselves and do not appear to induce further nutritional disorders (Burroughs and Sparks 1973 a,b). In dehydrated fruits and vegetables stored at room temperature, loss of thiamine after 28 days storage ranged from 26 to 38% of the total content (Quattrucci and Masci 1992). In the presence of manganese, oxygen and glycine at the pH of food systems, sulphur dioxide catalyses to cause a very rapid destruction of β-carotene (Peiser and Yang 1979). In spite of so many odds against chemical

additives, steeping preservation of fruits and vegetables with permissible chemical preservatives, has considerable scope for adoption in India (Siddappa 1980; Mudahar and Bhatia 1983; Sethi 1991).

Preservation by fermentation

Fermentation is a process of anaerobic, or partial anaerobic, oxidation of carbohydrates. Putrefaction is the anaerobic degradation of proteinaceous materials. The microorganisms used in fermentation, produce enzymes, which control chemical reactions in fermentation. The factors which control the fermentation process include pH, source of energy, availability of oxygen, temperature, and sodium chloride. An industrial type of alcoholic, and acetic fermentation has been described (Desrosier 1970) and briefly discussed below :

Alcoholic fermentation : Fresh fruit juice is inoculated by alcohol producing yeasts of which *Saccharomyces ellipsoideus* is the best example. The active starter is added to juice approximately 10% by volume. The conversion of sugar to alcohol should be completed in two weeks or less at an optimum temperature of 24-27°C. When fermentation is complete, gas evolution ceases, and the juice tastes dry or free of sugar. At this time, the barrels should be filled completely to prevent the growth of wine flowers on the exposed surface. The supernatant is siphoned out, and after aging, it can be used either as an alcoholic beverage or for vinegar manufacture.

Except for aeration during initial stage, the air is not necessary during alcoholic fermentation. The alcoholic fermentation should be conducted in containers in which the juice is not unduly exposed to air, for which the bunghole or mouth of a large bottle with cotton plug, or an air trap, is satisfactory. The container must not be sealed air tight as it may burst, due to the pressure of the gas produced. Room for frothing also must be allowed. Wine is the product of an alcoholic fermentation of juice of sound, ripe grapes. Dry wines are those containing little unfermentable sugar. Aging of wines improves the flavour and bouquet, due to oxidation and formation of esters. Aged wines may be polished by filtration to give a clear, bright appearance prior to bottling.

Acetic fermentation : To produce vinegar, the sugar is transferred first into alcohol by yeast, and then alcohol into acetic acid by vinegar bacteria. Acetic acid results from oxidation of alcohol by vinegar bacteria in the presence of oxygen. A suitable starter of *Acetobacter aceti* is added to the alcoholic juice in a vinegar generator. The best means of preventing the growth of undesirable organisms is to add strong, unpasteurized mother vinegar to the fermented juice. Vinegar bacteria grow in the liquid, and on the surface exposed to the air. The rate of conversion of alcohol to acetic acid depends on activities of the organisms, amount of alcohol present, temperature, and surface area exposed per unit volume. At a favourable temperature of 27°C, the limiting factor may be the surface area exposed. The time required for the slow barrel type process is about three months or more. In large scale generator, where surface area exposed to air is large, the time for fermentation is shortened to few hours. Acetic acid fermentation occurs most rapidly, when fermented juices contain 6 to 8% alcohol (Desrosier 1970).

In acetic acid fermentation, 100 parts of alcohol should yield 130 parts of acetic acid. Actually, because of evaporation losses and other causes, less than 120 parts of acetic acid are obtained. Hence, if one starts with 100 parts of sugar, it is possible to obtain 50 to 55 parts of acetic acid, under very favourable conditions. Therefore, to produce a vinegar of legally fixed minimum acid content of 4g/100 ml, it is necessary to use a juice, containing at least 8% of sugar (Desrosier 1970). Fermented products can be made from culled apples (Barwal 1991), mango processing waste (Ethiraj and Suresh 1992), mixed fruit juice of damaged guava and banana (Bhatt et al. 1987). Thus, it is evident that unmarketable fruits can be gainfully employed for the production of wine, and vinegar.

Conclusion

The fruits and vegetables can be preserved by treating with chemicals, and storing at refrigerated temperatures, in addition to the frozen forms. Refrigerated storage is the only dependable alternative to processing for preservation of the fresh produce. A short duration preservation by evaporative cooling has a great scope in future. Indirect solar drying, and low humidity with moderate air temperature dehydration has great prospects for better retention of original colour, and flavour of the produce. These preservation methods add value to the finished product, which is wholesome, nutritious, and available round the year. A judicious combination of these methods can reduce the cost of production and vending.

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Received 26 February 1994; revised 3 October 1994; accepted 5 October 1994

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 453-458

Effect of Treatments on Phytate Phosphorus, Iron Bioavailability, Tannins and *in vitro* Protein Digestibility of Grain Sorghum

POONAM AGRAWAL* AND UNNATI CHITNIS

Foods and Nutrition, Department of Home Science,
C.S. Azad University of Agriculture and Technology,
Kanpur-208 002, India.

Effect of different treatments on the phytate phosphorus, iron bioavailability, tannins and *in vitro* protein digestibility was assessed. It was found that soaking in water (16 h) and germination (72 h) of pre-soaked grains were effective in increasing the iron bioavailability upto 126%. A negative correlation ($r = -0.42$, $p < 0.01$) existed between phytate phosphorus content, and the iron bioavailability with respect to these two treatments. In four of the eight varieties tested, tannins were not detected. A significant positive correlation ($r = 0.99$, $p<0.01$) was observed between tannins and crude protein contents. A substantial decrease was observed in the tannin content of the treated sorghum grains, with a corresponding increase in the *in vitro* protein digestibility, excepting boric acid treatment. A significant negative correlation existed between tannin and *in vitro* protein digestibility. Steeping the grains in 0.2 N hydrochloric acid, 0.05 N sodium bicarbonate or germination increased the *in vitro* protein digestibility comparable to varieties, containing no tannins.

Keywords : Iron bioavailability, Phytate phosphorus, Sorghum, Nutritional improvement, *In vitro protein* digestibility, Germination.

Sorghum (*Sorghum bicolor* L Moench), popularly referred as *jowar* in some parts of India, is cultivated in all six continents (Wall and Ross 1970). It is a drought resistant crop and therefore, can give dependable, and stable yield in both *kharif* and *rabi* seasons. India accounts for about 16% of the world sorghum production (Salunkhe et al. 1984).

The nutritive value of sorghum grain is reported to be lower than that of maize and barley (Wall and Ross 1970). The presence of antinutritional factors, such as phytates and tannins, affects its nutritional quality (Prasad Rao and House 1972, Salunkhe et al. 1984, Chavan et al. 1980). Phytate is present in sorghum in appreciable amounts mainly in seed coat, and germ of seeds as phytic acid (Kadam and Chavan 1984). It is reported to form complexes with multivalent cations including iron, thereby decreasing their bioavailability (O'Dell 1969). Further, it is reported to interact with proteins, thereby forming insoluble complexes, many of which are unavailable to the human system under normal physiological conditions (Pawar et al. 1986). Nutritional quality of the grains is expected to improve, once the antinutritional factors are reduced or eliminated. Hence, the present investigation was undertaken to study the effect of various treatments on phytate phosphorus, tannins, iron bioavailability and *in vitro* protein digestibility of grain sorghum.

* *Corresponding Author*

Materials and Methods

Grain samples of six varieties of sorghum ('SU 52', 'SU 45', 'SPV 346', 'SPV 96', 'CSH 9' 'CSH 6') obtained from Genetics and Plant Breeding Department, Rajasthan College of Agriculture, Udaipur, were selected to study the effect of treatments on phytate phosphorus and iron bioavailability. Four of these varieties were found to have no tannins. Hence, for studies on tannins and *in vitro* protein digestibility, two more varieties '6449' (an improved cultivar from USA) and 'Kakri local' (a local variety), obtained from the same source, were also assessed.

After initial screening experiments, a) soaking the seeds in distilled water, 2:5 (w/v) ratio at room temperature for 16 h (T_1), b) germination for 72 h of pre-soaked grains (T_2), c) soaking in 0.05 N sodium bicarbonate for 48 h (T_3), d) soaking in 0.2 N hydrochloric acid for 48 h (T_4), and e) soaking in 2% boric acid for 48 h, followed by re-soaking in water for 16 h (T_5) were carried out for studies on phytate phosphorus and iron bioavailability effects. For studies on tannins and *in vitro* protein digestibility effects, the T_3, T_4 and T_5 were retained, based on preliminary screening. In addition, steeping in 0.005 N sodium hydroxide for 48 h (T_6) and in 2% boric acid for 48 h (T_7) was also included. Treatments T_1 and T_2 were included for their effects on *in vitro* protein digestibility. After a stipulated time, the grains were washed to make them free

of the chemicals, oven-dried at 105°C (AOAC 1965), and powdered using a Willey type grinding mill.

Phytate phosphorus content was estimated, using the method of Peach and Tracy (1955), ionizable iron by α-α dipyridyl method (AOAC 1965), iron bioavailability by the procedure, suggested by Narasinga Rao and Prabhavathi (1978), tannin content by vanillin hydrochloric acid method (Burns 1971), nitrogen content by the standard microKjeldahl procedure (AOAC 1965) and *in vitro* protein digestibility by the method given by Singh and Jambunathan (1981). The data were subjected to analysis of variance. Correlation coefficients between various related parameters were also calculated (Downie and Heath 1970).

Results and Discussion

The phytate phosphorus contents, expressed as mg per 100 g, ranged from 81.2 ('CSH-6') to 128.8 ('SPV-96') for untreated samples (Table 1). Earlier, Wang et al (1959) reported a range of 160-430, as against the value of 172-407 mg/100 g (dry wt) of whole grain sorghum (Doherty et al. 1982), while Gopalan et al (1991) documented a value of 172. The figures in the present study, when calculated on dry weight basis, are somewhat lower than those cited above, and can be attributed to varietal differences. Cultivar dependent variations in the phytate phosphorus levels are also evident from the literature (Doherty et al. 1982; Wang et al. 1959), and are also significant ($p < 0.01$) in this study.

The treatments decreased the phytate phosphorus contents of all sorghum cultivars (Table 1). Variety 'CSH 6' was most responsive to the treatments. Regardless of varieties, treating with hydrochloric acid (T_4) was most effective in lowering the phytate phosphorus content, possibly because phytate gets dissolved in aqueous solutions at lower pH values (Pawar and Parlikar 1990), and is thus eliminated. Germination of pre-soaked grains (T_2) also showed the effects, which are statistically comparable to those of T_4. The phytase activity is known to also increase in grains during soaking and germination (Pawar and Parlikar 1990). In view of the added nutritional advantages associated with germination, T_2 may be preferred over T_4 in which hydrochloric acid is to be used.

Soaking in water was reported to be effective in decreasing the phytate phosphorus content also in pearl millet (Pawar and Parlikar 1990). However, the decrease reported was much lower (14.8%, 15 h soaking, 28°C) than that observed in our stu dy for sorghum (about 57%, 16 h soaking, 37°C). A part of this variation may be due to differences in soaking temperatures, while some variations are also expected due to differences in the seed structure of the crops. A combined effect of dehulling and soaking in 0.2 N hydrochloric acid has been reported to be much more effective in pearl millet than soaking alone (Pawar and Parlikar 1990), while in sorghum, dehulling alone is reported to remove 40-50% of phytate phosphorus (Doherty

TABLE 1. PHYTATE PHOSPHORUS CONTENT (mg/100 g) AND PHYTATE (g/100 g) OF DIFFERENTIALLY TREATED SORGHUM VARIETIES*

Variety	Treatments						Variety** mean
	Control	T_1	T_2	T_3	T_4	T_5	
'SU 45'	112.0 **3.52**	47.6 (57.50) **0.95**	22.4 (80.00) **0.85**	50.4 (49.40) **1.42**	9.8 (91.42) **0.22**	28.0 (75.00) **0.68**	45.00[b]
'SU 52'	106.4 **3.40**	36.4 (65.79) **0.90**	16.8 (84.21) **0.70**	42.0 (60.53) **1.50**	5.6 (94.74) **0.20**	24.0 (77.44) **0.80**	38.53[b]
'SPV 96'	128.8 **3.88**	58.8 (54.35) **1.36**	42.0 (67.39) **1.00**	64.4 (49.84) **1.63**	28.0 (78.26) **0.50**	54.6 (57.61) **1.04**	62.77[a]
'SPV 346'	126.0 **3.60**	56.0 (55.56) **1.10**	35.0 (72.20) **0.80**	61.6 (51.10) **1.36**	21.0 (83.34) **0.45**	42.0 (66.67) **0.68**	56.94[a]
'CSH 6'	81.2 **2.10**	36.4 (55.17) **0.80**	15.4 (81.03) **0.37**	44.8 (44.83) **0.92**	8.4 (89.65) **0.20**	22.9 (72.41) **0.62**	34.76[b]
'CSH 9'	94.4 **2.80**	42.0 (54.54) **0.75**	22.4 (75.76) **0.45**	50.4 (45.45) **0.85**	14.0 (84.85) **0.35**	30.8 (66.67) **0.71**	42.00[b]
**Treat Mean	107.8 **3.21**	46.20[1] (76.77) **0.98**	25.67[2,3] (57.15) **0.70**	52.27[1] (50.19) **1.28**	14.44[3] (87.04) **0.32**	33.63[2] (69.30) **0.76**	

*Means of two independent estimations. Data in parentheses indicate per cent decrease as compared to the unprocessed.
** Significant at 1% level. CD for comparison of any two means (variety or treatment) is 11.78. Means with same superscript are at par. Figures in **bold** represent phytate content.

et al. 1982). In the present study, soaking in 0.2 N hydrochloric acid, without dehulling, resulted in about 87% removal of phytate phosphorus in case of sorghum. Addition of sodium bicarbonate in dough for preparation of wheat bread caused only minor removal of phytate, which was attributed to low phytate hydrolysis (Khan et al. 1986). Similar results are observed in this study for sorghum grains.

The ionizable iron contents of controls at pH 7.5 varied from 1.0-2.0 mg/100 g (Table 2). Treatments T_1, T_2 and T_5 increased the ionizable iron, while treatments T_3 and T_4 decreased it. All the treatments, which improved the ionizable iron, were also highly effective in removing phytate phosphorus, except for treatment T_4 with hydrochloric acid. This treatment decreased phytate phosphorus, but did not lead to corresponding increase in the ionizable iron, which may be because of leaching at acidic pH. The ionizable iron was taken into account for determining the iron bioavailability.

The iron bioavailability values of various control and treated sorghum cultivars are presented in Table 2. These values for control samples ranged from 0.94 '(SU-52') to 1.42 mg ('CSH-9') per 100 g, with an average of 1.2 2. Some of the values reported earlier for sorghum are 0.63 (Murthy et al. 1985), and 1.07 mg/100 g (Annapurani and Murthy 1985). Erdman and Forbes (1977) observed that dietary levels of 1% or more of phytic acid could interfere with mineral availability. In this study, phytate contents of all the varieties were higher than 1% (Table 1), and were reduced to less than 1% after treatments, except for treatment T_3, which did not improve the iron bioavailability. Except for treatments T_3 and T_4, all other treatments increased the iron bioavailability, which ranged from 1.42 to 3.3 mg/100 g. A non-significant correlation of –0.22 existed between phytate phosphorus and iron bioavailability. However, with respect to treatments T_1 and T_2, the correlation (–0.42) was significant ($p<0.01$).

While differences among varieties were non-significant, the treatments significantly differed ($p<0.01$) in influencing iron bioavailability. Irrespective of varieties, treatment T_1 (soaking), T_2 (germination) and T_5 (boric acid) were found to be the three most effective treatments for improving iron bioavailability from sorghum grains. However, during the treatment T_5, boron may be absorbed by the grains, and this may be hazardous to health (Windholz 1976).

Tannin content, expressed as catechin equivalent, ranged from 0.00 to 8.00 g/100 g samples for controls (Table 3). In literature, a similar range of 0.033 to 10.2 has been reported (Arora and Luthra 1974, Salunkhe et al. 1984). Such a wide variation suggests that, besides genotypes, the environmental factors such as rain during grain development may result in the accumulation of phenolic compounds (Salunkhe et al. 1984). In this study, tannins were not detected in varieties 'CSH 6', 'CSH 9', 'SPV 346' and 'SPV

TABLE 2. EFFECT OF TREATMENTS ON IONIZABLE IRON AND IRON BIOAVAILABILITY (mg/100 g) OF SORGHUM VARIETIES

Variety	Treatments						Variety mean
	Control	T_1	T_2	T_3	T_4	T_5	
'SU 45'	1.18 **1.5**	2.13 (80.51) **3.5**	1.89 (60.17) **3.0**	0.34 (-71.19) **1.5**	1.18 (0.00) **1.5**	1.65 (39.83) **2.5**	1.39
'SU 52'	0.94 **1.0**	1.48 (57.45) **2.0**	2.13 (126.60) **3.5**	0.94 (0.00) **1.0**	0.95 (1.06) **1.0**	3.30 (251.06) **6.0**	1.62
'SPV 96'	1.18 **1.5**	1.65 (39.83) **2.5**	1.42 (20.34) **2.0**	1.18 (0.00) **1.5**	0.95 (-19.49) **1.0**	1.42 (20.34) **2.0**	1.30
'SPV 346'	1.41 **2.0**	2.13 (51.06) **3.5**	1.65 (17.02) **2.5**	1.18 (-16.31) **1.5**	0.95 (-32.62) **1.0**	1.65 (17.02) **2.5**	1.49
'CSH 6'	1.18 **1.5**	1.89 (60.17) **3.0**	2.36 (100.00) **4.0**	1.29 (9.32) **1.8**	1.18 (0.00) **1.5**	1.42 (20.34) **2.0**	1.55
'CSH 9'	1.42 **2.0**	1.89 (39.47) **3.0**	1.89 (39.47) **3.0**	1.18 (-16.90) **1.5**	0.71 (-50.00) **0.5**	1.36 (-4.23) **4.0**	1.41
**Treat Mean	1.22[a] **1.58**	1.86[a] (52.46) **2.92**	1.89[a] (54.92) **3.0**	1.02[b] (-16.39) **1.47**	0.99[b] (18.85) **1.08**	1.97[a] (61.48) **3.17**	

*Means of two independent estimations. Data in parentheses indicate per cent decrease as compared to the controls.. ** Significant at 1% level. CD for comparison of any two treaments means is 0.67, Means with same superscript are at par. Figures in **bold** represent ionizable iron at pH 7.5.

TABLE 3. EFFECT OF TREATMENTS ON TANNIN AND CRUDE PROTEIN CONTENT OF GRAIN SORGHUM*

*Variety	Tannin (g/100g)							% Crude Proteins						
	Control	Treatment					Variety* Mean	Control	Treatment					Variety* Mean
		T_3	T_4	T_5	T_6	T_7			T_3	T_4	T_5	T_6	T_7	
'SU 45'	2.0	1.0	1.0	1.0	0.0	0.0	0.84^1	4.81	4.03	3.76	4.46	4.29	4.69	4.34^1
'SU 52'	4.0	2.0	2.0	0.0	0.0	0.0	1.34^1	8.23	7.18	7.09	7.88	7.44	8.05	7.65^2
'6449'	7.0	3.0	3.0	2.5	3.0	4.0	3.79^2	11.94	10.68	10.50	11.38	11.11	11.55	11.19^3
'Kakri local	8.0	4.5	4.0	3.5	4.0	5.0	4.84^3	12.69	11.20	11.02	11.90	11.81	12.25	11.81^4
*Treat Mean	5.25^b	2.60^a	2.50^a	1.75^a	1.75^a	2.25^a	*Treat Mean	9.42^a	8.27^c	8.09^c	8.90^{ab}	8.66^{bc}	9.13^{ab}	

* Means of two independent estimations
• Significant at 1% level
CD for comparison of any two varietal means is 1.33
CD for comparison of any two treatment means is 1.63
*Varieties 'SPV 96', 'SPV 346', 'CSH 6' and 'CSH 9' contained no tannin

* Means of two independent estimations
• Significant at 1% level
CD for comparison of any two treatment means is 0.63
CD for comparison of any two variety means is 0.52
Means with same superscript are at par

96'. For hybrid variety Radhakrishnan and Sivaprasad (1980) also recorded similar value of 0.06% tannin.

The five treatments selected (treatment T_3 to T_7) decreased the tannin content in all the varieties tested (Table 3), in some cases the reduction achieved being 100%. Earlier, a decrease of 75-80% in tannin content of sorghum was reported after soaking in 0.05 N sodium hydroxide for 24 h at room temperature (Chavan et al. 1976), whereas, in the present study, this treatment resulted in charring of the samples. However, soaking in 0.005 N sodium hydroxide for 48 h at room temperature lowered the tannins on an average by 76.76%. Soaking in 0.2 N hydrochloric acid resulted in removing about 55% tannin. Similar observation was made on pearl millet (Panwal and Pawar 1989; Pawar and Parlikar 1990).

The decrease in tannin content of sorghum cultivars as a result of different treatment was significant ($p<0.01$), and the five treatments were equally effective. The varieties also differed markedly ($p<0.01$) in their response to the treatments. On the whole, 'SU 45' was most responsive.

The percentage crude protein contents (Table 3) of the controls ranged from 4.81 ('SU 45') to 12.69 ('Kakri local'). The values are within the range of 4.4 to 21.0, as reported in literature (Arora and Luthra 1974; Salunkhe et al. 1984). The treatments slightly reduced the protein level, which may be attributed to leaching of some soluble fractions in the soaking solution. A high positive correlation of 0.99 ($p<0.01$) was observed between tannin and protein content of the control grains.

The per cent *in vitro* protein digestibility values of tannin- containing sorghum cultivars varied from 27.58 ('Kakri local') to 51.98 ('SU 45'), with an average of 40.31, while for those without tannin varieties, the values ranged from 66.4 ('CSH 6') to 84.58 ('SPV 346'). with a mean value of 74.60 (Table 4). The difference in the *in vitro* protein digestibility of the varieties, with or without tannin, was significant $p<0.05$). Malossini et al (1989) reported the values to be 12.2 and 69.3 for the high and

TABLE 4. *IN VITRO* PROTEIN DIGESTIBILITY OF DIFFERENTIALLY TREATED SORGHUM VARIETIES[X]

No tannin variety	IVPD	Tannin containing varieties	IVPD of tannin containing varieties								Variety ^mean
			Control	T_1	T_2	T_3	T_4	T_5	T_6	T_7	
'SPV 96'	72.31	'SU 45'	51.98	93.67	95.86	81.39	81.38	49.10	71.33	42.64	70.92^2
'SPV 346'	84.58	'SU 52'	50.51	62.50	69.28	63.93	70.94	41.62	64.65	48.94	59.05^2
'CSH 6'	66.40	'6449'	31.16	43.17	41.96	47.09	50.00	28.82	37.44	28.82	38.52^1
'CSH 9'	75.09	'Kakri local'	27.58	44.25	41.97	42.95	51.64	25.71	37.00	26.78	37.23^1
Mean	74.60	Treat *Mean	40.31^b	60.89^a	62.27^a	58.84^a	63.49^a	36.31^b	52.61^a	36.79^b	

[X]Means of two independent estimations, expressed as percentage of crude protein. • Significant at 1% level. CD for comparison of any two variety means is 10.13, CD for comparison of any two treatment means is 14.32. IVPD : *In vitro* protein digestibility.
Means with same superscript are at par

low tannin varieties of sorghum, respectively. A range of 49.6 to 95.6 has been recorded for sorghum, including the varieties with and without tannin (Arora and Luthra 1974), which corresponds well with the values obtained in the present study.

The effect of treatments on *in vitro* protein digestibility has been presented in Table 4. Treatments T_5 and T_7 involving boric acid, did not improve the *in vitro* protein digestibility, and remained statistically comparable to the control grains. Possibly, boron was absorbed during the treatments by grains, and was involved in some complex formation, thereby adversely affecting protein digestibility. However, treatments T_3 and T_4, involving sodium bicarbonate and hydrochloric acid, considerably increased the digestibility, and treatment T_6 was also comparable. Pawar and Parlikar (1990) observed increase in the *in vitro* protein digestibility of pearl millet, as a result of steeping (15 h) in 0.2 N hydrochloric acid. Positive effects of sodium bicarbonate supplementation in overcoming nutritional adversity of tannins have also been reported (Banda-Nyirenda and Vohra 1990). In the present study, soaking (T_1) and germination (T_2) were ineffective in reducing tannins. This finding is in concurrence with that reported by Malossini et al (1989) for sorghum. These treatments, nevertheless, caused significant improvement in the *in vitro* protein digestibility ($p<0.01$). This observation is supported by the findings of Bhise et al (1988). Increments in *in vitro* protein digestibility without reduction in tannin may be attributed to significant reduction ($P<0.01$) in the phytate phosphorus content (Pawar and Parlikar 1990).

Treatments T_1, T_2, T_3 and T_4 significantly improved the *in vitro* protein digestibility, as compared to control, and these four treatments produced comparable effects. One way analysis of variance indicated that after treatments, the digestibility of tannin-containing varieties became comparable to that of the no tannin varieties ($p<0.05$). A significant negative correlation ($r=-0.58$, $p<0.05$) was observed between tannin and the *in vitro* protein digestibility, which is supported by the findings of Mariami et al (1988), and Malossini et al (1988).

It may be concluded that tannin content of sorghum could easily be reduced by soaking in acidic or alkaline solution, thereby improving the *in vitro* protein digestibility. Further, steeping in water for 16 h at room temperature or germination of pre-soaked grains for 72 h improves the nutritional quality of sorghum grains, both in terms of iron and protein bioavailability.

Acknowledgement

The authors are thankful to the Head, Genetics and Plant Breeding Department, Rajasthan College of Agriculture, Udaipur, for providing the sorghum varieties and to the Head, Department of Foods and Nutrition, College of Home Science, for providing the necessary laboratory facilities.

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Received 22 July 1994; revised 26 June 1995; accepted 6 September 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 459-464

Kernel Infection and Aflatoxin Production in Peanut (*Arachis hypogaea* L.) by *Aspergillus flavus* in Presence of Geocarposphere Bacteria

H.K. CHOURASIA[1]

Department of Plant Pathology, Alabama Agricultural Experiment Station, Auburn University, Alabama - 36849-5409, USA.

Roots and pods of field-grown peanut were sampled at three stages of development and a total of eight microflora were isolated from the geocarposphere. *Aspergillus flavus* was grown on peanut extract agar, and on viable peanut kernels, either in pure culture or in dual culture with either of *Bacillus megaterium*, *B. laterosporus*, *Cellulomonas cartae*, *Flavobacterium odoratum*, *Phyllobacterium rubiacearum*, *Pseudomonas aurofaciens* and *Xanthomonas maltophila*. Aflatoxin production by *A. flavus*, its growth and interactions with other microorganisms were studied at three water activities (0.98, 0.95 and 0.90 a_w) and three temperatures (30, 25 and 20°C). Almost all bacteria stimulated growth and aflatoxin production by *A. flavus* on peanut kernels, especially at 30°C and 0.95 and 0.98 a_w. No aflatoxin was produced at 20°C and 0.90 a_w. *F. odoratum* showed inhibition of aflatoxin biosynthesis by *A. flavus*, as compared to cultures of *A. flavus* alone. The morphological interactions among *A. flavus* and other bacteria were also examined on peanut extract agar under similar controlled conditions.

Keywords : *Aspergillus flavus*, Aflatoxins, Peanut, Geocarposphere bacteria.

Peanuts (*Arachis hypogaea* L.) are frequently invaded by toxigenic *Aspergillus flavus* Link and *A. parasiticus* Speare, prior to harvest, especially in hot, dry conditions (Diener 1989). In recent past, a wide range of chemicals and bio-agents have been tested against pre- and post-harvest aflatoxin contamination of peanut kernels (Mixon et al. 1984). However, paucity of potential bio-competitive agents is striking (Kloepper and Bowen 1991), particularly among geocarposphere (zone around the subterranean pod) bacteria. Based on the results of previous study, which demonstrated that geocarposphere bacteria differed from rhizosphere bacteria (Joffe 1969; Kloepper and Bowen 1991), it was hypothesized that geocarposphere bacteria would be ideal candidates for protecting the developing peanut pods against aflatoxigenic fungi.

Environmental conditions, especially water activity (a_w, temperature, and composition of intergranular air (Magan and Lacey 1984) have been shown to influence succession of microorganisms on agricultural commodities. Components of this microflora interaction, may be responsible for transient or permanent changes within the population (Garren and Porter 1970; Wilson et al. 1985). Consequently, the interacting organisms may be inhibited or stimulated, on contact or at a distance, or unaffected. Studies on the production of aflatoxin by *A. flavus* have mostly been by pure culture technique (Mehan et al. 1991). Furthermore, most studies have used liquid media (Diener and Davis 1966), or autoclaved (non-viable) kernel (Fonseca et al. 1983; Elamin et al. 1988), and only rarely has viable kernel been used (Diener 1989).

The objective of the present study was to (i) isolate geocarposphere microflora from peanut pods with special reference to *A. flavus* and bacteria, (ii) study their growth, and interactions on peanut extract agar and viable peanut kernels, and (iii) the production of aflatoxins by *A. flavus* on viable peanut kernels either alone or in association with different bacteria under controlled conditions of water activities (0.90, 0.95 and 0.98 a_w) and temperatures (20,25 and 30°C).

Materials and Methods

Sampling procedures, isolation of microflora and microbial cultures : Eight microflora, viz., *Aspergillus flavus*, *Bacillus megaterium*, *B. laterosporus*, *Pseudomonas aurofaciens*, *Xanthomonas maltophila*, *Cellulomonas cartae*, *Flavobacterium odoratum* and *Phyllobacterium rubiacearum* were obtained from geocarposphere of peanut cv 'Florunner', taken from peanut research plots at the Alabama Agricultural Experiment Station, Auburn University, USA. The sampling dates representing different stages of physiological maturity (Boote 1982) were used and included (a) June 13, R3 stage (swelling of peg); (b) July 5, R5 stage (full size pod with visible seed cotyledon); and (c) July 26, R7 stage (testa colouration on seed).

[1]*Present Address : Plant Pathology Division, Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP Lucknow-226 015, India.*

Six replicate plants were selected at random from throughout the field trial (covering 10.5 hectares) on each sampling date. Six replicate light sandy and red sandy loam soil samples were also taken from non-planted buffer zones adjacent to the plots.

Three individual pods and three sub-samples of roots were removed from each replicate plant in the laboratory. Each replicate soil sample was mixed prior to removing three sub-samples. The sub-samples of pods, roots and soil were weighed, placed into 10 ml of 0.2 M phosphate buffer (pH 7.0) individually, and agitated for 1 h at 150 rpm on an orbital shaker. After agitation, root and pod samples were macerated with a sterile mortar and pestle, serially diluted in phosphate buffer to 10^{-4}, and plated with an automated plating system (Spiral SystemsInc; Bethesda, Maryland) onto test media. Bacterial populations were estimated by plating on 5% TSA (Bacto tryptone 17 g, Bacto soytone, 3 g, Bacto dextrose 2.5 g, sodium chloride 5 g, dipotassium phosphate 2.5 g, Bacto agar 20 g, and distilled water 1l, and PAF (Bacto peptone 10 g, proteose peptone 10 g, potassium phosphate 1.5 g, magnesium sulphate 1.5 g, Bacto agar 15 g, and distilled water 1l media. *Aspergillus flavus* was quantified by plating on M 351 B medium (Griffin and Garren 1974).

Plates for bacteria were incubated at 28°C for 24-96 h, and colonies were enumerated using a laser colony counter with bacterial enumeration software (Spiral System Instruments, Bethesda, Maryland). Plates for fungi were incubated upto 7 days at 25°C, and *A. flavus* was identified under a Nikon stereomicroscope.

Inoculum preparation : A. flavus was grown on potato-dextrose-agar (Difco), and stored at 4°C. Spores were harvested by flushing 5 days old cultures in a sterile 0.01% solution of Triton X-100. The spore concentration was standardized to $3x10^6$ spores/ml by using an improved Neubauer hemocytometer (BDH Ltd., Dagenham, England) with suitable dilutions.

All bacteria under study were grown on nutrient agar (beef extract 10 g, peptone 10 g, sodium chloride 5 g, agar 12 g, distilled water 1l and pH 7.5) slopes at 28°C. The cells were harvested in sterile distilled water, and shaken to obtain a homogeneous suspension. The cell concentration was determined by dilution plating on nutrient agar, incubating for upto 72 h at 28°C. Routinely, a suspension of 10^5 cells/ml was used as the inoculum.

Cultivation on peanut extract agar : Organisms were inoculated individually or in pairs on peanut extract agar adjusted to 0.90, 0.95 and 0.98 a_w with glycerol (Diener and Davis 1969). When grown individually, organisms were inoculated at the centre of each petri dish. For dual culture, the organisms were inoculated 1.75 cm from the centres, and diametrically opposite to one another (Johnson and Curl 1972). *A. flavus* cultures were inoculated with spores suspended in 0.1% water agar, while cells of each bacterium were transferred directly with sterile inoculating needles. Because of faster growth, each bacterium was inoculated 2 days after *A. flavus* inoculation. After inoculation, plates of the same a_w were stacked, sealed in microporous polypropylene bags, and put in controlled-environment cabinets adjusted to 90, 95 or 98% relative humidity and at 20, 25 or 30°C. The diameters of *A. flavus* colonies were measured daily from 2 to 10 days after inoculation. Growth of each bacterium was semi-quantitatively determined by dilution plate counting. Colony diameters in case of dual cultures, the gaps between the colonies were measured daily from 2 to 3 days after inoculation for upto 15 days. Microbial interactions on peanut extract agar were classified by the method of Johnson and Curl (1972). Treatments were replicated three times.

Treatment of peanut kernels : Sound, mature peanut kernels were surface-disinfested by agitating in 20% household bleach (Clorox; active ingredient 5.25% NaOCl, Clorox Co., Oakland, California) at 150 rpm on an orbital shaker for 1 h, followed by four to five rinses in sterile water, and drying at 25°C for 1 h in open petri dishes in a laminar flow hood. After sterilization, 1 kg of peanut was placed in each of three 1 litre sterilized flasks. The water content of the peanut was determined, and a_w was adjusted by the procedure recommended by Pixton and Warburton (1971). The a_w of peanut samples was first determined by using an electronic dew point meter (Protimeter DP 680) at 25°C, and then conditioned at 50°C for up to 14 days to 0.90, 0.95 and 0.98 a_w by adding sterile water.

Microbial culture in peanut kernels : Surface-sterilized peanut (50 g) at the required a_w was placed in a sterile 500 ml beaker, and immediately inoculated with either 1 ml of *A. flavus* spore suspension or, for dual culture, with 0.5 ml of *A. flavus* and 0.5 ml of either *B. megaterium*, *B. laterosporus*, *P. aurofaciens*, *X.maltophila*, *C. cartae*, *F. odoratum* or *P. rubiacearum*. An adjustable pipette (Gilson Co; Inc; Worthington, Ohio) with a disposable

tip was used for inoculation. The inoculated substrate was stirred briefly with a sterile glass rod, and then carefully transferred to a microporous film bag (Cuero et al. 1985). The bags were incubated in the controlled-environment cabinets at 20, 25 or 30°C and 90, 95 and 98% relative humidity, with daily shaking. Growth of *A. flavus* (portion of the kernel covered) and bacteria (cells/g peanut kernel) were assessed daily from 3 to 12 days of incubation.

Aflatoxin extraction and quantification : At the end of each experiment, aflatoxin was extracted from 50 g peanut by reversed phase high pressure liquid chromatography (Waters Associates Inc., Milford, Massachusetts) by the method of Stubblefield and Shotwell (1977). The mobile phase consisted of HPLC grade acetonitrile : tetrahydrofuran: water (10:6:84, v/v/v), adjusted to pH 3.9 with acetic acid. The flow rate was 2 ml/min. Detection was by fluorescence in a model 470 fluorescence detector (excitation at 360 nm and emission at 418 nm). Quantification of peak area was done using Baseline software (Millennium 2010, Millipore Corporation, Massachusetts). Standards of aflatoxin B_1, B_2, G_1 and G_2 (Sigma Chemical Co., St. Louis, Missouri) were run through the complete derivatization procedures.

Results

Growth of single organism on agar : A. flavus grew well at 30°C and 0.95 or 0.98 a_w on peanut extract agar, but grew much slowly at 0.90 a_w (Table 1). Among all bacteria isolated, maximum growth was found in case of *B. megaterium* at 25°C and 0.95 a_w, followed by the growth of *B.laterosporus, P. aurofaciens, P. rubiacearum* and *F odoratum* at the same temperature and a_w values. *X. maltophila* was the only bacterium, which showed the highest growth at 30°C and 0.95 a_w. There was no growth of bacteria at 20°C and 0.90 a_w.

Interactions on agar : A. flavus inhibited *B. megaterium, P. aurofaciens, X. maltophila* and *P. rubiacearum* on contact at 30°C and 0.95 a_w in peanut extract agar (Table 1). However, at 25°C and 0.95 a_w, the reverse was true and *B. megaterium, P. aurofaciens, X. maltophila* and *P. rubiacearum* inhibited *A. flavus* at a distance. At 25°C and 0.95 a_w, *B. laterosporus* and *F. odoratum* inhibited *A. flavus* and continued to grow. At 0.90 a_w, there was insufficient growth at all temperatures to determine microbial interactions.

Growth of single organism in peanut kernel : All organisms grew on peanut at all a_w values and temperatures. Maximum cell yield of *B. megaterium,*

TABLE 1. EVALUATION OF GROWTH AND INTERACTIONS OF *ASPERGILLUS FLAVUS* AND BACTERIA AT DIFFERENT A_w VALUES AND TEMPERATURES ON PEANUT EXTRACT AGAR

Organism(s)	Growth[a] and interactions[b] at temperature and a_w of								
	20°C			25°C			30°C		
	0.98	0.95	0.90	0.98	0.95	0.90	0.98	0.95	0.90
Aspergillus flavus	50.0	65.0	19.0	68.0	75.0	22.0	80.0	85.0	26.0
Bacillus megaterium	2.0	2.9	NG	2.4	3.5	0.5	2.7	3.0	0.4
B. laterosporus	0.9	2.0	NG	2.0	3.2	1.0	1.7	2.9	0.5
Pseudomonas aurofaciens	1.5	2.6	NG	2.5	3.2	1.0	1.8	3.0	0.8
Xanthomonas maltophila	0.6	2.5	NG	1.9	2.5	0.4	2.2	3.3	0.5
Cellulomonas cartae	0.4	1.7	NG	1.5	3.0	0.5	1.0	2.5	0.2
Flavobacterium odoratum	1.6	2.4	NG	1.4	2.5	0.2	1.0	2.2	NG
Phyllobacterium rubiacearum	1.2	2.5	NG	1.8	3.2	0.4	0.8	2.7	0.3
A. flavus/B. megaterium	3.0	2.0	-[c]	2.0	4.0[d]	-	2.0	4.0[e]	-
A. flavus/B. laterosporus	3.0	5.0[d]	-	2.0	5.0[d]	-	2.0	3.0	-
A. flavus/P. aurofaciens	2.0	2.0	-	3.0	4.0[d]	-	3.0	4.0[e]	-
A. flavus/X. maltophila	2.0	2.0	-	2.0	4.0[d]	-	3.0	4.0[e]	-
A. flavus/C. cartae	2.0	1.0	-	3.0	3.0	-	1.0	2.0	-
A. flavus/F. odoratum	2.0	2.0	-	2.0	5.0[d]	-	2.0	3.0	-
A. flavus/P. rubiacearum	3.0	3.0	-	2.0	4.0[d]	-	1.0	4.0[e]	-

[a]For *A. flavus*, growth was determined by measuring colony diameters (millimeters); for different bacteria, growth was semi-quantitatively determined by dilution plate counting (10^7 organisms per ml); NG : No growth. [b]Classifications - 1 : Mutual intermingling; 2 : mutual inhibition on contact, and space between two colonies is small; 3:mutual inhibition at a distance; 4: inhibition of one organism on contact and the antagonist continues to grow unchanged or at a reduced rate through the colony of the inhibited organism; 5: inhibition of one organism at a distance, and the antagonist continues to grow through the resulting clear zone at an unchanged or reduced rate. [c]Microbial interactions were not studied at 0.90 a_w because of insufficient growth. [d]Bacterium dominant. [e]*A. flavus* dominant

B. laterosporus and *P. rubiacearum* was at 25°C and 0.95 to 0.98 a_w. Growth of *A. flavus* at 30°C and 0.98 a_w was usually evident after 2 to 3 days, but growth was evident after 5 days at 25°C and 0.95 a_w. However, at 25°C and 0.90 a_w and at all a_w values at 20°C, little or no visible growth occurred in 12 days (data not presented).

Microbial interactions on kernel : In dual culture with *A. flavus*, *B. megaterium*, *B. laterosporus*, *P. aurofaciens*, *X. maltophila*, *C. cartae*, *F. odoratum* and *P. rubiacearum* grew moderately well over the whole a_w range at all temperatures (Table 2). Abundant mycelium was formed, when *A. flavus* interacted with *B. megaterium*, *B. laterosporus*, *C. cartae* and *P. rubiacearum* at 25°C and 30°C as well as 0.95 and 0.98 a_w. However, the growth of *A. flavus* decreased at 25°C and 30°C as well as 0.90 a_w.

Aflatoxin production in peanut kernels : Mean aflatoxin production by a single culture of *A. flavus* in sterilized peanut kernels is shown in Table 2. Maximum aflatoxin (1050 µg/kg) was produced at 30°C and 0.98 a_w, while in contrast, at 20°C and 0.98 and 0.95 a_w, only small amounts were formed, and none was produced at 20°C and 0.90 a_w.

When *A. flavus* was grown with different bacteria in dual culture on peanut, aflatoxin production increased in case of all bacteria under experimental conditions, except in case of *F. odoratum*, as compared to *A. flavus* in a single culture (Table 2). The highest aflatoxin production was noticed, when *A. flavus* and *B. megaterium* were grown together at 30°C (2175 µg/kg), 25°C (2005 µg/kg) and 0.98 a_w, thereby amounting to a two-fold increase over the single culture. A 10-fold increase in aflatoxin level was found at 30°C (1005 µg/kg, 25°C (1075 µg/kg), and 0.90 a_w with the same bacterium. Similarly, an increased level of aflatoxin production was observed in dual culture with *B. laterosporus*, *P. aurofaciens*, *C. cartae*, *X. maltophila* and *P. rubiacearum* at 30°C and 0.98 a_w, 0.95 and 0.90 a_w, followed by 25°C and 20°C, and at the same a_w values. Production was slightly different with *B. laterosporus*, as the level of aflatoxin

TABLE 2. GROWTH AND AFLATOXIN PRODUCTION BY *A. FLAVUS* IN SINGLE AND DUAL CULTURE WITH DIFFERENT BACTERIA IN STERILIZED PEANUT KERNELS

Organism(s)	Growth[a] and aflatoxin production[b] (µg/kg) at temperature and a_w of								
	20°C			25°C			30°C		
	0.98	0.95	0.90	0.98	0.95	0.90	0.98	0.95	0.90
A. flavus/	3	3	2	5	5	3	5	4	2
B. megaterium	3	3	2	5	5	2	3	4	4
A. flavus/	4	3	3	5	4	2	4	5	3
B. laterosporus	3	3	2	4	5	3	4	3	2
A. flavus/	4	2	2	4	3	3	2	3	4
P. aurofaciens	3	3	3	3	4	2	3	3	2
A. flavus/	4	3	4	4	4	3	4	2	3
X. maltophila	2	3	2	3	4	2	3	5	4
A. flavus/	4	4	2	4	4	3	5	4	3
C. cartae	3	3	2	4	4	3	3	3	2
A. flavus/	3	3	3	4	3	4	4	3	4
F. odoratum	2	3	2	3	4	3	2	3	2
A. flavus/	2	2	3	4	4	3	5	4	2
P. rubiacearum	3	3	2	5	5	4	3	3	3
A. flavus	270.0	100.5	NA[c]	1010.0	700.6	105.2	1050.0	940.0	100.5
A. flavus/B. megaterium	550.5	350.2	NA	2005.0	1500.2	1075.0	2175.0	1550.5	1005.0
A. flavus/B. laterosporus	450.2	207.7	NA	1700.5	1202.0	407.2	1900.7	1004.2	800.5
A. flavus/P. aurofaciens	420.5	217.2	NA	1505.0	909.9	505.0	1606.7	999.0	815.0
A. flavus/X. maltophila	350.2	189.0	NA	1050.5	5.5	200.0	1202.9	1111.0	740.5
A. flavus/C. cartae	400.6	175.0	NA	1300.2	990.0	350.0	1500.0	1202.5	850.0
A. flavus/F. odoratum	250.0	89.2	NA	850.0	505.5	90.2	917.2	705.3	400.5
A. flavus/P. rubiacearum	285.0	75.5	NA	1080.0	802.0	115.0	1102.0	1090.2	850.0

[a]Data on growth of individual organism are not presented but described in the text; Classifications for *A. flavus* (portion of the kernel covered) - 1: very little; 2: one-fourth; 3: one-half; 4: three-fourths; 5: whole of kernel covered. Growth of bacterium was assessed by dilution plating; Cells per gram of peanut kernel-1:10^1; 2:10^2; 3:10^3; 4:10^4; 5:10^5. [b]Mean level of six replicates. [c]Not detectable amount.

produced at 25°C and 0.95 a_w (1202 µg/kg) was greater than the amount of aflatoxin produced at 30°C and 0.95 a_w.

Discussion

The present results indicate that almost all geocarposphere bacteria, except *Flavobacterium odoratum*, stimulated the overall level of aflatoxin production by *A. flavus*, when grown in dual culture on sterilized peanut kernels under certain environmental conditions. The presence of *B. megaterium* significantly increased aflatoxin production, as compared to that by cultures of *A. flavus* alone. Despite a marked influence on total aflatoxin production, all test bacteria shifted the relative rates of aflatoxins B_1, G_1, B_2 and G_2.

Growth of *A. flavus* in the peanut extract agar and peanut kernels showed a specific pattern of sensitivity to the seven test bacteria, which might have resulted due to specific difference in the nature of the volatile bacterial metabolites produced and also from environmental changes such as alterations in the levels of oxygen or carbon dioxide. Fries (1973) suggested that volatile organic compounds may exert an effect on a fungus by influencing specific metabolic processes, rather than by gross inhibition of metabolism, growth and development. He proposed that volatile compounds could act, in small amounts, as metabolic regulators, by directing the metabolism through specific pathways. This suggestion could be of special importance, if the accumulation of fungal secondary metabolites such as mycotoxins, on a particular substrates, could be influenced by volatile metabolites from an associated bacterial flora.

The present results, with peanut as the substrate, suggest that the metabolic activity of all test bacteria altered the substrate, enhancing the growth of *A. flavus* or its ability to produce aflatoxin or both. It is possible that *B. megaterium*, *B. laterosporus*, *P. aurofaciens*, *X. maltophila*, *C. cartae* and *P. rubiacearum*, under our experimental conditions, could have enzymatically changed the substrates, making them more favourable for aflatoxin biosynthesis. Similarly, compounds from these organisms could have been released, leading to enhanced growth or enhanced toxin production or both. It is also possible that these six bacteria got attached to the hyphae of *A. flavus*, and travelled with hyphae into the tissue, thereby altering not only the substrate, but also the fungal wall-membrane structure, changing its diffusibility or increasing the rate at which metabolites, such as the aflatoxins, can leak through the membrane.

Results of dual culture tests with *Flavobacterium odoratum* on peanut substrate showed least production of aflatoxin by *A. flavus*, as compared to that by cultures of *A. flavus* alone. Studies with *A. flavus* and *Flavobacterium aurantiacum* (Mehan et al. 1991) and *A. parasiticus* with *Saccharomyces cerevisiae* (Weckbach and Marth 1977) in liquid culture also showed a decrease in aflatoxin production. Ciegler et al (1966) screened 1000 microorganisms for their ability to inhibit aflatoxin production, and found that only *Flavobacterium aurantiacum* removed aflatoxin from a nutrient solution. The mechanism proposed was that aflatoxin was degraded by intact and fragmented mycelium (Masimango et al. 1978).

The preferential association of fungi and bacteria with early development stages of the pod indicates that some microorganisms are well adapted for colonization of the geocarposphere, and these microorganisms could be of great microbiological significance.

Acknowledgement

The author is thankful to Prof. Barry J. Jacobsen, Head, Department of Plant Pathology, Alabama Agricultural Experiment Station, Auburn University, USA, for providing laboratory facilities. Financial support by Government of India, Department of Education, New Delhi in the form of Post-doctoral Fellowship is gratefully acknowledged.

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Received 4 April 1994; revised 6 July 1995; accepted 5 September 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 465-469

Chemical Determination of Nutritional and Antinutritional Properties in Tribal Pulses

V.R. MOHAN[1] AND K. JANARDHANAN*

Seed Physiology and Biochemistry Laboratory,
Department of Botany, Bharathiar University,
Coimbatore - 641 046, India.

Wild under-exploited edible legume seeds, *Cassia obtusifolia* and three germplasm of *Abrus precatorius* were investigated for their proximate composition, minerals, seed protein fractions, amino acid profiles, fatty acid composition and certain antinutritional substances. These contained crude proteins (16.1 to 22.8%), fat (8.8 to 12.7%) and carbohydrates (55.1 to 65.8%). The seed protein fractionation revealed that both albumins and globulins constituted predominant protein fractions. The data on amino acid profiles showed that the methionine content ranged from 1.7 to 2.5% in *A. precatorius*. The seed proteins of *C. obtusifolia* were found to be deficient in lysine, cystine, methionine and phenylalanine. The unsaturated fatty acids accounted for more than 60% of the lipids in *A. precatorius*, whereas in *C. obtusifolia* they accounted for 46%. The mineral composition showed that potassium formed the most predominant mineral in all the samples. Among the levels of the antinutritional factors investigated, total free phenols, tannins and L-DOPA were not particularly high. Only the globulin fraction of *A. precatorius* seed proteins exhibited strong agglutination with the erythrocytes from the blood groups B and O. In *C. obtusifolia*, the globulins exhibited strong agglutination against the erythrocytes of human blood group A only.

Keywords : Tribal pulses, *Abrus precatorius*, *Cassia obtusifolia*, Chemical composition, Antinutritional factors.

The tribal pulses, *Abrus precatorius* L. and *Cassia obtusifolia* L. grow in almost all parts of the tropical zones (Hooker 1973). During extreme food scarcity, boiled seeds of *A. precatorius* are known to be eaten by *onges* aborigines of Andamans (Bhargava 1990), and *Katkharis* of Pune District, Maharashtra, India (Gunjatkar and Vartak 1982). Similarly, the roasted seeds of *C. obtusifolia* are eaten by the tribal people living in the hilly region of Pune District, Maharashtra (Gunjatkar and Vartak 1982). Chemical composition and nutrient evaluation were carried out on the seeds of *A. precatorius* in an earlier investigation. (Rajaram and Janardhanan 1992). For the present study, three different accessions of the same tribal pulse, were collected from different agroclimatic regions, viz., Ayyanarkoil Forest, Kalakad Wildlife Sanctuary and Mundanthurai Wildlife Sanctuary. The tribal pulse, *C. obtusifolia*, was collected from Mundanthurai Wildlife Sanctuary, Tamil Nadu, South India.The nutrient and antinutritional properties of these two tribal pulses have been investigated and the results are reported in this paper.

Materials and Methods

The mature seeds of three germplasm of tribal pulse, *Abrus precatorius*, and other tribal pulse, *Cassia obtusifolia*, were collected from different agroclimatic regions in India, as mentioned above. The moisture content was determined by drying 50 transversely cut seeds in an oven at 80°C for 24 h. The oven-dried and air-dried seeds were powdered separately in a Willey mill (Scientific equipment works, Delhi, India) to 60 mesh size. Total nitrogen was determined by microkjeldahl method (Humphries (1956), and the crude protein content was calculated by multiplying the nitrogen content by 6.25. The contents of ether extracts, crude fibre and ash were determined by AOAC (1970) procedure. Nitrogen-free extractive was calculated by difference. The energy values of the seeds were determined by the method of Obsorne and Voogt (1978). Sodium, potassium, calcium, magnesium, zinc, manganese, iron and copper contents were estimated by using Perkin-Elmer, Model-5000 atomic absorption spectrophotometer following the method of Issac and Johnson (1975). Phosphorus content was estimated colorimetrically (Dickman and Bray 1940) from triple acid digested samples. The total proteins were extracted by the method of Rajaram and Janardhanan (1990), and estimated after cold 20% TCA precipitation as per the method of Lowry et al (1951).

The seed protein fractions, albumins and globulins, were extracted following the method of Murray (1979). From the remaining pellets, prolamins and glutelines were also extracted (Rajaram and Janardhanan 1990). The above mentioned protein fractions were estimated for protein content by the method of Lowry et al (1951). The fractionated total seed proteins were acid

* *Corresponding Author.* [1]*Present address : P.G. Department of Botany, V.O.C. College, Tuticorin, 628 008, India.*

hydrolyzed with 6 N hydrochloric acid at 110°C for 24 h *in vacuo* (Laurena et al. 1991). After flash evaporation, the dried residue was dissolved in citrate buffer (pH 2.2). Known aliquots were analyzed in LKB-Biochrome automated amino acid analyzer, Model-4151, Alpha plus (Rajaram and Janardhanan 1990). All the amino acids recovered are presented as g10 g^{-1} proteins.

The total lipids from the seed flours were extracted according to the method of Folch et al (1957). Fatty acid methyl esters were prepared from the parental lipids by the method of Metcalfe et al (1966). Fatty acid analysis was performed by gas chromatography (Shimadzu Model RIA), using a flame ionisation detector (Mohan and Janardhanan 1993).

The antinutritional factors like total free phenols (Bray and Thorne 1974), tannins (Burns 1971), and non-protein amino acid, L-DOPA (3,4-dihydroxyphenylalanine) (Brain 1976) were quantified. The haemagglutinating activity of albumin and globulin fractions of seed proteins (Liener 1976) was also assayed.

Results and Discussion

The chemical composition of germplasm seeds of *A. precatorius* and *C. obtusifolia*, are shown in Table 1. The crude protein content of the germplasm of *A. precatorius* is slightly higher, when compared with certain less known pulse crops (Amubode and Fetuga 1984; Ukhun and Ifebigh 1988). Similarly, germplasm of *A. precatorius* contained higher lipids than those in other two tribal pulses, *Acacia catechu* and *Parkinsonia aculeata* (Rajaram and Janardhanan 1991a). In contrast, crude proteins and lipids are higher in *C. obtusifolia* than in the commonly cultivated legumes like *Cicer arietinum* (Luz Fernandez and Berry 1988); *Cajanus cajan* (Oshodi and Ekperigin 1989); tribal pulses like, *Vigna umbellata* (Rajaram and Janardhanan 1990) and *Entada phaseoloides* (Mohan and Janardhanan 1993). Due to the lipid rich nature, the seeds of *A. precatorius* from all the germplasm and *C. obtusifolia* registered high food energy values than those of *Phaseolus vulgaris, P. limensis, Vigna unguiculata, Cicer arietinum, Pisum sativum, and Lens culinaris* (Meiners et al. 1976a).

Food legumes have been recognized as important sources of several minerals in Indian diets (Gopalan et al.1978). All the investigated germplasm of *A. precatorius* contained higher levels of sodium, potassium and calcium, when compared with other legumes, *Phaseolus vulgaris, P. limensis, Vigna unguiculata, Cicer arietinum, Pisum sativum* and *Lens culinaris* (Meiners et al. 1976b). In *C. obtusifolia* seeds, the minerals like potassium,

TABLE 1. CHEMICAL COMPOSITION OF THE SEEDS OF *ABRUS PRECATORIUS* AND *CASSIA OBTUSIFOLIA*[1]

	A. precatorius (Ayyanarkoil Forest)	*A. precatorius* (Kalakad Wildlife Sanctuary)	*A. precatorius* (Mundanthurai Wildlife Sanctuary)	*C. obtusifolia*
		Proximate composition (g 100 g^{-1} seed flour)		
Moisture	8.0 ± 0.1	7.7 ± 0.0	8.3 ± 0.1	7.9 ± 0.0
Crude proteins	16.2 ± 0.1	16.1 ± 0.1	18.1 ± 0.2	22.8 ± 0.2
Crude lipids	8.2 ± 0.0	9.4 ± 0.1	9.2 ± 0.1	12.7 ± 0.1
Crude fibre	6.2 ± 0.2	5.8 ± 0.1	6.1 ± 0.0	4.5 ± 0.1
Ash	3.0 ± 0.0	3.1 ± 0.0	4.2 ± 0.0	4.9 ± 0.1
Nitrogen free extractives (NFE)	65.8	65.6	62.3	55.1
*Calorific value (Kcal 100 g^{-1} DM)	407	412	405	425.5
		Mineral composition (mg 100 g^{-1} seed flour)		
Sodium	22.9 ± 0.2	22.8 ± 0.3	23.0 ± 0.4	68.5 ± 0.1
Potassium	1608.7 ± 0.5	1971.8 ± 0.9	1828.2 ± 1.2	2171.6 ± 4.1
Calcium	243.5 ± 0.3	243.7 ± 0.1	244.3 ± 0.5	521.2 ± 0.6
Magnesium	62.6 ± 0.1	62.4 ± 0.2	62.8 ± 0.2	813.0 ± 1.0
Phosphorus	173.9 ± 0.3	184.2 ± 0.5	196.3 ± 0.3	336.6 ± 0.5
Iron	2.8 ± 0.1	2.8 ± 0.1	3.1 ± 0.1	3.5 ± 0.1
Copper	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	1.0 ± 0.1
Zinc	1.3 ± 0.0	1.5 ± 0.0	1.1 ± 0.0	1.3 ± 0.1
Manganese	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	1.3 ± 0.1

[1]Values are means of triplicate determinations. * Calculated ± Denotes standard error.

TABLE 2. AMINO ACID (g/100 g protein) PROFILES OF ACID-HYDROLYZED, (PURIFIED) TOTAL SEED PROTEINS OF *ABRUS PRECATORIUS* AND *CASSIA OBTUSIFOLIA*

	A. precatorius (Ayyanarkoil Forest)	*A. precatorius* (Kalakad Wildlife Sanctuary)	*A. precatorius* (Mundanthurai Wildlife Sanctuary)	*C. obtusifolia*	WHO/FAO (1973) Provisional pattern
Glutamic acid	15.0	15.0	14.3	15.3	-
Aspartic acid	10.0	12.0	12.5	12.5	-
Serine	2.7	3.3	3.0	5.1	-
Threonine	3.0	2.5	2.6	3.6	4.0
Proline	4.0	3.3	3.2	4.1	-
Alanine	6.0	6.1	5.8	5.7	-
Glycine	5.7	5.4	5.3	4.2	-
Valine	7.1	7.9	7.5	4.2	5.0
Crystine	Trace	Trace	Trace	Trace	
Methionine	1.7	2.5	2.2	Trace	3.5
Isoleucine	5.6	4.4	5.0	8.4	4.0
Leucine	8.4	9.5	9.7	8.7	7.0
Tyrosine	2.0	2.2	1.6	2.2	
Phenylalanine	4.5	4.7	4.8	4.7	6.0
Lysine	5.8	6.1	6.3	4.1	5.5
Histidine	1.0	1.2	1.1	1.2	-
Tryptophan	ND	ND	ND	1.0	-
Arginine	6.3	6.0	5.9	5.2	-

ND - Not detected

calcium, magnesium and phosphorus, are higher than those in other legumes (Meiners et al. 1976b).

In *A. precatorius*, the total (true) protein content in the germplasm ranged between 13.6 and 14.2%, whereas in *C. obtusifolia*, it was 20.5%. The albumin protein fractions in all the three accessions of *A. precatorius* were in the range of 37.7 to 39.1%; whereas in *C. obtusifolia* it was 33.1%. Similarly, the globulin protein fractions in all the three accessions of *A. precatorius* ranged from 44.8 to 45.8%, whereas in *C. obtusifolia*, it was 49.9%. The seed protein fractionation data revealed that both albumins and globulins constituted the predominant protein fractions as in other tribal pulses, *Phaseolus lunatus* (Vijayakumari et al. 1993) and *Xylia xylocarpa* (Siddhuraju et al. 1994).

The investigated germplasm seed proteins of *A. precatorius* were rich in most of the essential amino acids (Table 2), except for threonine and cystine, as compared to those of WHO/FAO (1973) provisional pattern. In *C. obtusifolia*, the seed proteins are limiting in cystine, methionine, phenylalanine and lysine. The concentrations of the other essential amino acids, i.e., isoleucine, leucine, threonine and valine were higher than the WHO/FAO (1973) provisional pattern.

Fatty acids, like palmitic, oleic and linoleic, were the major fatty acids of lipids of all the germplasm of *A. precatorius* and *C. obtusifolia* (Table 3). The levels of palmitic and linoleic acids were higher than those in cultivated legumes like *Vigna radiata* and *V. mungo* (Salunkhe et al. 1982).

Food legumes are important sources of dietary proteins in the developing countries, but their

TABLE 3. FATTY ACID COMPOSITION OF THE SEED LIPIDS OF *ABRUS PRECATORIUS* AND *CASSIA OBTUSIFOLIA*[1]

Fatty acid %		*A. precatorius* (Ayyanarkoil Forest)	*A. precatorius* (Kalakad Wildlife Sanctuary)	*A. precatorius* (Mundanthurai Wildlife Sanctuary)	*C. obtusifolia*
Palmitic acid	(C16:0)	18.2	21.4	23.2	34.4
Stearic acid	(C18:0)	11.3	4.6	3.5	15.1
Oleic acid	(C18:1)	36.7	39.3	40.2	19.6
Linoleic acid	(C18:2)	23.6	30.2	26.3	26.4
Linolenic acid	(C18:3)	8.3	3.1	6.3	4.5
Others (unidentified)		1.8	1.4	0.5	-

[1]All values are means of two determinations.

TABLE 4. ANTINUTRITIONAL FACTORS OF *ABRUS PRECATORIUS* AND *CASSIA OBTUSIFOLIA*

Component	*A. precatorius* (Ayyanarkoil Forest)	*A. precatorius* (Kalakad Wildlife Sanctuary)	*A. precatorius* (Mundanthurai Wildlife Sanctuary)	*C. obtusifolia*
Total free phenols (%)	0.7[a] ± 0.0	0.8[a] ± 0.1	0.7[a] ± 0.0	0.5[a] ± 0.0
Tannins (%)	0.3[a] ± 0.0	0.3[a] ± 0.0	0.3[a] ± 0.0	0.2[a] ± 0.0
L-DOPA (%)	0.1[a] ± 0.0	0.2[a] ± 0.0	0.5[a] ± 0.0	3.7[a] ± 0.1
Phytohaemagglutinating activity[b]				

Name of the protein fraction	Erythrocytes from the human blood group	Haemagglutinating acitivity			
Albumins	A	–	–	–	+
Albumins	B	+	–	–	+
Albumins	O	+	–	–	+
Globulins	A	–	–	–	++
Globulins	B	++	++	++	+
Globulins	O	++	++	++	+

a. Mean of triplicate determination expressed on dry weight basis. b. Values of two independent experiments. – No clumping, pellet disperses easily. + Some clumping pellet disperses partially. ++ No dispersion of pellet. ± Denotes standard error.

acceptability and utilization have been limited due to the presence of relatively high concentrations of certain antinutritional factors (Nowacki1980). The antinutritional factors, like total free phenols and tannins were present in all the three germplasm seeds of *A. precatorius* and *C. obtusifolia* (Table 4), but their concentrations were lower as compared to those in other pulses consumed by tribals, such as *Acacia catechu* (Rajaram and Janardhanan 1991a) and *Vigna umbellata* (Rajaram and Janardhanan 1990).

The content of the non-protein amino acid, L-DOPA, was found to be lower, when compared with the pulses reported earlier from our laboratory (Janardhanan and Lakshmanan 1985; Rajaram and Janardhanan 1991b; Arulmozhi and Janardhanan 1992; Mary Josephine and Janardhanan 1992). The phytohaemagglutinating activity of albumins of all the samples investigated in the present study generally appeared to be weak against the erythrocytes of the human blood groups A,B and O. Nonetheless, the phytohaemagglutinating activity of globulins of all the germplasm of *A. precatorius* exhibited strong agglutination with the erythrocytes from the blood groups B and O, whereas the globulins of *C. obtusifolia* exhibited strong agglutination against the erythrocytes of human blood group A only.

It may concluded that both the tribal pulses, *A. precatorius* and *C. obtusifolia* are useful as good sources of proteins, lipids, energy, some essential amino acids, fatty acids and some minerals. All the antinutritional factors reported, except L-DOPA, are heat labile and hence the protein quality may be enhanced by wet or dry thermic treatments. In an earlier study, it has been demonstrated that the L-DOPA content can be significantly reduced by repeated soaking and boiling of the seeds in water (Jabadhas 1980). Therefore, it may be possible to reduce the activity of all the antinutritional substances in both the pulses.

Acknowledgement

The first author thanks the University Grants Commission, New Delhi for a FIP fellowship.

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Received 9 December 1994; revised 17 May 1995; accepted 12 July 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 470-476

Storage Properties of Whole Egg Powder Incorporated Biscuit

T.S. SATYANARAYANA RAO*[1], M.N. RAMANUJA, N. ASHOK AND H.S. VIBHAKAR

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

Protein-rich biscuits containing spray dried egg powder and flavours were packed in paper-aluminium (0.012 mm) foil polyethylene laminate pouches, biaxially oriented polypropylene and metallised polyester, for evaluating storage life at different temperatures. The changes in moisture, peroxide value, free fatty acids and TBA values during storage at different temperatures in different packaging materials were insignificant, as compared to those in freshly made biscuits. The biscuits with vanillin plus orange and vanillin plus pineapple flavours were found to be more stable at different temperatures, and were highly acceptable for a period of 6 months at 37°C, ambient temperature (19-26°C) and at 4°C, as compared to those with orange flavour alone. Among packaging materials used, paper-aluminium foil polyethylene laminate pouch packed samples were more stable and acceptable, when compared to other packaging materials like metallised polyester and biaxially oriented polypropylene. The combinations of the above flavours were found to efficiently mask the egg flavour in the biscuits.

Keywords : Protein-rich biscuits, Whole egg powder, Packaging materials, Flavours, Storage temperature, Acceptability.

Extensive studies have been carried on the enrichment of cereal-based foods by plant proteins (Chandrashekara et al. 1962; Kwon et al. 1976; Rajput et al. 1988; Diwan et al. 1982; Prabhakaran and Srinivasan 1971; Haridasa Rao and Surpalekar 1976) and animal proteins (Chari and Srinivasan 1980; Sen et al. 1969). However, protein enrichment of wheat-based snack foods, such as biscuits and crackers, has not received much attention. In recent years, the consumption of biscuits has increased in most of the countries, as these serve as important sources of nutrients (Ranhotra et al. 1980). Biscuit is also one of the versatile snack food items used by Armed Forces and is equally liked by children and adults.

Protein calorie malnutrition is widely prevalent amongst the lower income population in India. Conventional biscuits contain low levels of the proteins of poor quality (Rajor et al. 1989). Townsend and Buchanan (1967) have developed a high protein milk biscuit, which had not only a long shelf-life, but also facilitated easy transport, storage and distribution, thereby leading to its use as an emergency food at the time of natural disasters. Egg proteins possess all the desirable nutritional and functional properties (Satyanarayana Rao et al. 1987), and therefore, is used in the form of a variety of food products, such as omelette, egg salad, scrambled egg and boiled egg (Satyanarayana Rao et al. 1995). Armed Forces are entitled to two eggs/ person/ day, and transportation of fresh eggs to remote and far-off places is not logistically feasible (Satyanarayana Rao and Sharma 1987), and hence the Armed Forces have been provided with convenient products such as egg powder, omelette mix (Jayaraman et al. 1976), and scrambled egg mix (Satyanarayana Rao and Sharma 1987), in lieu of fresh egg. These mixes require further preparation before consumption, and thus add on the cost of the final product. In order to overcome these limitations, egg in the form of egg powder can be incorporated into biscuits for use as a snack food.

The present paper reports results of incorporation of whole egg powder for obtaining protein-rich biscuits and evaluation of different packaging materials for achieving longer shelf life. Results of studies on the incorporation of food flavours in such biscuits to mask the egg flavour and, in turn, improve the organoleptic qualities of the biscuits are also reported.

Materials and Methods

Materials : Refined wheat flour (*maida*) was obtained from International School of Milling Technology, Central Food Technological Research Institute, Mysore. Commercially available hydrogenated fat (*vanaspati*), cane sugar and spray-dried milk powder were procured from local market. Whole egg powder was prepared, after desugaring of the egg melange from an initial level of 400 mg to 20 mg % sugar by using baker's wet yeast at 600 mg % (Satyanarayana Rao and Murali 1985). The desugared melange was pasteurized at 64°C for 3 min, chilled to 4°C and dried in a spray drier (Model Zahn-Ravo-Rapid, Universal Laboratory, Germany) (Satyanarayana Rao et al. 1987). Double acting baking powder, ammonium bicarbonate,

* *Corresponding Author 'Present address: 59/D5, ARKA, 2nd Main, Yadavagiri, Mysore-570 020, India*

sodium bicarbonate, vanillin and orange as well as pineapple flavours were of standard quality. Paper-aluminium (0.012 mm) foil-polyethylene (PFP) laminate, biaxially oriented polypropylene (BOPP) (35μ) and metallised polyester (MP) (12μ) were from reputed manufacturers.

Preparation of biscuit : Recipe (100 g based) used for the preparation of protein-rich biscuit included *maida* 50.5, powdered sugar 24.2, hydrogenated fat 12.1, milk powder 1.8, baking powder 0.18 ammonium bicarbonate 0.6, sodium bicarbonate 0.3, whole egg powder 10.0, vanillin 0.03, and pineapple or orange flavour 0.03. Distilled water used was 12-16 ml depending on moisture levels of other ingredients.

Crystal sugar was pulverized in a hammer mill (Kamas, Salling, Switzerland) to pass through BS 100 mesh sieve, and stored in a moisture-proof container. Before use, hydrogenated fat and flavours were creamed in a mixer (Hobart, Model No. 50, U.K.) for 2-3 min. Egg powder, milk powder and baking powder were added to the *maida* and blended well. Sodium bicarbonate and ammonium bicarbonate were dissolved in water, which was used for dough making. The whole contents were mixed in the same mixer for 2-3 min. The dough was sheeted on a specially made aluminium platform, using a wooden rolling pin to obtain a uniform thickness of 3 mm. The sheet was cut into circular pieces of 5.0 cm diam with round cutter, and 40 pieces were equally distributed on an aluminium tray of 40 x 16 cm size. The trays were loaded in baking oven (APV Ltd. Baker Parkins, Australia), with a rotating base, for baking at 205°C for 7-8 min.

Biscuits, so obtained, were cooled to room temperature (19-26°C), packed (10 biscuits/pack) in PFP, BOPP as well as MP pouches, and stored in air tight containers until used. Moisture, total fat, total proteins (N x 6.75), free fatty acids, peroxide value, total ash, acid insoluble ash and crude fibre were determined as per AOAC (1970) methods. Thiobarbituric acid value was determined as per the method of Kwon and Norgaard (1966), and expressed as mg of malonaldehyde/kg sample. Total and reducing sugars were determined by the methods described by Ranganna (1986). Colour, texture, appearance, taste and the overall acceptability of the product were evaluated by a taste panel (8 judges), and expressed as scores on a 9 point Hedonic scale (Satyanarayana Rao 1979).

Moisture sorption isotherm characteristics were determined at ambient temperature (19-26°C) by exposing weighed quantities of the samples in the glass petri dishes to relative humidity, ranging from 0 to 97%, by using appropriate saturated salt solutions (Rockland 1960). Samples were periodically weighed, till they attained a constant weight or showed signs of fungal growth.

The packed samples were stored at 37°C, ambient temperature (19-26°C), and control (4°C) for 6 months. These were analyzed initially and at an interval of 2 months for changes in physico-chemical and organoleptic properties.

Results and Discussion

Standardization of composition : The chemical composition of protein-rich biscuits e.g., 10% spray dried whole egg powder, 3.50% moisture, 0.85% total ash and 0.09% acid insoluble ash were within the limits prescribed for protein-rich biscuits by BIS (1974). However, the total fat (19.5%), sugar and proteins (19.5%) were at higher levels, as compared to other biscuits, due to use of higher levels of egg powder used in the recipe. The crude fibre and carbohydrates were 0.13 and 56.6%, respectively. The biscuits gave a slightly bitter taste, attributed to egg powder, if the sugar concentration is not higher. Due to the increased fat, proteins and carbohydrates, the total calorific value of the biscuits increased to 480 K cal. The biscuits had 5.8-6.0 cm average diam, 1.0-1.5 cm thickness and 9.5-10.0 g weight.

Moisture isotherm characteristics of protein-

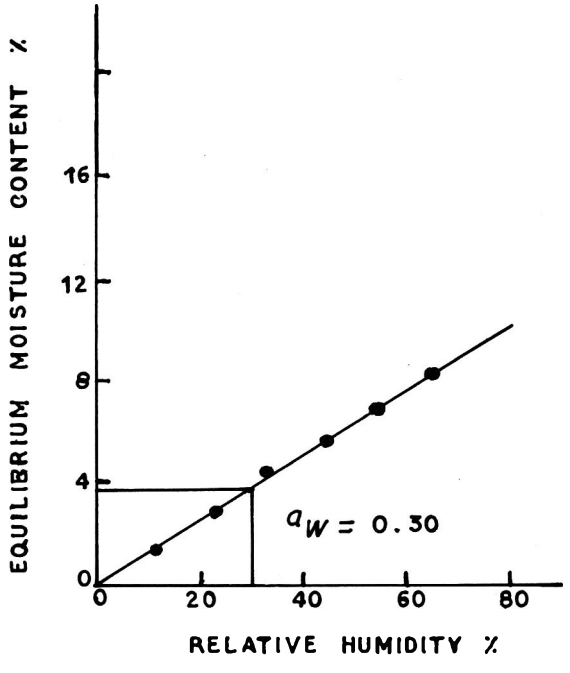

Fig. 1. Sorption isotherm for egg powder based protein biscuit

rich biscuits are presented in Fig. 1. The protein-rich biscuits had 3.27% initial moisture content, which equilibrated at 30% RH. It is evident that the biscuits pick up moisture rapidly above 30% RH, thereby indicating that the product is highly hygroscopic, and requires immediate packaging.

Table 1 gives the changes in moisture content of differently flavoured protein-rich biscuits, when packed in different packaging materials and stored at various temperatures. It is seen that biscuits had different initial moisture contents, ranging from 3.27 to 3.70%, except for 2.15% in one of the batches, mainly due to the batch-wise variation, and also the different sources of *maida* used for the preparation of biscuits. The biscuits packed in MP or BOPP had higher moisture contents, than those packed in PFP at the end of 6 months storage at 37°C. A slight increase in moisture was noted at ambient temperature, and at 4°C in case of flavoured biscuits. This shows that MP and BOPP are not good barriers to moisture, thereby rendering unsuitable for storing biscuits for more than 4 months. PFP was found to be a better barrier for moisture and efficient in improving the shelf life of biscuits to 6 months at all temperatures. Moreover, the moisture content has not exceeded more than 6%, the limit prescribed by BIS (1974), at any time during the entire storage period.

Changes in peroxide values of biscuits, packed in different packaging materials, and stored at different temperatures are also presented in the same Table. The peroxide values increased in samples packed in BOPP and MP after 4 months storage at 37°C. A further increase was noticed in the samples packed in PFP, MP and BOPP in all the flavoured biscuits. The formation of peroxide at ambient temperature was not higher in case of all the packaging materials, probably due to moderate storage temperature, and presence of antioxidants like lecithin in the egg powder (Satyanarayana Rao 1979). A similar trend was also observed in differently flavoured biscuits, which were packed in different packaging materials and stored at 4°C.

Free fatty acid contents of differently flavoured biscuits, which were packed in different packaging materials, and stored at different temperatures are also presented in Table 1. The free fatty acid contents were found to increase in orange-flavoured biscuits at 37°C, when packed in MP, PFP and BOPP. The samples stored at ambient temperature in different packaging materials recorded a lower free fatty acid value at the end of 6 months storage period, with the use of all the flavouring compounds and their combinations. The formation of free fatty acids is, thus, independent of different packaging materials and storage temperatures.

The effect of packaging materials on the changes in thiobarbituric acid values are also given in Table 1. The formation of thiobarbituric acid was highest in BOPP-packed material at the end of 6 months storage at 37°C, but the level was less than the values reported earlier (Thakur and Arya 1990; Satyanarayana Rao et al. 1990 a, b; 1991) at ambient temperatures. In contrast, formation of thiobarbituric acid was less in all other packaging materials and control (4°C) samples. The MP and BOPP packaging materials recorded higher values at 37°C at the end 4 and 6 months storage. The samples stored in PFP showed the least increase at all the storage periods. These values were also lower in biscuits stored at ambient temperature and 4°C (control) during the entire storage period, and with the use of all flavouring compounds. Such increase was also reported by Thakur and Arya (1990). The extents of changes were almost similar in all packaging materials and also during the entire storage period. This shows that paper-aluminium-foil polyethylene laminate pouch has got lower oxygen permeability, when compared to commercially used MP or BOPP. Though the PFP packaging material is slightly costlier than the other two packaging materials, it may prove cost-competitive, when considered in terms of longer keeping quality of the biscuits.

The changes in organoleptic quality of flavoured protein-rich biscuits, when packed in different packaging materials, and stored at different temperatures, are shown in Table 2. The initial overall acceptability score on a 9 point Hedonic point scale was about 7.4 ± 0.7

The colour, aroma, taste and texture are the important characteristics for acceptability, and also these are good indicators for the adverse physico-chemical changes during storage (Satyanarayana Rao 1979). It was observed that the PFP - packed samples retained nearly the same score during the entire storage at 37°C. Control samples (4°C) showed better results than the others at two temperatures studied. The samples stored in MP and BOPP showed lower acceptability during storage at 37°C at the end of 6 months, with respect to colour, texture and overall acceptability, though the levels were within the acceptable limit of 6 (Fryd

TABLE 1. CHANGES IN MOISTURE, PEROXIDE VALUE, FREE FATTY ACIDS AND TBA CONTENT OF EGG POWDER BASED PROTEIN RICH BISCUITS CONTAINING DIFFERENT FLAVOURS AND STORED IN DIFFERENT PACKAGING MATERIALS AND AT DIFFERENT TEMPERATURES

Flavour/s	Initial moisture, g/100 g	Packaging materials	37°C			Ambient temp,. 19°-26°C			Control 4°C		
			2 m	4 m	6 m	2 m	4 m	6 m	2 m	4 m	6 m
					Moisture						
Orange	3.27	PFP	3.20	3.86	4.19	3.64	3.87	4.04	3.18	3.76	3.87
		MP	3.61	4.56	5.80	4.54	4.64	4.92	3.48	3.80	3.90
		BOPP	4.15	5.06	6.67	4.90	5.64	5.93	3.56	3.94	4.06
Orange	3.62	PFP	3.66	4.02	4.55	4.11	4.31	4.46	3.20	4.04	4.11
+		MP	4.07	4.74	5.49	4.50	5.10	5.57	3.84	4.07	4.15
Vanillin		BOPP	4.11	5.72	6.99	4.78	5.13	5.66	3.68	4.22	4.35
Vanillin	2.15	PFP	2.33	4.29	3.79	2.34	3.63	5.17	2.29	2.02	2.74
+		MP	4.07	4.57	5.36	3.45	4.63	4.99	2.41	3.19	3.29
Pneapple		BOPP	3.68	4.89	5.69	4.94	5.25	5.52	3.42	3.92	4.13
					Peroxide Value						
Orange	0.75	PFP	2.07	3.15	5.37	1.61	2.31	3.27	1.27	1.42	1.51
		MP	2.36	4.70	6.37	2.11	2.70	3.68	1.01	1.57	1.88
		BOPP	2.04	4.24	6.68	1.77	2.60	3.79	1.22	1.45	1.53
Orange	0.79	PFP	2.16	3.28	5.26	1.72	2.18	3.28	1.40	1.89	2.06
+		MP	2.28	4.52	6.87	1.90	2.42	3.44	1.03	1.39	1.60
Vanillin		BOPP	2.17	4.17	6.72	2.15	2.94	3.62	1.19	1.50	1.95
Vanillin	0.77	PFP	3.14	6.94	7.79	2.02	4.71	5.21	1.74	1.94	2.14
+		MP	2.08	4.45	6.71	1.80	2.44	3.30	0.98	1.13	1.43
Pneapple		BOPP	1.96	4.66	6.61	1.76	2.85	3.97	1.25	1.39	1.39
					Free fatty Acids						
Orange	4.04	PFP	0.11	0.14	0.14	0.06	0.08	0.11	0.05	0.07	0.09
		MP	0.07	0.13	0.15	0.06	0.08	0.10	0.08	0.08	0.10
		BOPP	0.07	0.12	0.14	0.06	0.06	0.08	0.07	0.09	0.10
Orange	0.04	PFP	0.12	0.16	0.16	0.07	0.09	0.11	0.06	0.08	0.10
+		MP	0.07	0.15	0.19	0.07	0.09	0.11	0.09	0.09	0.11
Panillin		BOPP	0.09	0.13	0.15	0.07	0.09	0.13	0.07	0.10	0.12
Vanillin	0.04	PFP	0.05	0.08	0.09	0.06	0.07	0.07	0.07	0.08	0.08
+	0.05	MP	0.07	0.17	0.17	0.04	0.10	0.15	0.05	0.07	0.10
Pineapple		BOPP	0.05	0.18	0.17	0.06	0.07	0.15	0.03	0.05	0.07
					TBA						
Orange	0.13	PFP	0.22	0.27	0.33	0.15	0.17	0.19	0.13	0.14	0.15
		MP	0.25	0.32	0.39	0.16	0.18	0.20	0.14	0.16	0.17
		BOPP	0.29	0.35	0.45	0.17	0.20	0.21	0.15	0.17	0.20
Orange	0.15	PFP	0.22	0.27	0.29	0.16	0.18	0.19	0.15	0.16	0.17
+		MP	0.27	0.32	0.41	0.18	0.19	0.21	0.17	0.18	0.18
Vanillin		BOPP	0.31	0.36	0.48	0.17	0.22	0.26	0.18	0.20	0.20
Vanillin	0.16	PFP	0.23	0.26	0.28	0.21	0.23	0.24	0.16	0.18	0.19
+	0.17	MP	0.29	0.32	0.38	0.19	0.21	0.22	0.17	0.18	0.19
Pineapple		BOPP	0.30	0.36	0.44	0.20	0.23	0.29	0.18	0.19	0.20

PFP : Paper aluminium foil (0.012 mm) polyethylene laminate pouch. MP : Metallised polyester (12 μ); MP : ; BOPP : Biaxially oriented polypropylene (35 μ); m : Month.

TABLE 2. CHANGES IN ORGANOLEPTIC QUALITY OF PROTEIN RICH BISCUITS PREPARED WITH DIFFERENT FLAVOURS, PACKED IN DIFFERENT PACKAGING MATERIALS AND STORED AT DIFFERENT TEMPERATURES

Packaging material	Organoleptic quality	37°C			Ambient temp., 19°-26°C			Control 4° C		
		2 m	4 m	6 m	2 m	4 m	6 m	2 m	4 m	6 m
					Vanillin + Pineapple					
	Colour	7.1 ± 1.4	7.3± 0.7	7.4± 1.1	7.4± 0.5	7.3 ± 1.1	7.6± 0.5	7.5± 0.5	7.6 ± 0.8	7.4 ± 0.9
	Aroma	7.2 ± 1.4	7.2± 1.0	7.6± 0.9	7.5± 1.0	7.6 ± 0.7	7.7± 0.7	7.7± 0.8	8.1 ± 0.8	7.5 ± 1.4
PFP	Taste	7.2 ± 1.4	7.1± 1.1	7.4± 1.2	7.2± 1.4	7.6 ± 0.7	7.7± 0.7	7.5± 0.5	8.1 ± 0.8	7.3 ± 1.4
	Texture	7.3 ± 0.7	7.0± 1.3	7.4± 0.9	7.0± 1.2	7.5 ± 0.9	7.5± 0.7	7.5± 0.5	8.0 ± 0.9	7.4 ± 0.9
	OAA	7.2 ± 1.0	7.3± 1.1	7.5± 1.0	7.2± 1.1	7.5 ± 0.8	7.8± 0.6	7.5± 0.8	8.1 ± 0.8	7.6 ± 1.1
	Colour	7.0 ± 0.8	7.1± 0.8	6.5± 1.1	6.9± 0.9	7.1 ± 0.3	7.2± 0.4	7.8± 0.7	7.0 ± 0.7	7.1 ± 0.8
	Aroma	7.2 ± 0.6	7.2± 1.0	7.2± 0.9	7.6± 0.7	7.4 ± 0.5	7.4± 0.7	7.5± 0.8	7.3 ± 0.7	7.8 ± 0.5
MP	Taste	7.0 ± 0.8	7.2± 0.7	7.0± 0.8	7.0± 1.1	7.2 ± 0.8	6.9± 0.8	7.4± 0.7	7.6 ± 0.5	7.6 ± 0.5
(35 μ)	Texture	7.0 ± 1.1	7.0± 1.1	6.4± 1.0	7.0± 0.8	6.6 ± 1.0	6.8± 1.0	7.1± 1.4	7.5 ± 0.5	7.0 ± 0.9
	OAA	7.0 ± 0.7	7.3± 0.9	6.8± 1.0	7.0± 1.1	7.3 ± 0.8	7.1± 0.8	7.4± 0.9	7.5 ± 0.5	7.5 ± 0.5
	Colour	7.1 ± 0.6	7.0± 0.7	7.2± 1.0	7.1± 0.6	7.4 ± 0.5	7.3± 0.7	6.9± 1.1	7.0 ± 0.7	7.1 ± 0.8
	Aroma	7.3 ± 0.7	7.6± 0.5	7.1±0.9	7.3± 0.7	7.6 ± 0.5	7.5± 1.1	7.5± 0.5	6.9 ± 0.7	7.6 ± 0.5
BOPP	Taste	7.6 ± 0.7	7.3± 0.5	7.0± 0.8	7.3± 0.7	7.2 ± 0.9	7.5± 0.9	7.3± 0.7	7.2 ± 0.6	7.5 ± 0.5
(12 μ)	Texture	7.3 ± 0.5	7.1± 0.6	6.7± 0.7	6.8± 0.9	6.8 ± 0.8	6.8± 1.1	7.1± 0.7	7.1 ± 0.6	7.1 ± 0.6
	OAA	7.5 ± 0.9	7.5± 0.5	6.9± 0.7	7.1± 0.9	7.2 ± 0.9	7.2± 0.8	7.1± 0.9	7.1 ± 0.9	7.6 ± 0.7
					Orange					
	Colour	7.7 ± 0.5	7.4± 0.8	6.8± 0.9	7.0± 0.5	7.3 ± 0.5	7.2± 0.6	7.4± 0.5	7.0 ± 0.5	7.4 ± 0.5
	Aroma	7.6 ± 0.5	7.4—0.7	6.8± 0.9	7.6± 0.7	7.1 ± 0.6	7.1± 0.6	7.5—0.5	6.9 ± 0.7	7.3 ± 0.5
PFP	Taste	7.4 ± 0.7	7.2± 0.8	6.8± 0.8	7.4± 0.5	7.2 ± 0.6	7.4± 0.7	7.6± 0.5	7.2 ± 0.5	7.4 ± 0.5
(0.012 mm)	Texture	7.6 ± 0.5	7.3± 1.0	6.8± 1.0	7.1± 0.7	7.1 ± 0.7	7.2± 0.6	7.6± 0.5	6.9 ± 0.3	7.5 ± 0.5
	OAA	7.5 ± 0.7	7.2± 0.6	6.8± 0.8	7.4± 0.7	7.2 ± 0.6	7.2± 0.8	7.6± 0.5	7.0 ± 0.7	7.3 ± 0.5
	Colour	7.1 ± 0.9	7.1± 0.6	7.2± 0.6	7.0± 0.9	7.2 ± 0.4	7.0± 0.7	7.5± 0.5	7.2 ± 0.4	6.8 ± 1.0
	Aroma	7.0 ± 0.9	6.9± 1.1	7.0± 0.7	7.1± 1.0	7.0 ± 0.7	6.9± 0.8	7.1± 0.8	7.1 ± 0.7	6.8 ± 0.7
MP	Taste	7.1 ± 0.7	6.8± 1.2	7.1± 0.7	7.3± 1.0	7.0 ± 0.7	6.8± 0.7	7.1± 0.8	7.1 ± 0.6	6.6 ± 1.1
(35 μ)	Texture	7.1 ± 0.7	7.2± 1.0	6.5± 0.9	7.0± 1.1	6.6 ± 0.8	6.4± 0.7	7.4± 0.7	7.2 ± 0.6	7.0 ± 0.8
	OAA	7.0 ± 0.9	6.8± 1.0	7.0± 0.8	7.4± 1.0	7.1 ± 0.7	7.0± 0.9	7.3± 0.7	7.2 ± 0.6	6.8 ± 1.2
	Colour	7.1 ± 0.4	7.2± 0.4	7.3± 1.1	7.2± 0.8	7.2 ± 0.6	7.2± 1.0	7.6± 0.5	7.2 ± 0.8	7.1 ± 0.6
	Aroma	6.6 ± 0.9	7.6± 0.7	7.0± 0.9	6.9± 0.7	6.8 ± 0.9	7.1± 0.2	6.7± 1.2	7.3 ± 0.8	7.0 ± 0.5
BOPP	Taste	6.8 ± 0.7	7.2± 0.6	6.8± 0.8	7.0± 0.7	6.7 ± 1.0	7.1± 0.9	7.6± 0.7	7.1 ± 0.7	7.0 ± 0.5
(12 μ)	Texture	7.1 ± 0.6	7.1± 0.6	6.6± 0.8	6.9± 0.7	6.8 ± 0.8	6.7± 1.1	7.4± 0.7	7.2 ± 0.6	7.0 ± 0.5
	OAA	7.0 ± 0.8	7.4± 0.7	6.8± 0.8	6.8± 0.6	7.0 ± 0.8	6.8± 0.8	7.3± 0.7	7.3 ± 0.7	7.0 ± 0.8
					Orange + Vanillin					
	Colour	7.2 ± 1.0	7.4± 0.8	7.4± 0.5	7.5± 0.5	7.1 ± 0.6	7.6± 0.5	6.8± 0.9	7.4 ± 0.5	7.4 ± 0.5
	Aroma	7.1 ± 0.9	7.4± 0.8	6.9± 0.7	7.4± 0.5	7.3 ± 0.5	7.2± 0.8	7.3± 0.9	7.1 ± 0.9	7.5 ± 0.5
PFP	Taste	6.9 ± 1.2	7.4± 0.8	7.2± 0.6	7.5± 0.5	7.4 ± 0.7	7.6± 0.5	7.1± 0.6	7.2 ± 0.8	7.4 ± 0.5
(0.012 mm)	Texture	7.3 ± 0.5	7.4± 0.8	6.8± 0.9	7.3± 0.8	7.0 ± 0.7	7.0± 0.7	7.5± 0.8	7.0 ± 0.5	7.5 ± 0.5
	OAA	7.0 ± 1.2	7.4± 0.7	7.1± 0.6	7.5± 0.5	7.5 ± 0.5	7.3± 0.7	7.0± 0.5	7.2 ± 0.8	7.6 ± 0.5
	Colour	7.3 ± 1.0	7.3± 0.5	7.4± 0.7	7.3± 0.7	7.5 ± 0.5	7.3± 0.5	7.3± 1.2	7.1 ± 1.2	7.1 ± 0.6
	Aroma	7.2 ± 0.8	7.3± 0.9	6.9± 0.7	7.0± 0.7	7.1 ± 0.7	6.8± 1.0	7.4± 0.7	7.0 ± 0.7	6.9 ± 0.4
MP	Taste	7.4 ± 0.7	7.0± 1.0	6.9± 0.6	7.2± 0.8	7.0 ± 0.5	6.4± 0.7	7.3± 0.9	6.9 ± 0.6	7.0 ± 0.5
(35 μ)	Texture	7.2 ± 0.6	7.0± 1.0	6.9± 0.6	7.2± 0.8	7.0 ± 0.5	6.4± 0.7	7.3± 0.9	6.9 ± 0.6	7.0 ± 0.5
	OAA	7.3 ± 0.7	7.1± 1.1	6.9± 0.6	7.1± 0.6	7.2 ± 0.6	6.6± 0.9	7.3± 0.7	7.1 ± 0.6	6.8 ± 0.7
	Colour	7.3 ± 0.5	7.1± 0.3	7.5± 0.5	7.2± 0.6	7.4 ± 0.8	7.2± 0.8	7.3± 0.7	6.8 ± 0.8	7.1 ± 0.6
	Aroma	7.1 ± 0.4	7.0± 0.5	7.2± 0.8	6.8± 0.4	7.2 ± 0.8	7.0± 0.9	7.2± 1.0	6.8 ± 0.8	7.0 ± 0.5
BOPP	Taste	7.4 ± 0.5	7.0± 0.5	6.8± 0.8	7.1± 0.7	6.9 ± 0.9	7.0± 1.1	7.4± 0.8	6.9 ± 0.9	7.1 ± 0.6
(12 μ)	Texture	7.1 ± 0.6	7.1± 0.3	6.7± 0.7	6.9± 0.6	6.8 ± 1.0	6.7± 1.1	7.2± 0.6	7.1 ± 0.6	6.6 ± 0.7
	OAA	7.4 ± 0.5	7.0± 0.5	6.8± 0.8	7.1± 0.6	7.1 ± 0.6	6.8± 0.6	6.9± 0.6	6.8 ± 0.9	7.0 ± 0.8

Initial score 7.4±0.7; OAA - Overall acceptability

and Hanson 1944). However, these samples did not show any off flavour or taste during storage. It is interesting to note that the biscuits containing vanillin + pineapple flavour were highly acceptable, during six months storage at both ambient and control temperatures. The PFP - packed samples retained highest colour, aroma, taste and texture, thereby indicating that the biscuits packed in PFP were highly acceptable.

Table 2 also indicates the changes in colour, aroma, taste, texture and overall acceptability of protein-rich biscuits, with orange flavour, and packed in different packaging materials as well as stored at different temperatures. The samples packed in PFP packaging material had a lower score at the end of 6 months storage with respect to colour, aroma, taste, texture and overall acceptability, as compared to the biscuits containing vanillin + pineapple. The data indicate that the use of only one flavour does not help to mask the egg flavour entirely. The retention of colour, aroma, texture, taste and overall acceptability in the product with respect to packaging materials are in the order of PFP, MP and BOPP, both at ambient temperature and 4°C. However, the panel judges could not identify any egg flavour in any of these biscuits during the course of study.

To mask the egg flavour and also to have a more acceptable product, another combination of flavour, i.e., orange + vanillin was incorporated in the biscuits, and storage studies were performed. This combination of flavours gave almost the same results as those of vanillin+pineapple (Table 2), when packed in PFP and stored at 37°C for 6 months. In case of samples packed in MP and BOPP, the acceptability characteristics were slightly lower, but no adverse comments were made by the judges. The variations observed may be due to varied responses by the judges. The samples packed in PFP and stored at ambient temperature and 4°C were well within the acceptable range, with respect to their colour, aroma, taste, texture and overall acceptability up to a period of 6 months storage. The samples packed in BOPP and MP showed a lesser degree of acceptance at the end of 6 months storage at ambient temperature. There were not much changes in all the samples packed in PFP, MP and BOPP at 4°C for a period of 6 months.

Acknowledgement

The authors thank Dr. (Mrs.) R. Sankaran, Director, and Dr. K.S. Jayaraman, Additional Director, for valuable suggestions.

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Received 12 February 1994; revised 30 August 1995; accepted 9 September 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 477-481

Comparison of the Effect of Gamma Irradiation, Heat-radiation Combination, and Sulphur Dioxide Generating Pads on Decay and Quality of Grapes

PAUL THOMAS*, BRIJ BHUSHAN AND M.R. JOSHI

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

Effect of gamma irradiation, heat-radiation combination and in-package sulphur dioxide fumigation on fungal decay, and quality of seedless grape cultivars, 'Thompson', 'Sonaka' and 'Tas-A-Ganesh' was evaluated under different storage regimes. Irradiation at 2 kGy or a combination of hot water dip (50°C, 5 min), plus irradiation (1 kGy) showed less spoilage due to *Rhizopus* spp. and *Botrytis* spp. in grapes packaged in tissue paper lined boxes and stored at 4°, 15° and 25°C. Storage in polyethylene lined boxes increased the fungal rot. In-package sulphur dioxide generating pad was most effective for control of decay in polyethylene lined boxes stored at 10° and 20°C, but caused berry bleaching. Irradiation at 2.5 or 3.5 kGy controlled decay at 10°C, but not so effectively at 20°C. Organoleptic quality, berry firmness, and soluble solids were not affected by irradiation, but decreases in titratable acids and ascorbic acid were recorded. Packaging in polyethylene lined boxes retained berry turgidity, while slight shrivelling occurred in tissue paper lined boxes. The results indicate that gamma irradiation has potential as an alternative to sulphur dioxide fumigation for decay control during shipping and storage.

Keywords : Gamma irradiation, Sulphur dioxide fumigation, Hot water dip, Seedless grapes, Packaging, Fungal rot, Storage regimes.

Production and export of Indian table grapes have rapidly expanded in recent years (Muchrikar 1992; Chadha 1992; Anon 1993). India's export of fresh grapes was 4738 MT in 1988-89, which increased to 11150 MT in 1992-93, resulting in an increase of export earnings from 6.4 to 21 crore rupees (Anon 1993).

Grey mould, caused by *Botyrtis cinerea*, is the most important rot, causing losses in the post-harvest storage and marketing of grapes, whereas under the tropical ambient conditions, considerable losses also occur due to rots caused by *Rhizopus*, spp., *Penicillium* spp. and *Aspergillus* spp. (Thomas 1986). Fast cooling of the produce and in package sulphur dioxide generating pads (Ladania and Dhillon 1989; Sandhu and Randhawa 1992) are used for control of fungal rot, particularly in export marketing, when grapes are to be ocean-transported for extended periods (Arve 1992; Chadha 1992).

Recently, some countries, including the U.S.A. have expressed concern about sulphur dioxide residue in table grapes. In the U.S.A., the generally regarded as safe (GRAS) status of sulphating agents for use on fresh and frozen foods has been revoked (Anon 1986a), and a 10 μg/g tolerance for fumigated table grapes has been established (Anon 1986b). Also, the use of methyl bromide, as a fumigant for quarantine treatment against insect pests in fruits entering international trade, is to be phased out due to its listing as an ozone depleting agent (Marcotte 1993). The environmental protection agency (EPA) of the U.S.A. has also decided to ban methyl bromide by year 2000 (Marcotte 1993). Therefore, there is a need for developing non-chemical alternatives to control fungal rot and for quarantine purposes.

Results of a comparative study on the efficacy of sulphur dioxide generating pads and gamma irradiation, either alone or in combination with hot water dip, for the control of post-harvest fungal rot in table grapes are reported in the present paper.

Materials and Methods

Three seedless grapes cultivars, 'Thompson', 'Sonaka' and 'Tas-A-Ganesh' grown in Pune district, Maharashtra State and harvested at commercial maturity, were supplied by Maharashtra Grape Growers Association, Pune. Produce packed in 2 or 4 kg cardboard cartons were received in the laboratory within 24 to 36 h of harvest.

Irradiation : Grapes in 2 kg cartons, lined with polyethylene (150 gauge), were irradiated in the same packages without further handling. Grapes received in 4 kg cartons, lined with tissue paper, were repacked in 10"x10"x10" sized cartons that can fit into the aluminium boxes for irradiation. Following irradiation, they were transferred into the original cartons for storage. Irradiation was carried out in a cobalt-60 package irradiator (Atomic Energy of Canada Limited, Ottawa) at a dose rate

* Corresponding Author

of 0.05 kGy/min (Padwal-Desai et al. 1973). Dosimetry was performed by Fricke's dosimeter (Fricke and Hart 1966). The ratio of maximum to minimum dose received in the container box was 1.3.

Sulphur dioxide generating pads : Uvas quality grape guard (IMAL LTD A.Av. Bulnes 98, Santiago, Chile) was used for in-package sulphur dioxide fumigation. The two strips of grape guard, containing anhydrous sodium bisulphite as an active ingredient, were placed on top of each 2 kg box with polyethylene lining. The polyethylene liners were then folded on the top of the grapes and the boxes closed.

Hot water dip : Grape bunches were placed inside a large perforated stainless steel container, and immersed for 5 min in water maintained at 50±1°C in a jacketted open steam kettle. The bunches were drained and dipped in 30% aqueous ethanol, and air-dried overnight at 28°C. Aqueous ethanol dip was given to facilitate rapid and proper drying of the bunches (Brodrick 1982).

Texture measurements : Individual berry firmness was measured by two methods, namely compression test (non-destructive), and skin puncture test (destructive), using an Instron Universal testing machine, table model (Instron Engineering Corporation, 2500 Washington Street, Canton, Massachusetts, U.S.A.). The compression test measured the resistance force of a berry, when subjected to vertical compression for a constant distance (5 mm) by a metal disc fixed to the moving cross head (Bourne et al. 1966). The skin puncture test measured the force required to puncture the skin by a 7 mm diam probe fixed to the moving cross head (Bourne et al. 1966). The Instron was calibrated each day of the test, and identical cross head speed setting of 2 cm/min and chart speed of 5 cm/min were employed every time. Each of the tests was performed on 10 separate berries picked at random.

Decay evaluation : Infected and decayed berries were separated and sound berries weighed at each observation period and expressed as percentage of initial weight of the stored produce.

Organoleptic evaluation : A panel of 10 judges was employed. Scoring was done on a Hedonic scale of 1-9 (Jellinek 1985). A score of 5 and above was considered as acceptable quality.

Chemical analysis : Total soluble solids were determined using a hand refractometer (range 0-32° Brix, National Instrument Co., Inc. Baltimore, Maryland, U.S.A.). Titratable acidity was determined by titrating the aliquot to pH 8.1 with 0.1 N sodium hydroxide and expressed as percentage tartaric acid. Ascorbic acid was determined by visual titration, using 2,6-dichlorophenol indophenol dye (AOAC 1980).

Results and Discussion

Losses due to fungal rot, occurring under different storage regimes in 3 seedless grape cultivars subjected to gamma irradiation at a dose of 2 kGy and a combination of irradiation (1 kGy) plus hot water dip (50°C, 5 min), are shown in Table 1. Both the treatments were equally effective in reducing fungal rot during storage, particularly at 25° and 15°C. Although the differences between untreated and treated samples stored at 4°C were not as marked as at the higher storage temperatures, the percentages of sound berries after 67 days storage were higher in treated lots, whereas fungal rot at 15° and 25°C was mostly caused by *Rhizopus* spp., *Botrytis* rot predominated at 4°C (Thomas 1986). The efficacy of low dose irradiation in combination with hot water dip treatment on the control of fungal rot corroborates similar findings by other workers (Padwal-Desai et al. 1973; Brodrick 1982).

The changes in texture and other quality parameters in cv 'Thompson', subjected to irradiation and combination treatment are shown in Table 2. A reduction in the firmness of berries was discernible. Those subjected to the combination treatment showed comparatively lower firmness values, than those irradiated at 2 kGy. Differences in soluble solids and titratable acids were not apparent between different treatments. However, slightly lower contents of ascorbic acid were recorded in irradiated and combination-treated samples, as compared to control. Similar results were recorded in 'Sonaka' and 'Tas-A-Ganesh' (data not shown), though slight shrivelling of berries was noted in these two cultivars, particularly in combination-treated samples stored for extended periods at 4°C. This could be due to increased transpiration loss in boxes lined with tissue paper. Both irradiated and combination-treated berries were acceptable to a taste panel, and any changes attributable to the treatments were not detected (Table 1).

Since hot water immersion involved additional handling and drying operations, thus limiting its commercial potential, a second series of experiments were undertaken to evaluate the efficacy of irradiation at higher dose levels in comparison to the standard in-package sulphur dioxide fumigation,

TABLE 1. EFFECT OF GAMMA IRRADIATION AND COMBINATION OF HOT WATER DIP AND IRRADIATION ON DECAY AND QUALITY OF GRAPES UNDER DIFFERENT STORAGE REGIMES

Cultivar/ Treatment	Sound berries after storage, %						Organoleptic score •			
	25°C		15°C			4°C	Appearance	Odour	Taste	Texture
	Days		Days			Days				
	11	15	18	25	30	67				
'Thompson'										
Control	86	0	90	87	78	87	6.28 ± 1.89	6.57 ± 1.27	7.00 ± 1.15	6.28 ± 1.11
Heat + 1 kGy	94	85	98	87	84	91	7.00 ± 1.00	6.85 ± 1.21	7.00 ± 0.57	7.00 ± 1.00
2 kGy	91	50	98	94	87	88	6.70 ± 1.49	6.85 ± 0.89	7.30 ± 0.95	6.85 ± 1.06
'Sonaka'										
Control	65	0	85	80	75	82	7.20 ± 1.14	7.30 ± 0.94	7.50 ± 0.97	7.20 ± 0.91
Heat + 1 kGy	92	50	95	92	92	95	6.80 ± 1.22	6.90 ± 1.10	6.40 ± 0.96	6.60 ± 0.84
2 kGy	90	50	95	90	85	96	6.80 ± 1.03	7.10 ± 0.73	7.10 ± 0.73	7.10 ± 0.73
'Tas-A-Ganesh'										
Control	77	30	90	74	70	91	8.00 ± 0.81	7.80 ± 0.98	7.50 ± 0.79	7.60 ± 1.12
Heat + 1 kGy	95	25	98	90	90	96	7.70 ± 0.75	7.30 ± 0.98	7.50 ± 0.90	7.70 ± 0.91
2 kGy	92	50	95	91	85	94	7.61 ± 0.86	7.20 ± 0.83	7.30 ± 0.62	7.10 ± 0.77

Each value represents mean of 4 to 6 boxes, each containing 2 kg of grapes. Boxes were lined with tissue paper.

• Grapes stored at 4°C for 67 days were used.

using sulphur dioxide generating pads. Untreated grapes stored at 20°C showed complete spoilage after 13 days due to *Rhizopus* rot, which could be attributed to the high humidity, and moisture condensation inside the polyethylene lined cartons and consequent favouring of rapid growth of the fungus (Table 3). Irradiation, depending on the dose, reduced the rotting in 'Sonaka' but not in 'Tas-A-Ganesh', whereas sulphur dioxide fumigation effectively controlled the fungal rot under these conditions. At 10°C, losses were minimal in untreated grapes during the first month, but a rapid rise in fungal rot occurred during extended storage. Both sulphur dioxide generating pads and irradiation effectively controlled fungal rot during the first month. However, sulphur dioxide fumigation provided better decay control during extended storage.

These results indicated that the use of sulphur dioxide generating pads was more effective than gamma irradiation in controlling fungal rot in grapes stored in boxes with polyethylene liners during extended storage at 10° and 20°C (Table 3). However, more than 75% of the berries showed sulphur dioxide injury, as evidenced by bleaching of the pedicel-end half. This could be attributed

TABLE 2. PHYSICO-CHEMICAL CHANGES IN 'THOMPSON' SEEDLESS GRAPES DURING STORAGE

Treatment	Compression force, kg	Total soluble solids, %	Titratable acidity, % tartaric acid	Ascorbic acid, mg%
Initial	1.05 ± 0.17	20.5 ± 1.49	3.37 ± 0.13	3.56 ± 0.13
		After 13 days at 25°C		
Control	0.94 ± 0.24	20.4 ± 0.9	3.37 ± 0.13	2.90 ± 0.03
Heat + 1 kGy	0.86 ± 0.15	19.8 ± 1.9	3.30 ± 0.10	2.53 ± 0.01
2 kGy	0.95 ± 0.17	20.2 ± 1.1	3.39 ± 0.14	2.06 ± 0.06
		After 34 days at 15°C		
Control	1.04 ± 0.18	19.8 ± 1.1	2.66 ± 0.11	3.50 ± 0.07
Heat + 1 kGy	0.86 ± 0.16	19.6 ± 1.8	2.92 ± 0.15	1.41 ± 0.06
2 kGy	0.94 ± 0.10	18.2 ± 1.6	2.40 ± 0.06	2.07 ± 0.06
		After 60 days at 4°C		
Control	0.89 ± 0.14	20.6 ± 0.88	2.43 ± 0.06	1.02 ± 0.13
Heat + 1 kGy	1.04 ± 0.26	20.8 ± 1.32	2.44 ± 0.03	0.72 ± 0.08
2 kGy	1.22 ± 0.31	20.4 ± 1.33	2.51 ± 0.06	0.73 ± 0.03

Each value represents the mean of 3 replicates except compression force which is the mean of 10 replicates.

TABLE 3. EFFECT OF SULPHUR DIOXIDE GENERATING PAD AND GAMMA IRRADIATION ON DECAY OF GRAPES

Cultivar and treatment	Percentage of healthy berries after storage			
	20°C	10°C		
	13 days	14 days	33 days	48 days
'Sonaka'				
Control	0	98	97	80
Sulphur dioxide generating pad*	95	100	99	99
2.5 kGy	57	99	98	91
3.5 kGy	72	99	97	89
'Tas-A-Ganesh'				
Control	0	96	90	66
Sulphur dioxide generating pad*	94	98	97	92
2.5 kGy	0	98	93	61
3.5 kGy	20	99	94	55

Each value represents mean of percent healthy berries in 4 to 6 polyethylene lined boxes.

* Over 75% berries exhibited bleaching due to sulphur dioxide injury.

to the condensation of moisture in the polyethylene lined boxes and the formation of sulphurous acid (Jooste 1987). Bleaching of berries by sulphur dioxide has been reported, when sulphur dioxide generating pads were used in cardboard boxes lined with unvented polyethylene (Mustonen 1992). Polyethylene liners were necessary to prevent excessiv moisture loss during extended storage and to facilitate the release of sulphur dioxide (Morris et al. 1992).

The physico-chemical changes in grapes subjected to higher doses of gamma irradiation are given in Table 4. A reduction in berry firmness, as measured by skin puncture test, was recorded in irradiated samples, but the changes in compression force were minimal in comparison to control. It has been reported that compression test is a more precise indicator than skin puncture test for measuring textural changes in Bell peppers (Hampshire et al. 1987). Present data suggest that irradiation may not significantly affect the berries' capacity to withstand mechanical stress during handling and shipping. This also reflected in the taste panel assessment (Table 1), which indicated an inability to detect any changes in the textures of irradiated grapes. Total soluble solids and ascorbic acid levels did not show marked changes, though titratable acidity values were comparatively lower in irradiated samples. Textural and other quality parameters of sulphur dioxide fumigated samples were not determined, since berries exhibited unacceptable bleaching. However, the extension of the green colour of the rachis was better in sulphur dioxide fumigated samples, as compared to irradiated grapes. Practically, no shrivelling was noted in berries stored in the polyethylene lined boxes.

In the present studies, grapes were irradiated at ambient temperature (25-27°C), and then transferred to lower storage temperatures. This resulted in free water accumulation in the polyethylene lined boxes due to moisture condensation, which provided optimal conditions for growth and proliferation of surviving fungal populations. The effect of pre-cooling of grapes closer to the final holding temperature, and

TABLE 4. PHYSICO-CHEMICAL CHANGES IN 2.5 AND 3.5 kGy IRRADIATED GRAPES DURING STORAGE

Cultivar and storage regime	Treatment	Firmness value, kg		Total soluble solids, %	Titratable acidity, % tartaric acid	Ascorbic acid, mg %
		Skin penetration force	Compression force			
'Sonaka'	Control	-	1.99 ± 0.34	20.2 ± 0.96	0.54 ± 0.01	1.88± 0.09
1 day after	2.5 kGy	-	1.47 ± 0.23	20.3 ± 0.84	0.44 ± 0.01	2.13± 0.04
irradiation	3.5 kGy	-	1.24 ± 0.23	19.5 ± 1.20	0.45 ± 0.01	2.22± 0.04
After 56	Control	1.17± 0.14	1.59 ± 0.48	18.6 ± 1.41	0.51 ± 0.01	1.76± 0.11
days at	2.5 kGy	1.76± 0.08	1.40 ± 0.33	19.5 ± 1.10	0.45 ± 0.02	1.53± 0.03
10°C	3.5 kGy	0.77± 0.07	1.27 ± 0.27	19.7 ± 0.71	0.42 ± 0.01	1.52± 0.03
'Tas-A-Ganesh'	Control	-	1.50 ± 0.25	16.7 ± 0.75	0.50 ± 0.02	1.95± 0.14
1 day after	2.5 kGy	-	1.17 ± 0.25	16.5 ± 1.70	0.45 ± 0.01	1.82± 0.10
irradiation	3.5 kGy	-	1.10 ± 0.26	17.4 ± 2.07	0.42 ± 0.01	1.76± 0.14
After 56	Control	1.17± 0.10	1.06 ± 0.21	16.2 ± 2.58	0.51 ± 0.01	0.85± 0.02
days at	2.5 kGy	0.74± 0.09	1.29 ± 0.26	16.5 ± 1.58	0.41 ± 0.02	0.92± 0.05
10°C	3.5 kGy	0.75± 0.08	1.18 ± 0.27	17.9 ± 1.56	0.40 ± 0.01	0.94± 0.07

Firmness values represent mean of 10 independent determinations and values for chemical parameters are mean of 3 replicates.

irradiating at the same temperature needs to be studied for better control of fungal rot during extended refrigerated storage.

It is concluded that in-package sulphur dioxide generating pads provide the most effective and cheaper means of controlling fungal decay in grapes. However, considering the possibility of berry bleaching, which can adversely affect marketability, irradiation has potential as alternative to sulphur dioxide fumigation. With proper post-harvest management practices, its efficacy can be further improved. Moreover, irradiation can also replace methyl bromide fumigation as a quarantine treatment, as doses required for this purpose are below 1 kGy (Thomas 1986).

Acknowledgement

The authors thank the Maharashtra Grapes Growers Association, Pune, for *gratis* supply of grapes as well as Uvas grape guard pads and Shri G.S. Ghanekar for technical assistance.

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Received 29 August 1994; revised 2 September 1995; accepted 9 September 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 482-485

Langostilla (*Pleuroncodes planipes*), Stimpson: Mineral Content Under Different Preservation Procedures

M.I. CASTRO-GONZALEZ*[1] C.M.C. CALVO[1], L.R. SANCHEZARMAS[1], R.F. PEREZGIL[1] AND D. AURIOLES-GAMBOA[2]

[1] Department Nutricion Animal, Instituto Nacional de la Nutricion "Salvador Zubiran". Vasco de Quiroga 15 Tlalpan, 14000. Mexico DF.

[2] Departamento Recursos Marinos, Centro de Invetigaciones Biologicas de B.C.S. A.P. 128, La Paz, Baja California Sur, 23000, Mexico.

After freezing, pressing, boiling, boiling-pressing, sun-drying and oven-drying procedures, minerals such as zinc, copper, sodium, potassium, calcium, iron and manganese were quantified in red crab (*Pleuroncodes planipes*) meal. The results indicated that each treatment affected the content of the majority of the minerals studied, excepting phosphorus, copper, zinc and iron. Calcium and potassium were showing a major statistical significant variation. The oven-drying method preserved the quantities of most of the minerals, and it can be used in a wide range without affecting the mineral content, specially for use of this marine resource in animal feeding, and for ration formulation with a slight margin of error.

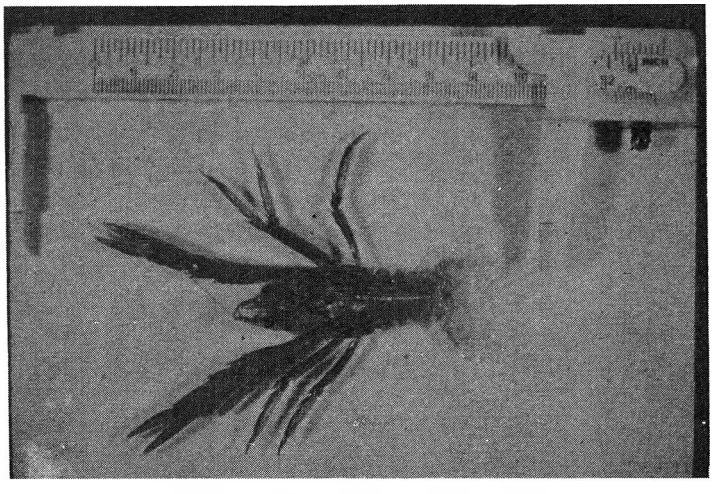
Keywords : *Pleuroncodes planipes*, Red crab, Langostilla, Minerals, Preservation procedures

Langostilla (*Pleuroncodes planipes*), a member of the crustacea decapoda known also as pelagic red crab, forms part of abundant (662 kg/ha) fisheries in the Gulf of California, and the Western coast of Baja California (Allsopp 1985; Blackburn 1969; Guzman and Aurioles 1991; Hendricks 1985). Pelagic red crab belongs to a group of small lobster-like crabs, which have abdomen and tail fins well adapted for swimming, while their long first pair of legs are armed with pincers (chelipeds) (Kato 1974). It has been qualified as a potentially important marine resource for industries such as food, dyes, pharmaceuticals, cosmetics, etc. (Castro 1993; Guzman and Aurioles 1991; Jimenez 1978; Karnicki 1981; Rocha et al. 1988; Spinelli 1974; Wilkie 1972). Given the great autolytic activity of this organism, simple alternative technologies could prolong its useful life without altering its chemical composition, as has been studied by Castro (1993) and Garcia-Carreno (1992).

Most of the scientific publications on chemical composition of red crab, reported high ash content (35%). Therefore, mineral content should serve to quantify the quality.

Langostilla was collected on board the R/V "EL PUMA", on the Western coast of South Baja California, Mexico, during September 1990. Samples were obtained from one catch, using a stern trawling equipment. The net used was the same as that for typical shrimp fishery collection. Catch was composed of adult 10 cm long red crabs (150 kg), males and females. Once the catch was discharged on board, langostilla was separated from the other faunistic species, weighed and mixed, getting 120 kg of red crab free of organic materials. Samples were randomly obtained, washed with sea water, drained, and divided into four lots (30 kg each).

Four preservation methods were employed on board, in an attempt to reduce bacterial activity, as well as the enzymic reaction time. In case of freezing the samples were kept in perfectly closed plastic bags, and placed at -20°C in a freezer room (Alvarez 1992; Brennan et al. 1979). In case of pressing, to reduce body water content, and volume for overland shipping, a manual stainless steel press was used, and the samples were frozen at -20C. Boiling inactivates enzymes or destroys enzyme-substrate complexes, and avoids decomposition reactions, and



Langostilla (lPeuroncodes *planipes*) stimpson.

* *Corresponding Author*

pathogens proliferation (Brennan et al. 1979; Guerrero 1992). For boiling treatment, a stainless steel container with sea water was heated to 90 C over a gas stove, and a metal strainer containing langostilla was submerged in it for 5 min. Water was drained, and red crab was placed in plastic bags for immediate freezing. The treatment involving boiling-pressing, combined the individual effects of both these operations, with great success.

Once the samples were unloaded, they were thawed at room temperature, and sun-dried for two days or oven-dried at 60 C for 24 h, to obtain product with 10% moisture content. Finally, the samples were ground, using a crushing, and grinding mill, to a particle size of 0.5 mm.

Mineral content was determined according to AOAC (1990) analytical procedures, and after wet digesting as per AOAC (1984). Final solutions were read by atomic absorption spectrophotometry (Perkin Elmer Model 2380). The instrument setting, and other experimental conditions were in accordance with the manufacturer's specifications. The results of each determination were subjected to an ANOVA with a random block design (Steel and Torrie, 1988). The Tukey test with a significance level of $P<0.05$ was employed for comparison among means (Steel and Torrie 1988).

Ash content varied statistically ($P<0.05$), ranging between 32.0 and 38.4%, with the oven-dried samples, showing a greater variation. The pressed samples gave the highest ash levels independent of the drying method. Ash in the boiled-pressed samples was lowest. The frozen-sun- dried, boiling-pressing-sun- dried, and boiling-pressing-oven-dried samples were very similar ($P<0.05$). Table 1 shows the results of the most important minerals in langostilla. Calcium content showed greatest variations ($P<0.05$), the values ranging from 0.97 to 1.70 g/100 g. Phosphorus contents varied between 1.15-1.33 g/100 g. Manganese values were between 0.012 and 0.024 g/100 g for frozen-sun-dried, and pressing-oven dried samples, respectively. Copper contents were almost equal in three of the sun-dried samples. Regardless of the drying method, boiled, and frozen samples showed no statistical difference in copper contents. Zinc values showed slight statistical variations. Nevertheless, all the oven-dried samples had greater numerical values of zinc with frozen-oven-dried having the highest values ($P<0.05$).

Sodium content ranged from 2.56 to 4.13 g/100 g, with no difference ($P<0.05$) amongfrozeng-oven-dried, frozen-sun-dried, pressing-sun dried, boiling-pressing-sun dried treatments, and the boiling-oven-dried, boiling-pressing-oven-dried, and boiling-sun-dried group. In the case of potassium, there were differences ($P<0.05$) in all the sun-dried samples, with values frozen from 0.65 to 1.40 g/100 with the oven dried samples not differing significantly. Magnesium contents were lower in frozen-sun-dried, and boiling-pressing-sun-dried samples, and no differences ($P<0.05$) were found in pressed or boiled samples with different drying techniques. Iron values had greatest numerical variations ($P<0.05$), with values between 0.05 and 1.36 g/100 g, except for frozen-oven-dried, and pressing-oven-dried samples, which did not show any significant difference ($P<0.05$) among them.

Biede et al (1980), reported that blue crab meat subjected to the key system process, which involves halving blanched crabs, squeezing meat from the shell, and cooking the extracted meat at 100°C in water or by steam, had lower contents of soluble minerals (sodium, potassium, calcium, copper, magnese and zinc) in comparison with hand-picked crab meat, probably because of the amount of water

TABLE 1. ASH AND MINERAL CONTENTS IN LANGOSTILLA *(Pleuroncodes planipes)* SUBJECTED TO EIGHT PRESERVATION PROCEDURES (g/100g dry basis)

Preservation procedures	ASH	Ca	P	Mn	Cu	Zn	Na	K	Mg	Fe
				Oven-drying						
Freezing	33.7^{a}	1.25^{a}	1.25^{ab}	0.019^{a}	0.034^{a}	0.185^{a}	3.22^{a}	1.17^{a}	1.51^{a}	1.09^{a}
Pressing	36.8^{b}	1.70^{b}	1.33^{a}	0.024^{b}	0.023^{b}	0.275^{b}	2.56^{d}	1.04^{b}	1.77^{b}	1.36^{a}
Boiling	35.7^{c}	1.08^{c}	1.27^{ab}	0.014^{cd}	0.045^{c}	0.062^{c}	4.21^{c}	1.11^{ab}	1.54^{a}	0.05^{b}
Freezing-pressing.	33.8^{a}	1.36^{d}	1.17^{b}	0.019^{a}	0.043^{c}	0.031^{c}	3.90^{bc}	1.10^{ab}	1.76^{b}	0.07^{b}
				Sun-drying						
Freezing	34.3^{d}	0.97^{e}	1.15^{b}	0.012^{d}	0.036^{a}	0.033^{c}	3.23^{a}	0.65^{c}	1.19^{c}	0.58^{ab}
Pressing	38.3^{e}	1.38^{d}	1.23^{ab}	0.019^{a}	0.034^{a}	0.029^{c}	3.54^{ab}	1.40^{d}	1.64^{ab}	0.08^{b}
Boiling	33.8^{a}	1.23^{af}	1.32^{a}	0.018^{ac}	0.045^{c}	0.027^{c}	4.13^{c}	1.18^{a}	1.66^{ab}	0.09^{b}
Freezing-pressing.	32.0^{f}	1.15^{cf}	1.26^{ab}	0.014^{cd}	0.029^{ab}	0.022^{c}	3.43^{a}	0.87^{e}	1.23^{c}	0.06^{b}

^{abcdef} Means with the same letter do not differ significantly at 0.05 level

used for the extraction. This study showed that most minerals in samples treated by freezing, pressing, boiling-pressing were higher in oven-dried samples than in the sun-dried ones, which might be due to the loss of minerals during sun-drying which took longer time to dry than oven-drying. In boiling samples, all minerals, excepting calcium, were not statistically different for both types of drying methods. Lopez et al., (1981) have reported that the boiled, steamed, and pasteurized crab meats are excellent sources of copper, phosphorus, sodium and zinc and good sources of calcium, iron, magnesium and potassium. Minerals in the intercellular liquid are present as sodium, potassium, calcium and phosphorus, forming insoluble compounds like carbonates and phosphates (calcium phosphate or pyrophosphate), which are integral parts of the exoskeleton (Dall and Moriarty 1983; Rainbow 1988). Minerals with less statistical difference among treatments were phophorus, zinc, iron and copper. King et al (1990) have reported that shelfish, dungeness crab (*Cancer magister),* and pink shrimps (*Pandalus borialis* and *P. jordani*) are good sources of zinc, manganese and iron. Sundarrao et al (1991) reported significant concentrations of copper and zinc in mud crabs (*Scylla serrata*) captured in Papua, New Guinea. Iron concentrations in crustaceans are extremely variable and high iron concentrations often have been associated with sediment particles in the gut or elsewhere in the body (Rainbow 1988). In spite of the fact, that there were statistical differences among treatments, it can be concluded that the preservation procedures studied can be used in a wide range without affecting the mineral content, specially when one wishes to use this marine resource in animal feeding, for ration formulation with a slight margin of error.

The authors express their gratitude to the CONACYT for partial financing, and to the Captain, and the crew of the R/V "EL PUMA" (which belongs to the UNAM) for help during the EP 9009 oceanographic campaign.

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Received 6 June 1994; revised 24 June 1995; accepted 6 September 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 486-488

Preparation of Acceptable Traditional Dehydrated Lentil Soup at Small Scale

KHALIL I. EREIFEJ

Faculty of Agriculture,
Jordan University of Science and Technology, P.O. Box 3030, Irbid, Jordan.

A process for the preparation of an acceptable, and nutritious traditional lentil soup cubes has been developed. The process consists of cleaning, dehulling, frying onion cuts with olive oil, addition of salt, lentil splits and water, cooking, moulding, and drying. Results showed that lentil cultivars ('Jor-1', 'Jor-2, 'Jor-3' and 'Balady') produced acceptable soup cubes. The variety 'Jor-1' was superior to commericial soup with respect to protein content and energy value.

Keywords : Lentil processing, Dehydrated soup cubes, Chemical composition, Minerals, Organoleptic evaluation, Acceptability.

Food legumes, such as lentil have made a significant contribution to human diets since ancient times (Bhatty 1988). Lentil provides more than double the amounts of dietary proteins than cereals can supply (Bhatty 1988). Lentil is grown on 2.5 million ha in the world and the Indian Sub-continent alone produces 38% of the world production (Bhatty 1988). Jordan produced 3389 metric tonnes on 7372.3 ha in 1992 (Anon 1993). Use of lentil in dehydrated form for preparation of lentil soup can offer a unique opportunity for its consumption, providing varieties, and value addition apart from protecting it from insect damage. Therefore, a process for the preparation of soup cubes has been developed from four lentil varieties, and their characteristics studied.

Whole, and sound seeds of 'Balady landrace', and three newly developed lentil cultivars ('Jor-1', 'Jor-2' and 'Jor-3') were used. The process flow chart for preparing traditional dehydrated lentil soup cubes is shown in Fig. 1. The product was stored in glass jars, until the time of analysis. A commercial lentil soup powder (lentil flour, salt, wheat flour, potato starch, vegetable oil, onion, flavours, spices, Na-benzoate, and citric acid) was bought from Irbid supermarkets for use as a reference standard.

Proximate and mineral analysis: Moisture, proteins (N X 6.25), fat, ash, and fibre were determined according to AOAC (1984) procedures, while carbohydrates were calculated by difference. Energy values were calculated by multiplying proteins, fat, and carbohydrates by factors of 4, 9 and 4, respectively (Khan et al. 1986). Calcium, sodium, potassium, iron, zinc, manganese, magnesium, copper contents of the dehydrated lentil soups were determined (Leece and Short 1967) by atomic absorption spectrophotometer (Model SP9, Pye Unicam Ltd. Cambridge, England). Phosphorus was determined according to Watanabe and Olsen (1965), using wet digestion method.

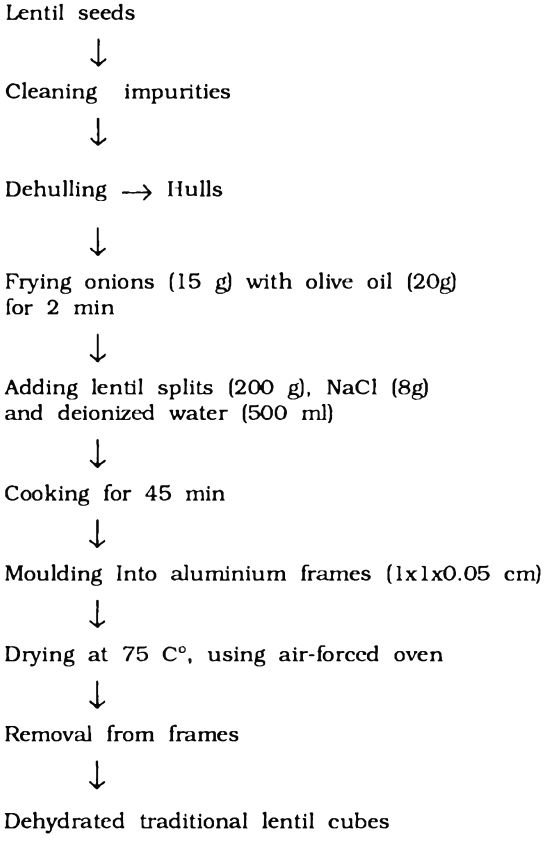

Fig. 1. Process flow chart for producing dehydrated lentil soup cubes.

Cooking characteristics: The cooking parameters (g/100 seed, ml/100 seed, specific gravity, and water absorption %) were done on the seeds according to Sharma et al (1989). Increase (%) in cooked wet, and volume were done for the soups with their ingredients, the % increase in cooked

* *Corresponding Author*

weight over the initial weight was recorded as percentage (Sharma et al. 1989).

Soup evaluation : The traditional dehydrated soups were evaluated for acceptability by 29 untrained panelists for appearance, flavour, texture, and taste. Each quality trait was given a numerical value on a 10-point Hedonic scale, 10 being liked the most (Faridi and Rubenthaler 1984).

Statistical analysis : The data were statistically analyzed according to Steel and Torrie (1980). The least significant difference among the means was calculated. Data on organoleptic evaluation were analyzed according to BSI (1975).

Data on proximate analysis are given in Table 1. The dehydrated lentil soups varied significantly in their chemical composition. Soup from 'Jor-1' was the highest in protein content (29.2%), whereas the commercial soup powder had the lowest value (18.3%). It was expected that the 'landrace' ('Balady'), and the cultivars will have higher fat content than the commercial sample, because olive oil is added to the prepared soups, but this will not be true for the ash content, as salt is part of the commercial soup. Fibre content was highest in the commercial

TABLE 1. PROXIMATE ANALYSIS, ENERGY VALUES, MINERAL CONTENT, MEAN SENSORY SCORES, COOKING CHARACTERISTICS OF DEHYDRATED LENTIL SOUP AND PHYSICAL PROPERTIES OF A LANDRACE AND THREE LENTIL CULTIVARS.[a]

	Type of soup				
	'Balady'	'Jor-1'	'Jor-2'	'Jor-3'	Commercial
Proximate analysis, %					
Proteins	27.6^{c}	29.2^{a}	28.3^{b}	26.8^{d}	18.3^{e}
Fat	11.1^{a}	11.2^{a}	9.3^{b}	11.5^{a}	2.1^{c}
Ash	5.2^{c}	3.6^{d}	7.3^{b}	3.6^{d}	16.5^{a}
Fibre	1.6^{d}	1.5^{d}	2.7^{b}	2.3^{c}	3.1^{a}
Carbohydrates	54.4^{c}	54.5^{c}	52.4^{d}	55.8^{b}	60.0^{a}
Energy value, Kcal/100 g					
Energy value	428.4^{b}	435.5^{a}	406.8^{c}	434.2^{a}	332.1^{d}
Mineral content, (mg/100g)					
Ca	125.0^{a}	71.2^{c}	76.6^{bc}	86.8^{b}	121.1^{a}
Na	1067.0^{c}	605.4^{d}	1300.0^{b}	617.0^{d}	1649.0^{a}
K	2496.0^{a}	2119.0^{b}	2017.0^{c}	1813.0^{d}	1286.0
Fe	6.0^{a}	5.6^{b}	6.0^{a}	4.9^{c}	3.5^{d}
Zn	3.9^{a}	3.4^{c}	3.9^{a}	3.5^{b}	1.9^{d}
Mg	83.4^{a}	64.9^{d}	66.6^{c}	68.5^{b}	55.8^{e}
Mn	0.9	1.0	1.0	1.1	0.9
Cu	0.9	0.8	1.0	0.8	0.4
P	439.5^{b}	432.9^{b}	470.3^{a}	433.3^{b}	253.0^{c}
Cooked soup mix					
Increased cooked volume (%)	86.3^{ab}	88.2^{ab}	85.5^{b}	90.0^{ab}	90.7^{a}
Increased cooked weight (%)	310.0^{ab}	308.3^{ab}	301.3^{b}	311.5^{ab}	318.7^{a}
Sensory scores					
Appearance	6.8^{b}	6.8^{b}	7.0^{b}	6.7^{b}	8.5^{a}
Flavour	6.6^{b}	6.4^{b}	7.0^{b}	6.7^{b}	8.5^{a}
Texture	6.7^{bc}	6.6^{bc}	7.2^{b}	6.5^{c}	8.1^{a}
Taste	7.2^{a}	7.0^{a}	7.2^{a}	7.0^{a}	7.4^{a}
Lentil seeds					
Seed weight (g/100)	3.8^{c}	4.5^{b}	5.0^{a}	4.7^{b}	ND
Seed volume (ml/100)	3.0^{b}	3.8^{a}	4.0^{a}	4.0^{a}	ND
Specific gravity (g/ml)	1.3^{a}	1.2^{a}	1.2^{a}	1.2^{a}	0.8^{b}
Water absorption (%)	92.8^{d}	95.6^{d}	99.2^{a}	96.7^{b}	ND

[a] Values are average of three determinations on dry basis. Means within rows having different superscripts are significantly different according to least significant difference (LSD) at $P< 0.05$

ND : Not determined.

TABLE 2. ANALYSIS OF VARIANCE OF TASTE PANEL SCORES FOR THE CHARACTERISTICS OF THE DEHYDRATED LENTIL SOUPS.

Source of variation	Degrees of freedom (n-1)	Mean square of scores			
		Appearance	Flavour	Texture	Taste
Soup	4	17.71 (0.000)	6.99 (0.002)	12.53 (0.000)	0.77
Judges	28	4.30 (0.000)	2.82 (0.012)	3.02 (0.023)	2.92 (0.068)
Error	112	1.378	1.515	1.735	1.933

P values are in parenthesis

soup (3.1%), probably because it was prepared from undecorticated lentils. The variations were reflected on carbohydrates, and energy values. 'Balady', and cultivars soups had the lowest energy values, the range was from 406.8 ('Jor-2') to 435.5 Kcal/100g ('Jor-1'), with the commercial having the lowest value (332.1 Kcal/100g).

Mineral contents varied significantly except for manganese, and copper (Table 1). Soups prepared from 'Balady', and cultivars had higher concentrations of potassium, iron, zinc, magnesium, and phosphorus than those in commercial powder soup. On the contrary, the commercial soup had the highest levels of sodium, and calcium, because NaCl was a major ingredient.

Table 1 shows the cooking characteristics of the soups. There were significant variations in seed weight, seed volume, seed specific gravity, water uptake, increased cooked volume, and increased cooked weight. The increased cooked volume % ranged from 85.5 ('Jor-2') to 90.7% (commercial), the values being comparable to those reported on lentil strains grown in India (Sharma et al. 1989). The increased cooked weights ranged from 301.3 ('Jor-2') to 318.7% (commercial soup), these values being higher than those reported previously (Sharma et al. 1989), probably due to genetic variation.

'Balady', 'Jor-1', 'Jor-2' and 'Jor-3' soups had similar acceptable appearance, and flavour, but the commercial soup scored higher for appearance due to its powder form. The flavour of the commercial soup scored slightly higher than the others, probably due to the uncooked lentil smell. It was more acceptable than the new dry soup cubes, but all these soups had the same taste. The organoleptic scores of the quality characteristics of the traditional dehydrated soup versus the commercial soup were above the minimum acceptable scores of five (Table 1). Analysis of variance (Table 2) of the test panel scores for each quality traits of the different soups, revealed that there were variations, in appearance, flavour, and texture, though the taste was the same.

The author is indebted to the Deanship of Research at Jordan University of Science and Technology for financing the project (Grant # 31/91).

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Received 4 April 1994; revised 27 July 1995; accepted 5 September 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 489-492

Physico-Chemical Properties of Fruits of Four Open Pollinated Tomato (*Lycopersicon esculentum* Mill) Cultivars Grown Under Rainfed Conditions in Jordan

RIDA A. SHIBLI, KHALIL I. EREIFEJ*, MOHAMMED A. AJLOUNI AND ALTAF HUSSAIN

Faculty of Agriculture,
Jordan University of Science and Technology, P.O. Box 3030, Irbid, Jordan.

Fruits of open pollinated tomato cultivars ('Pello', 'E. Mich', 'Pakit' and 'Riogrande') were evaluated for physical characters, chemical composition, and mineral contents. Results indicated that the physical properties were comparable to other tomato cultivars, except for the size of the tomato fruits, which was smaller than other cultivars, but the pulps were as high as 92.4% ('Riogrande'). Data on the chemical composition varied among cultivars, and were comparable with other varieties except for the % total soluble solids, which ranged from 8.0 ('Pello') to 8.8% ('Pakit'). Results of mineral analysis showed that all minerals were comparable with other varieties except for K content, which was very high, ranging from 567.2 ('Pello') to 801.0 mg/100 g ('Pakit'). The open pollinated tomatoes can be used to replace the imported seeds.

Keywords : Tomato, Cultivars, Physical properties, Chemical composition, Minerals.

Consumer's preference for fresh tomatoes is influenced mostly by its appearance, colour, sensory quality, and nutritional value (Frenkel and Jen 1989). Tomato is a leading crop in Jordan and the total annual production in 1992 was 621.3 thousand metric tonnes (Anon 1993). Several investigators have screened tomato cultivars, and hybrids from different genetic resources, which exhibited immense range of variation in fruit quality (Ibarbia and Lambeth 1971), physical properties (Suwwan and Abubaker 1986), chemical composition (Abubaker 1984), fruit size (Emery and Munger 1970), the shape (Abubaker 1984), specific gravity of the tomato fruits (Suwwan and Abubaker 1986), acidity (Abubaker 1984), pH (Shibli and Suwwan 1987), total soluble solids (Young et al. 1993), ability to develop seeds (Abubaker 1984), the number of locules per fruit (Suwwan and Abubaker 1986), and presence or absence of seeds (Johnson and Hall 1954).

However, as new genetic resources are continuously produced, a new focus on open pollinated cultivars has been adopted in the country in order to reduce the cost of cultivation, using imported seeds. The purpose of this study was to evaluate the physico-chemical properties of four open pollinated tomato cultivars grown under rainfed conditions.

Seeds of four open pollinated tomato cultivars ('Pello', 'E. Mich', 'Pakit', 'Riogrande') were germinated in polystyrene trays in a 1:1 peat: perlite mixture. Seedlings (35 days old) were planted through black plastic mulch on March 20, 1993. Plastic mulch was spread (at a circle of 1 m diam) around the plant one week before planting. Plastic mulch was made in a slope around the plant to allow the rain to flow to the root zone of the plant. The field was treated two months before planting with 20 tonnes/ha sheep manure. A compound fertilizer (Mikafoze, 18 nitrogen: 18 phosphorus : 6 potassium + 0.15 zinc oxide, 0.15 boric oxide, 0.15 copper oxide, 1.05 magnesium oxide) was side-dressed at a rate of 25 g/bed at planting. Plants were irrigated with two litres of water at the time of planting, and repeated after three weeks. Each cultivar was replicated four times (10 plants per replicate) in a randomized complete block design (RCBD). Plants were sprayed once with the insecticide lanate (methomyl: S-methyl-N-[(methylcarbomy) oxy] thioacetimidate) one week before the first harvest. The field received a total of 210 mm rainfall.

The harvest season was commenced on June, 1993, and terminated on Aug. 1, 1993. At the peak of the harvest season (in the mid harvest), representative composite samples of red-ripe fruits were collected, and kept in nylon bags either in a refrigerator or a freezer until the time of analysis.

Physical properties: Tomato fruits were washed thoroughly, dried at room temperature, and the average weight of individual (25 fruit) was recorded per each replicate. Fruits were cut open, and the average diameter (in cm) was measured. The fruit shape was determined by measuring the ratio of minor, and major axes (Abubaker 1984). The fruit volume (size) was determined by water displacement method. The specific gravity was determined on

* Corresponding Author

representative samples of ten fruits per replicate, using the platform scale method (Mohsenin 1970). Fruits were cut open, and the number of locules counted. The thickness of pericarp was measured using a vernier calliper.

The tomato fruits were blanched for 3 min at 100°C to remove the peels, which were weighed, and percent peel was calculated. The average pulp weight was recorded after seeds, and peels were removed. Juice was prepared by blending tomato on a Waring blender for 3 min, the macerate was passed through a double layer cheese cloth (40 mesh), the resulting juice was weighed, and juice percentage was recorded. The juice was filtered through Whatman filter paper No. 541, and the pH of the filtrate was measured. The titratable acidity was determined by titrating an aliquot of the serum with 0.1 N NaOH to pH 8.3 (Shibli 1986). Abbe stage refractometer was used to determine the total soluble solids at room temperature.

Chemical analysis: Moisture, proteins (N x 6.25), crude fibre, crude fat, and ash were determined according to AOAC (1984) procedures. Total carbohydrates were calculated by difference, and reducing sugars were determined by Lane-Eynon method (AOAC 1984). The titratable acidity was calculated as mg citric acid monohydrate per 100 ml serum.

Mineral analysis: Ash was analyzed for calcium, sodium, potassium, zinc, copper, iron, manganese, and magnesium contents, using an atomic absorption spectrophotometer (Model Sp9, Pye Unicam, England) (Ereifej and Shibli 1993). Each sample (3g) was digested in a Kjeldhal flask with a mixture of nitric, sulphuric, and perchloric acids (10:1:4), until all the organic matter was oxidized, the resulting clear liquid was filtered through Whatman filter paper No. 42, made up to known volume with distilled deionized water, and introduced to the atomic absorption spectrophotometer. The final volume of calcium, and magnesium contained 1 percent lanthanum oxide to overcome interferences mainly by phosphates. Phosphorus was determined according to the method described by Watanabe and Olsen (1965), using a CECIL CE 1020 spectrophotometer.

Statistical analysis: The data were analyzed according to Steel and Torrie (1980). Means were separated according to least significant difference (LSD), using MSTATC program (Michigan State University).

The physical properties of tomato fruits obtained from 'Pello', 'E. Mich', 'Pakit', and 'Riogrande tomato cultivars are shown in Table 1. The fruit weight values were lower than those of tomato fruit reported by Ibarbia and Lambeth (1971). Their diameters were also small, and the sizes of the fruits varied because of genetic properties, environmental, and cultural practices (Suwwan and Hamayel 1982). There were no significant differences in peel percentage. Seeds, and number of locules per fruit varied significantly.

Pericarp thickness was the same (0.6 cm) in all cultivars. Juice content did not vary statistically while the specific gravity of the fruits ranged from 0.96 ('Riogrande') to 1.0 g/ml ('Pello' and 'E. Mich').

Ibarbia and Lambeth (1971) reported that tomato fruit weights varied among cultivars, and decreased with the season. The average fruit weight in the present case was lower than the lowest value reported by Ibarbia and Lambeth (1971), probably, low rainfall contributed to lower fruit weight. Fruit shape is characteristic of the tomato cultivars according to Kasrawi et al (1981).

The cultivars investigated showed high pulp, and juice %, low peel and pericarp thickness as shown in Table 1. Different tomato cultivars differ in their tendency to develop seed in their locules according to Dempsey and Boynton (1965), and Johnson and Hall (1954).

Data on the chemical composition of the tomato fruits are shown in Table 1. There were no significant differences in proteins, crude fat, and fibre contents. There were statistical variations in ash, and carbohydrate contents. The reducing sugar, and vitamin C contents did not vary significantly. Titratable acidity, and total soluble solids showed no significant differences. Data on proteins, fat, and carbohydrates were comparable to values reported on raw tomatoes (Anon 1975), but fibre content was found to be slightly lower than that reported (Anon 1975) on raw tomatoes. On the other hand, ash contents were slightly higher than those reported previously (Frenkel and Jen 1989).

The reducing sugars (Table 1) represent almost 50% of the TSS% of the tomatoes, and this is in agreement with the values reported by Goose et al (1964). The values of vitamin C contents per fruit, were comparable with those reported previously (Anon 1975; Frenkel and Jen 1989). The titratable acidity, and pH (Table 1) were comparable with data reported previously (Abubaker 1984; Shibli 1986; Young et al. 1993).

TABLE 1. PHYSICAL PROPERTIES, CHEMICAL COMPOSITION AND MINERAL CONTENTS OF RIPE FRUITS OF FOUR TOMATO CULTIVARS GROWN UNDER RAINFED CONDITIONS[a]

	Tomato cultivar			
	'Pello'	'E. Mich'	'Pakit'	'Riogrande'
Physical properties				
Fruit weight (g)	19.4[ab*]	19.0[b]	24.5[ab]	29.7[a]
Diameter (cm)	4.1[ab]	4.1[ab]	3.8[b]	4.7[a]
Shape	oblate	oblate	spherical	oblate
Colour	red	red	red	red
Size (cm^3)	19.2[b]	18.9[b]	25.0[ab]	31.0[a]
Sp. g (g/ml)	1.0[a]	1.0[a]	0.97[a]	0.96[a]
Locules/fruit	2.2[ab]	2.3[a]	2.1[ab]	2.0[b]
Peel %	9.6[a]	10.0[a]	9.6[a]	7.6[a]
Pulp %	90.4[a]	89.9[a]	90.4[a]	92.4[a]
Seed/fruit	56.9[a]	58.8[a]	54.9[a]	35.0[a]
Pericarp thickness (mm)	0.6[a]	0.6[a]	0.6[a]	0.6[a]
Juice %	61.8[a]	61.4[a]	65.3[a]	66.1[a]
Chemical composition				
Proteins % (N X 6.25)	1.9[a]	1.9[a]	2.1[a]	1.0[a]
Fat %	0.1[a]	0.1[a]	0.1[a]	0.1[a]
Fibre %	0.7[a]	0.6[a]	0.5[a]	0.7[a]
Ash %	0.8[b]	0.8[b]	0.9[a]	0.8[b]
Carbohydrates %	6.5[a]	6.7[a]	6.4[a]	6.5[a]
Reducing sugars %	3.5[a]	3.4[a]	3.1[a]	3.8[a]
Vit. C. mg/100 g	6.0[a]	7.9[a]	10.4[a]	11.5[a]
TA %	0.3[a]	0.3[a]	0.3[a]	0.3[a]
TSS %	8.0[a]	8.2[a]	8.8[a]	8.4[a]
pH (juice)	4.1[b]	4.1[b]	4.1[b]	4.3[a]
Minerals, mg/100g				
Ca	6.9[a]	9.6[a]	8.2[a]	7.2[a]
Na	23.1[b]	36.2[a]	28.5[ab]	29.9[ab]
K	567.2[a]	608.3[a]	801.0[a]	730.3[a]
Zn	0.2[b]	0.2[b]	0.3[a]	0.2[b]
Mn	0.1[a]	0.2[a]	0.1[a]	0.1[a]
Mg	12.3[a]	13.8[a]	13.3[a]	11.8[a]
Fe	1.4[a]	1.8[a]	1.6[a]	1.7[a]
Cu	0.1[a]	0.1[a]	0.1[a]	0.1[a]
P	70.6[a]	46.5[a]	56.8[a]	55.8[a]

[a] : All values are based on 90% moisture content.

* Numbers within rows having same superscripts are significantly not different according to the least significant difference (LSD) at $P<0.05$.

The mineral contents of the tomato fruits are presented in Table 1. Calcium, and sodium contents were in agreement with data reported by Frenkel and Jen (1989), except for 'E. Mich' cultivar, which was slightly higher than the maximum value reported previously. Potassium content was noticeably very high, and even higher than the range reported previously (Frenkel and Jen 1989), probably due to uptake of potassium from rich soil. Zinc, manganese, copper, and magnesium contents were in the ranges as reported by Frenkel and Jen (1989) for these minerals. Iron, and phosphorus contents were higher than the ranges reported (Frenkel and Jen 1989). The variations of mineral contents might be due to environment, and cultural practices conditions.

This project was funded by the Deanship of Research (J.U.S.T), project No. 25/92, whose support is gratefully acknowledged.

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Received 9 March 1994; revised 27 July 1995; accepted 5 September 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 493-496

Evaluation of Laminated and Co-extruded Films for Packing Potable Water

S. NADANASABAPATHY*, A.N. SRIVATSA AND R. SANKARAN

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

Laminated, and co-extruded plastic films such as paper/aluminium foil/low density polyethylene, polyester/aluminium foil/low density polyethylene, metallised polyester high-low density polyethylene have been evaluated for their suitability to pack potable water. Potable water packed in polyester/aluminium foil/low density polyethylene showed a minimum increase in total solids and total hardness, both of which were within the permissible limits. No plastic odour or taste was observed in the water sample packed, and a shelf life of 12 months was achieved.

Keywords : Laminated films, Co-extruded films, Potable water, Open top sanitary cans, Shelf life.

In recent years, the focus in packaging has been on the development of multilayer polymeric films for a variety of applications within the food industry (Hotchkiss 1982). Institutional, and food service users have shown keen interest in food products packaged in flexible pouches, because of lower costs, and added convenience (Erickson 1990). Storage, supply, and distribution of potable water are essential for both civilian, and Defence services. At present, potable water is commercially available in blow moulded semi-rigid plastic containers, mainly for the use by tourists, and travellers. However, not much scientific data are available in this regard. In Defence services, potable water is being supplied in metal jerry cans, fabric-based *chaguls*, and in open top sanitary cans, during operational conditions. The disadvantage of using metal cans is its weight, and impartment of metallic taste, which restricts the shelf life significantly (Brooks 1964).

Furthermore, unit packaging of potable water in flexible packaging materials is found essential during off-shore responsibilities, and special missions undertaken by maritime services (Anon 1985). Therefore, attempts were made earlier to evaluate the shelf life of potable water packed in semi-rigid poly vinyl chloride, and high density polyethylene containers (Anon 1977; Satyanarayana Rao et al. 1993).

However, the suitability of indigenously available laminated, and co-extruded flexible films to pack potable water has not been yet reported. Hence, this study was undertaken to determine the effect of storage time, and temperature on various physical, chemical, microbiological, sensory quality of potable water packaged in laminated, and co-extruded films, in comparison to control samples consisting of water, packed in glass bottles. The packaging materials used are described in Table 1.

The physico-mechanical properties of the packaging materials were determined as per ASTM (1982) procedures.

Water sample : Local supply of drinking water was used. As the pH of the water was above 8.40, it was adjusted to 6.50 by adding citric acid-sodium citrate buffer. The water was pasteurized at 80°C for 5 min. Approximately, 150 ml water in the ratio of 1 ml/sq cm surface contact area was filled in the above packaging materials, and stored at ambient (26°-32°C, 65% RH), and accelerated hot humid (37°C, 90% RH) conditions. Control samples were stored in a refrigerator maintained at 5°C, till the end of the experiment. During storage, water sample was analyzed for pH, total solids, total hardness (total alkalinity), chloride, and substances causing odours, and tastes as per APHA (1965) methods. Microbiological examination was carried out as described by England and Wales (1958). Sensory evaluation was carried out on a 9- point Hedonic scale by a panel of 7 trained personnel (Piggot 1988).

Physico-mechanical characteristics of the packaging materials are shown in Table 2. On storage, these properties did not change significantly. Changes in the physico-chemical, and sensory properties of the packed water samples are shown in Table 3. In all the packaging materials, the contact layer was polyethylene. BIS (1982) has listed various constituents of polyethylene in contact with foodstuffs, pharmaceuticals, and drinking water. The stability, and migration of these constituents are dependent on storage time, and conditions (Labuza 1988).

At 37°C. and 90% RH, all parameters were found to be higher in all test materials. On storage,

* *Corresponding Author*

TABLE 1. SPECIFICATIONS OF THE PACKAGING MATERIALS USED FOR PACKING OF POTABLE WATER

Abbreviation	Composition	Type and dimension of the pack.
PFP	Paper (100 gsm)/aluminium foil (20μ)/low density polyethylene (37.5μ)	Rectangular, 7 X 5 X 9 cm
PET/Al. foil/ LDPE	Polyester (12μ)/aluminium foil (20μ)/ low density polyethylene (37.5μ)	Pillow pouch, 15 X 20 cm
MPP	Metallised polyester/high-low density polyethylene, total thickness 100μ	Pillow pouch, 15 X 20 cm
HDLD	High density polyethylene/low density polyethylene, total thickness 100 μ	Pillow pouch, 15 X 20 cm
Glass bottle	Borosil	Straight side, wide mouth, 16 oz capacity

there was a small but distinct increase in pH of the water sample, and changes in pH value of the samples stored in different packaging materials were in the order: PFP > HDLD > MPP >PET/Al. foil/LDPE. Similarly, total solids of the stored water samples had increased in all the test materials, and the increase followed the order: PFP > MPP > HDLD> PET/Al. foil/LDPE. It has been reported that the increased in pH, and total solids were due to migration of polymeric residues, non-polymeric additives, and adhesive compounds from the packaging materials (Crompton 1979). Therefore, migration of constituents from the packaging materials is reported as total solids as described by BIS (1986). Also, during storage, total hardness of the stored water samples increased in the order similar to that of the total solids (Table 3). This may be attributed to the migration of non-polymeric additives. Initial chloride content of the water sample was 60 mg/l, and it did not change significantly after the storage.

TABLE 2. PHYSICO-MECHANICAL CHARACTERISTICS OF THE FILMS

Properties		Packaging films			
		Paper/ Al. foil/ LDPE	PET Al. foil/ LDPE	MPP	HDLD
Tensile strength, KN/M	MD	2.14	3.25	2.76	2.01
	TD	1.26	2.90	2.42	1.88
Elongation, %	MD	6.30	9.50	7.70	8.90
	TD	3.80	7.50	4.90	5.40
Bursting strength, kg/cm		2.10	2.80	1.90	1.30
Tearing strength, g	MD	50	20.60	26.00	22.00
	TD	55	25.50	23.00	26.00
Seal strength, kg/10 mm	MD	0.78	1.10	1.26	1.66
	TD	0.36	1.20	1.48	1.77
Oxygen transmission rate (flat sheet), ml/M²/24 h at 27°C		0.10 to 0.90	0.25 to 0.75	0.20 to 0.80	1.10 to 1.40
Water vapour transmission rate (flat sheet), g/M²/24 h at 37°C and 90% RH		< 0.10	0.40 to 0.70	0.10 to 0.30	3.10 to 3.30

MD = Machine direction, TD = Transverse direction

The water sample packed in PFP had picked up an objectionable plastic odour, and taste, after 8 months of storage, thereby rendering the water unacceptable. On further storage, the intensity of the plastic odour, and taste further increased. In MPP, a similar behaviour was observed after 10 months of storage. In HDLD also, this behaviour was observed in the samples stored at 37°C and 90% RH. Migration of polymeric additives may have contributed to the development of plastic odour, and taste, as described by Shepherd (1982). However, no such plastic odour, and taste were observed in water samples packed in PET/Al. foil/LDPE and stored at ambient, and accelerated conditions. The probable reason may be the grade of low density polyethylene used for lamination in PET/Al. foil/ LDPE, which was different from the grade of low density polyethylene used in other packaging materials.

Absence of microorganisms, such as faecal *streptococci, Clostridium perfringens*, coliform and *E. coli*, indicated that the water sample was microbiologically acceptable. At the end of 12 months storage under ambient condition, water stored in PET/Al. foil/LDPE scored the sensory rating of 7.2, whereas that in HDLD, MPP and PFP scored 3.4, 3.4 and 2.8, respectively. At 37°C and 90% RH, the sensory rating was 7.0 for the sample stored in PET/Al. foil/LDPE, as against 3.8, 3.8 and 2.1 for the samples stored in MPP, HDLD and PFP, respectively. Thus, the water sample packed in PET/Al. foil/LDPE, and stored for a period of 12 months at ambient condition was found acceptable. No significant changes were observed during 12 months storage in the water stored in glass bottles at 5°C, which served as control for comparison.

TABLE 3. CHANGES IN THE PHYSICO-CHEMICAL CHARACTERISTICS OF THE POTABLE WATER.

Packaging material	Storage condition	Storage period, months	Quality changes				Odour and taste
			pH	[a]Total solids mg/l	[b]Total hardness, mg/l $CaCO_3$	[c]Sensory score	
	Initial value	0	6.50	465 ± 1	18.6 ± 0.2	9.0 ± 0.2	A
Paper/Al.foil/LDPE	Ambient	3	6.54	478 ± 1	19.4 ± 0.2	8.2 ± 0.2	A
		6	6.62	478 ± 1	20.6 ± 0.2	7.6 ± 0.2	A
		9	6.67	504 ± 1	21.2 ± 0.3	4.0 ± 0.2	NA
		12	6.71	524 ± 2	21.5 ± 0.2	2.8 ± 0.3	NA
	37°C	3	6.58	480 ± 1	19.8 ± 0.2	8.0 ± 0.2	A
	and	6	6.66	488 ± 2	21.1 ± 0.4	7.4 ± 0.3	A
	90 % RH	9	6.74	516 ± 1	21.8 ± 0.4	4.2 ± 0.2	NA
		12	6.80	536 ± 1	22.4 ± 0.1	2.1 ± 0.2	NA
PET/ Al.foil/LDPE	Ambient	3	6.51	468 ± 1	18.8 ± 0.1	8.8 ± 0.2	A
		6	6.58	472 ± 2	19.1 ± 0.1	8.4 ± 0.3	A
		9	6.63	482 ± 2	20.2 ± 0.2	7.6 ± 0.3	A
		12	6.67	490 ± 2	20.5 ± 0.2	7.2 ± 0.3	A
	37°C	3	6.54	470 ± 1	19.2 ± 0.1	8.2 ± 0.2	A
	and	6	6.61	475 ± 1	19.6 ± 0.2	8.2 ± 0.2	A
	90 % RH	9	6.66	487 ± 1	20.6 ± 0.3	7.4 ± 0.3	A
		12	6.70	496 ± 2	20.9 ± 0.1	7.0 ± 0.3	A
MPP	Ambient	3	6.52	470 ± 1	19.2 ± 0.1	8.4 ± 0.4	A
		6	6.60	480 ± 2	19.5 ± 0.2	7.8 ± 0.3	A
		9	6.65	484 ± 2	20.8 ± 0.2	6.4 ± 0.3	A
		12	6.69	508 ± 1	21.2 ± 0.2	3.4 ± 0.2	NA
	37°C	3	6.55	472 ± 1	19.5 ± 0.3	8.2 ± 0.3	A
	and	6	6.64	485 ± 2	20.6 ± 0.1	7.6 ± 0.4	A
	90 % RH	9	6.70	498 ± 2	21.0 ± 0.1	4.4 ± 0.2	NA
		12	6.73	524 ± 1	21.6 ± 0.2	3.8 ± 0.4	NA
HDLD	Ambient	3	6.53	468 ± 1	19.0 ± 0.1	7.8 ± 0.3	A
		6	6.61	476 ± 2	19.4 ± 0.1	6.6 ± 0.2	A
		9	6.67	485 ± 1	20.4 ± 0.2	6.2 ± 0.3	A
		12	6.70	496 ± 2	21.0 ± 0.2	3.4 ± 0.4	NA
	37°C	3	6.56	474 ± 2	19.4 ± 0.2	7.4 ± 0.3	A
	and	6	6.65	488 ± 1	19.9 ± 0.2	6.2 ± 0.2	A
	90 % RH	9	6.71	504 ± 2	20.9 ± 0.1	4.2 ± 0.3	NA
		12	6.74	514 ± 1	21.4 ± 0.2	3.8 ± 0.4	NA

a, b, c = Mean ± SD; A = Acceptable ; NA = Non-acceptable

It may be concluded that PET/Al. foil/LDPE shows minimum increase in total solids, and total hardness during 12 months storage. These values are within the permissible limits prescribed by ICMR (1975) and WHO (1971). Further, it does not contribute any objectionable plastic odour, and taste to the water sample. The easy availability, and reasonable cost make this laminate, a suitable alternative to open top sanitary cans for unit packaging of potable water.

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Received 20 June 1994; revised 20 July 1995; accepted 5 September 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 497-500

Effects of Processing on Total Phenols and Proximate Composition of Pigeonpea (*Cajanus cajan*) and Climbing Bean (*Vigna Umbellata*)

SYLVESTER O. IGBEDIOH,*[1] STELLA SHAIRE [1] AND BABATUNDE J.I. ADERIYE [2]

[1] College of Food Technology
University of Agriculture, PMB 2373, Makurdi, Nigeria.
[2]Department of Applied Microbiology
Ondo State University, Ado-Ekiti, Nigeria

Effects of processing, such as dehulling, sprouting, soaking, cooking, roasting, and autoclaving, on total phenols, and chemical constituents in pigeonpea (*Cajanus cajan*) and climbing bean (*Vigna umbellata*), cultivated in Nigeria, were investigated. All the processing methods reduced the levels of total phenols in both the legumes to varying extents. Cooking of soaked, and dehulled seeds had the most pronounced lowering effect on the total phenolic contents, of pigeonpea, and climing bean, by 49 and 50%, respectively. Sprouting of the legume seeds increased the protein content, and significantly reduced the carbohydrate contents ($P<0.05$). These processing methods altered the composition of both the legumes, without effecting any change in carbohydrates.

Keywords : Processing, Total phenols, Proximate composition, Pigeonpea, Climbing bean.

Pigeonpea, and climbing bean are nutritionally important legumes as sources of proteins to poor communities in many tropical, and sub-tropical regions of the world (Salunkhe et al. 1982; Singh 1984). In Nigeria, pigeonpea, and climbing bean are consumed after soaking, and cooking or roasting (Igbedioh 1993 unpublished data). They are eaten with sauce or mixed with other foods, along with spices, and condiments.

Legumes contain antinutritional factors such as polyphenols (Salunkhe et al. 1982). Phenolic compounds are mostly present in the seed coat of grain legumes (Singh and Jambunathan 1981), and inhibit the activity of digestive as well as hydrolytic enzymes, such as alpha-amylase, trypsin, chymotrypsin, and lipase (Salunkhe et al. 1982). Polyphenols also decrease the digestibility of carbohydrates, and the availability of vitamins, and minerals (Rao and Deosthale 1982), and interact with proteins to make them insoluble (Singh 1984). Available information indicates that the levels of polyphenols in legumes can be reduced by simple processing methods, such as soaking, dehulling, and roasting. (Rao and Deosthale 1982; Deshpande and Cheryan 1983; Jood et al. 1987).

This communication reports the results of an investigation on the effect of combined processing methods like soaking, dehulling, roasting, germination, and autoclaving, on polyphenolic contents, and chemical constituents of pigeonpea, and climbing bean.

* Corresponding Author

The seed samples of pigeonpea (*Cajanus cajan*), and climbing bean (*Vigna umbellata*) were purchased at the Makurdi local market, and divided into eight lots for different processing treatments.

Soaking and soaking plus dehulling : Healthy seeds (3 kg), free from dust, and other foreign materials, were soaked in 5 volumes (w/v) of tap water for 24h, washed twice with ordinary water, and then with distilled water. Thoroughly washed seeds were dried to a constant weight in a hot air oven at 70°C. In an another experiment, part of the soaked seeds were manually dehulled to remove the seed coat, and dried as above.

Cooking : Dry seeds (100 g) were boiled in 3 volumes (w/v) of distilled water in a beaker, until soft, as judged by pressing them between the thumb, and the fingers. The cooked seeds were dried as above.

Germination : The soaked seeds (500 g) were placed in sterile petri dishes, lined with wet filter paper, and were allowed to germinate for 3 days at 32°C with frequent watering. The sprouted seeds were dried as above.

Autoclaving : The seeds (500 g) were autoclaved at a temperature of 121°C for 15 min, and further processed as above.

Roasting : Undamaged, and healthy seeds (500 each), free from dust, and other foreign materials, were roasted individually, in an aluminium pot at a temperature of 160°5C for a period of 15, 30 and 45 min.

Chemical analysis : Total phenols were analyzed

using the Prussian blue method of Budini et al (1980). Proteins, fat, moisture, ash, and crude fibres were determined according to AOAC (1984) methods.

Soaking of pigeonpea, and climbing bean in tap water reduced the total phenol contents ($P<0.05$) in both legumes (Table 1). These decreases in the phenolic contents may be due to the leaching of the polyphenols into the soaking water (Jood et al. 1987). Significant decreases in the polyphenol due to soaking in water of chickpea, and blackgram (Jood et al. 1987), *mung* bean (Barroga et al. 1985) dry bean (Deshpande and Cheryan 1983) and winged bean (Sathe and Salunkhe 1981; King and Puwastein 1984) have been reported. The decline in the phenolic content of the pigeonpea (30%), and climbing bean (37%), due to dehulling of the soaked seeds, suggests the location of the significant proportions of the total phenols in the seed coat. Rao and Deosthale (1982) also reported 90% decline in polyphenol in pigeonpea upon dehulling. Similarly, Ogun et al (1989) observed that all the total phenols in cowpeas were located in the seed coat, as no detectable total phenols were found in dehulled cowpeas.

TABLE 1. EFFECTS OF SOAKING, DEHULLING, COOKING, GERMINATION, AUTOCLAVING AND ROASTING ON TOTAL PHENOLS OF PIGEONPEA AND CLIMBING BEAN

Seed treatments	Total phenols in mg catechin g^{-1}	
	Pigeon pea	Climbing bean
Unsoaked, control	1.04 ± 0.05	1.09 ± 0.30
Soaked, 24 h	0.08 ± 0.04 (23)	0.76 ± 0.02 (30)
Soaked and dehulled	0.71 ± 0.01 (32)	0.69 ± 0.02 (27)
Cooked unsoaked	0.62 ± 0.02 (40)	0.62 ± 0.04 (43)
Cooked soaked	0.59 ± 0.01 (43)	0.58 ± 0.01 (47)
Soaked, dehulled and cooked	0.53 ± 0.03 (49)	0.54 ± 0.01 (50)
Sprouted, soaked	0.97 ± 0.02 (7)	0.91 ± 0.01 (07)
Soaked, dehulled and sprouted	0.89 ± 0.03 (14)	0.87 ± 0.01 (30)
Sprouted cooked	0.71 ± 0.02 (32)	0.74 ± 0.02 (32)
Unsoaked and autoclaved	0.92 ± 0.04 (11)	0.93 ± 0.02 (15)
Soaked and autoclaved	0.89 ± 0.03 (14)	0.90 ± 0.01 (15)
Soaked, dehulled and autoclaved	0.78 ± 0.02 (25)	0.83 ± 0.02 (24)
Unsoaked and roasted		
15 min	0.76 ± 0.03 (27)	0.76 ± 0.2 (02)
30 min	0.68 ± 0.02 (36)	0.71 ± 0.1 (35)
45 min	0.65 ± 0.03 (50)	0.54 ± 0.2 (0)

Values are means ± S.D. of four determinations expressed as mg/catechnin g^{-1} sample. Figures in parenthesis represent loss in polyphenol content expressed as percentage of control values.

Cooking, without prior soaking, also brought about a decrease in the phenolic contents of both the legumes, probably due to the leaching of the phenols into the water during cooking and changes in chemical reactivity (Jood et al. 1987). It was also reported that cooking reduced the polyphenolic contents of cowpeas (Sathe and Salunkhe 1981) and *mung* beans (Barroga et al. 1985). The relatively higher effect of cooking on the phenolic contents of soaked seeds, over the unsoaked seeds, in the two legumes may be due to the fact that soaking could have permitted the initial losses of some of the polyphenols, and the remaining amount was further removed, when the soaked seeds were cooked. Comparatively, these losses in phenolic contents were slightly higher in the climbing bean than in pigeonpea.

Factors such as leaching of polyphenols into the soak water (Jood et al. 1987), and the action of polyphenol oxidase in causing enzymic hydrolysis (Subbulakshmi et al. 1976; Rao and Deosthale 1982) may have been responsible for the losses of phenols during germination (Table 1). Comparatively, the losses in polyphenols in the two legumes on sprouting were equal to those in soaking plus dehulling treatment. Roasting, and autoclaving involved the application of heat, and the results showed that the polyphenols in the two legumes decreased by 17 to 39% due to these treatments. The results indicate that roasting of soaked, and dehulled seeds resulted in greater loss of the phenols, followed by roasting of soaked seeds, and roasting of unsoaked seeds in both the legumes (Table 1). A similar pattern was observed for the autoclaved seeds. (Table 1). However, roasting had a greater lowering effect on the total phenol contents than autoclaving, and this effect was time dependent. The reason for the greater loss on autoclaving is due to the combined effect of high temperature, and the leaching out of phenolic compounds during the preliminary process of soaking and/or soaking and dehulling. Comparatively, slightly greater losses of total phenols were observed in the climbing bean than in pigeonpea.

The reason for the observed reduction in protein content in the roasted legumes is difficult to explain. However, Cheftel (1979) reported that roasting of proteins resulted in destruction of amino acids such as methionine, lysine, and cysteine. These are broken down into fractions of various molecular weights (Cheftel 1979). In addition, severe heating of moist foods with low carbohydrate

TABLE 2. EFFECTS OF PROCESSING ON PROXIMATE COMPOSITION OF PIGEONPEA AND CLIMBING BEAN.

Seed treatments	Proteins, %	Ash, %	Fat, %	Moisture, %	Crude fibre, %	Carbohydrates, %
			Pigeonpeas			
Raw	20.30 ± 0.14	3.5 ± 0.08	1.5 ± 0.11	12.0 ± 0.03	4.4 ± 0.16	58.00 ± 0.03
Sprouted	21.40 ± 0.14 (5)	3.0 ± 0.29 (14)	0.6 ± 0.9 (60)	50.0 ± 3.65 (76)	4.6 ± 0.14 (5)	21.00 ± 0.10 (64)
Roasted						
15 min	10.56 ± 0.10 (48)	3.3 ± 0.29 (0)	1.0 ± .02 (17)	10.0 ± 0.02 (17)	2.0 ±0 .08 (55)	74.49 ± 0.34 (18)
30 min	9.75 ± 0.25 (52)	3.0 ± 0.29 (12)	0.8 ± .14 (33)	30.0 ± 1.24 (75)	2.0 ± 0.80 (55)	83.05 ± 0.34 (32)
45 min	8.50 ± 0.22 (58)	3.0 ± 0.22 (12)	0.6 ± .09 (50)	2.5 ± 0.29 (79)	2.0 ± 0.12 (55)	85.22 ± 0.52 (36)
Autoclaved	12.90 ± 0.04 (36)	3.0 ± 0.14 (14)	0.8 ± .14 (46)	11.0 ± 0.14 (8)	4.0 ± 0.11 (9)	68.03 ± 1.15 (17)
			Climbing bean			
Raw	18.50 ± 0.22	2.5 ± 0.14	13.5 ± 0.33	13.5 ± 0.24	4.0 ± 0.16	60.20 ± 0.49
Sprouted	19.20 ± 0.48 (4)	2.0 ± 0.08 (20)	0.6 ± 0.19 (50)	55.0 ± 0.34* (75)	4.2 ± 0.21 (5)	19.00 ± 0.23*(68)
Roasted						
15 min	14.41 ± 0.40 (24)	1.3 ± 0.17 (8)	1.0 ± 0.16 (17)	10.0 ± 0.22 (26)	2.5 ± 0.33 (37)	73.43 ± 1.15 (14)
30 min	12.20 ± 0.41 (35)	2.4 ± 0.16 (4)	2.4 ± 0.16 (17)	3.0 ± 0.22 (78)	2.1 ± 0.26 (47)	81.05 ± 5.32 (27)
45 min	10.00 ± 0.22 (46)	2.2 ± 0.14 (12)	0.8 ± 0.14 (33)	2.5 ± 0.29 (81)	2.0 ± 0.26 (50)	84.30 ± 0.24 (31)
Autoclaved	14.00 ± 0.21 (24)	2.0 ± 0.14 (20)	1.0 ± 0.25 (23)	10.0 ± 0.21 (26)	3.5 ± 0.22 (13)	79.50 ± 0.42 (32)

Values are means ± S.D. for four independent determinations. Figures in parenthesis represent loss (-) or increase (+) in constituents expressed as percentage of control values. *Significant at 5% level of probability.

content may cause a marked destruction of cysteine, together with a decrease in the availability of many amino acids, (Evans and Butts 1949). This process is called desulphurization. Similarly, the reductions in fat, and moisture content, upon roasting, could have been due to conversion of fats to fatty acids, and glycerol which was further hydrolyzed to acetate (King and Puwastein 1987). The loss in moisture was caused by the application of high temperature, which resulted in vapourization of water in the legume seeds.

The breakage, and subsequent release of some of the carbohydrates, hitherto bonded, (Ngoddy and Ihekoronye 1985) are probably responsible for the observed increase in the carbohydrate contents of both the legumes during roasting (Table 2).

The slight increase in the protein content, on sprouting of two legumes ($P>0.05$), may be due to the synthesis of new proteins (King and Puwastein 1987), while the reduction in fat may have been due to the breakdown of fat by beta-oxidation (King and Puwastein 1987). The significant increase in the moisture content of the sprouted legumes is probably due to imbibition of water by the seed microphyle on soaking in water prior to sprouting. Autoclaving at 121°C for 15 min decreased both the protein, and fat contents of pigeonpea, and climbing bean, the values being similar to those in roasted seeds at 160°C (for 15-45 min), since the two processes involved application of high temperature.

Soaking, and dehulling prior to cooking of the legumes before consumption are suggested as a means of removing the larger amounts of total phenols in the two legumes investigated.

The authors thank Dr. M.A. Akpapunam, Prof. I. Onyido and Mr. Onyeje for permission to use facilities, and Mrs. C.U. Inyang for the gift of the standard catechin.

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Received 14 July 1994; revised 3 July 1995; accepted 6 September 195

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 501-503

Effects of Roasting on Protein Quality of Chickpea (*Cicer arietinum*) and Peanut (*Arachis hypogaea*)

CHARANJEET K. HIRA* AND NUPUR CHOPRA

Department of Foods and Nutrition,
Punjab Agricultural University, Ludhiana-141 004, India.

Raw, and roasted chickpea as well as peanut were analyzed for proximate principles, methionine, available lysine, and trypsin inhibitor activity. The results indicated that roasting decreased available lysine and trypsin inhibitor activity considerably, and improved true digestibility, relative nitrogen utilization, and protein efficiency ratio values. Biological value of both chickpea, and peanut decreased significantly ($P<0.05$) on roasting, but the decrease in net protein utilisation was not significant.

Keywords : Chickpea, Peanut, Protein quality, Roasting, Available lysine.

Legumes enjoy the distinction of being protein-rich foods, and form an important constituent of the diets of the people living in developing countries (Rao and Sastry 1991). Grain legumes are appropriate supplements to cereals, due to their high lysine contents (Hulse 1991). In addition to consumption of boiled legumes, the roasted legumes also form popular snack items, which are liked by all (Khan 1991; Kadam and Chavan 1991). Roasting gives a crunchy form to the grains, and makes them easy to masticate, and reasonably palatable (Khan 1991). Roasting might affect the nutritional quality of grain due to high temperature treatment. The present study reports the effect of roasting on protein quality of chickpea, and peanut.

Samples of chickpea, and peanut were procured from local market. Chickpea was roasted in sand at 220–280°C for 2-2.5 min. Peanuts were roasted in sand along with pods at same temperature for 4-5 min. Raw samples were used as such, while roasted samples were dehusked. Raw, and roasted peanuts were defatted in locally used oil extractors at small scale, where crushing is done to extract oil. The samples were finely ground, using tecator cyclotech 1093 sample mill, and sieved through 60 mesh.

Samples were analyzed for proximate principles, using AOAC (1985) methods. Methionine was estimated according to Horn et al (1946), after hydrolysis of fat-free samples with 6 N hydrochloric acid. Available lysine was determined by the method of Booth (1971). Antitryptic activity was estimated by measuring the rate of hydrolysis of casein by reading the absorbance of the split products at 280 nm, based on the modified method of Kakade et al (1972). All samples were analyzed in duplicate.

The diets for rat feeding experiments were prepared at 10% protein level, using chickpea, and peanut as protein sources. Crude fibre, and fat were adjusted to 5 and 10%, respectively, by adding non-nutritive cellulose, and refined groundnut oil. Vitamin mixture (Chapman et al. 1959) and mineral mixture (Hawk and Oser 1965) were added at 1 and 4% levels, respectively, and corn starch was used to make it 100 g. The ingredients were thoroughly mixed, and sieved for uniform distribution of ingredients. Skimmed milk powder was used as a reference protein. The diets were fed to 24 days old male albino 'Wistar' rats (average weight 29.2 g), and protein efficiency ratio as well as nitrogen utilization were calculated, as described by Evans and Witty (1978). Relative nitrogen utilization was calculated, using skim milk as standard. True digestibility, biological value, and net protein utilization were estimated (Evans and Witty 1978) from the same diets fed to adult 'Wistar' rats of 350 g average weight. A non-protein group was also run to calculate endogenous losses. Carcass method as described by Evans and Witty (1978), was followed for the determination of net protein utilization. The data were tested for their significance using t-test (Walter 1955).

The results revealed that proteins, fat, and fibre contents of chickpea (Table 1) were comparable to the values reported by Singh and Jambunathan (1981). Trypsin inhibitor activity of chickpea was also within the range of 37.4-60.4 TIU/mg proteins as reported by these authors. Taking fat extraction of peanut into consideration, protein, and fibre contents of the defatted samples were within the range of literature values (Gopalan et al.1980). Roasting of both the legumes did not affect methionine content (Table 1). Kapoor and Gupta (1977) have shown that roasted soy protein

* Corresponding Author

TABLE 1. EFFECT OF ROASTING ON CHEMICAL CONSTITUENTS AND PROTEIN QUALITY OF CHICKPEA AND PEANUT

Parameters	Chickpea		Peanut, defatted		Skim milk
	Raw grains	Roasted dehusked	Raw grains	Roasted dehusked	
Moisture, %	9.67	6.36	3.20	2.98	-
Proteins, %	19.35	22.01	37.82	39.32	-
Crude fat, %	5.27	5.88	3.40	3.31	-
Crude fibre, %	3.92	3.86	5.26	5.91	-
Methionine, g/16 g N	1.36	1.26	1.10	0.99	-
Available lysine, g/16 g N	5.84	9.64	3.37	2.59	-
Trypsin inhibitor activity, TIU/mg proteins	43.00	9.21	38.09	8.29	-
Protein efficiency ratio	1.71 ± 0.34	1.94 ± 0.18	1.60 ± 0.16	2.04 ± 0.29	3.00 ± 0.28
Relative nitrogen utilization	72.60 ± 16.32	78.84 ± 18.12	67.82 ± 9.12	74.20 ± 15.40	100
True digestibility	85.16 ± 4.67	90.80** ± 3.30	79.91 ± 1.47	87.99** ± 1.98	91.50 ± 2.09
Biological value	66.52 ± 10.49	57.87* ± 7.99	60.98 ± 7.57	51.59* ± 12.50	76.64 ± 6.51
Net protein utilization	56.56 ± 8.65	53.43 ± 7.39	48.75 ± 6.17	43.25 ± 10.67	69.30 ± 5.09

All samples were analyzed in duplicate. Values for protein quality parameters are mean ± SD for 8 rats in each group.

* Significantly different from corresponding raw grains at 5% level.

** Significantly different from corresponding raw grains at 1% level.

concentrate did not affect its methionine content, in contrast to slight decrease in methionine of roasting of faba beans as reported by Rani and Hira (1993). Available lysine contents of raw chickpea, and peanut were comparable to the earlier reports (Rama Rao 1974; Jambunathan 1991), and decreased considerably on roasting. Losses of available lysine during heat treatments in chickpea, and peanut have also been reported by Rama Rao (1974), and of faba beans by Rani and Hira (1993). Trypsin inhibitor activity on roasting has been reduced to almost one fifth of the original value (Table 1). Destruction of trypsin inhibitor activity in chickpea by heat has also been reported by Weder and Link (1993).

Protein efficiency ratio, and relative nitrogen utilization values increased on roasting of chickpea, and peanut, but the effect was statistically significant ($P<0.05$) only for peanut (Table 1). Protein efficiency ratio values of 1.75 for raw groundnut, and 1.66 for raw *Bengal*gram have been reported by Rama Rao (1974). A slightly higher value of 83.27 for relative nitrogen utilization of chickpea flour has been reported by Zamora and Fields (1979). Increase in protein efficiency ratio on roasting could be due to the fact that lysine, which was adversely affected by heat, may not be the limiting amino acid (Hulse 1991). In contrast, methionine, which is limiting amino acid in legumes (Zamora and Fields 1979), was not affected by heat. In addition, reduction in antinutritional factors in roasted products could have enhanced the nutritional quality. Kaur and Hira (1988) reported that trypsin inhibitor activity was negatively correlated with protein efficiency ratio. Improvement in protein efficiency ratio, and relative nitrogen utilization of faba beans on roasting have also been reported by Rani and Hira (1993).

The digestibility of chickpea, and peanut increased significantly ($P<0.01$) on roasting, because high temperature had destroyed the antinutritional factors, which otherwise would have hindered the protein absorption of raw diets (Hulse 1991). An increase in digestibility of roasted faba beans, compared to raw faba beans, has also been reported by Rani and Hira (1993). The biological value of legumes decreased significantly ($P<0.05$) on roasting, and biological value of raw peanut was comparable to the range reported by Jambunathan (1991). Geervani and Theophilus (1980) have reported that the moist heat is more efficient in enhancing the biological value, than the dry heat. A decrease in biological value of chickpea from 60.8 to 56.8 on heating was also reported by Buss and Goddard (1948). The net protein utilization of legumes decreased on roasting, but the differences were not significant. A decrease in net protein utilization of soybean from 58.3 to 44.0% has also been reported by Kapoor and Gupta (1977).

It may be concluded that roasting improves the digestibility of legumes, and does not adversely affect the net protein utilization. Thus, it can be used to combat protein energy malnutrition in young children.

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Received 1 July 1994; revised 26 June 1995; accepted 6 September 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 504-506

An Orange Juice-borne Diarrhoeal Outbreak due to Enterotoxigenic *Escherichia coli*

B.R. SINGH*[1] S.B. KULSHRESHTHA AND K.N. KAPOOR

Division of Veterinary Public Health
Indian Veterinary Research Institute, Izatnagar, 243 122, India.

During diarrhoeal outbreak at Faridpur in Bareilly District in June 1992, the patients were interviewed and samples of orange juice, sugarcane juice, and water were collected as the suspected vehicle, along with stool samples of the patients. Enterotoxigenic *Escherichia coli*, and enterotoxigenic *Klebsiella pneumoniae* sub sp. *aerogenes* were isolated from all juice samples, but not from the water samples. From all the patients with cholera-like syndrome, enterotoxigenic *E.coli* were isolated from their stool samples. All the enterotoxigenic *E. coli* isolates from patients, and one of the orange juice samples had the same antibiogram, showing resistance to cotrimoxazole, carbenicillin doxycycline, streptomycine, tetracycline, and sulphonamides as well as sensitivity to neomycin, gentamicin, nitrofuradentoin, polymyxin B, nalidixic acid and kanamycin. All isolates of *E. coli* remained untypable with available specific O-antisera, except for one isolated from sugarcane juice, which belonged to O:134 serotype. Isolation of enterotoxiic *E. coli* from orange juice sample is of public health significance.

Keywords : *Escherichia coli* O:134, *Klebsiella pneumoniae* sub sp.*aerogenes*, Multiple drug resistance, Diarrhoea, Orange juice. Enterotoxigenic *E. coli*.

Food and waterborne outbreaks of cholera, and cholera-like diseases are common in Indian sub-continent (NICD 1986, 1988; Banerjee 1991), and a variety of foods and drinks has been incriminated in such outbreaks (Pal 1991; Mittal et al. 1991; Baird Parker 1992; Singh 1994; Sandhu 1994; Singh et al. 1995). Major causes of the diarrhoeal outbreaks were diarrhoeal strains of *Escherichia coli, Vibrio cholerae, Salmonella, V. parahaemolyticus, Bacillus cereus, Shigella*, and some other members of enterobacteriaceae (Jay 1987; Tauxe et al. 1987; Sandhu 1994; Singh 1994). Recently, some studies have shown that the contamination of salad, juice, fruits, and vegetables is due to unsanitary cultivation, and marketing practices (Geldreich and Bordner 1971; Khan et al. 1992). This study on a diarrhoeal outbreak is an additional proof of contamination of drinks due to unhygienic practices by the vendors.

In an outbreak of diarrhoea at Faridpur (Bareilly District) in June 1992, many people suffered from diarrhoea, out of which, six patients were hospitalised, and interrogated for details of the foods/drinks consumed within last 48 h. All of them reported consumption of orange juice as well as sugar cane juice from a nearby juice shop about 18-32 h before they fell ill, i.e., about 42 h before investigation. Many of the other residents were also questioned about illness in the locality. Among them, 18, 24 and 8 reported that-they had consumed orange juice, sugarcane juice, and both juices, respectively. Only those, who consumed either orange juice or both juices, reported 1-3 purging or mild laxative effect after an average of 24 h incubation period, but they all recovered naturally. There were 4 roadside vendors in the locality, among whom 2 were selling orange juice, and the other 2 sugar-cane juice. Dust blowing with each passing vehicle on the road was usual, and one of the orange juice shops was just 6 m away from the garbage heap.

The orange juice, sugarcane juice, ice, and water samples were collected aseptically in sterilized water sampling bottles from each vendor, along with water samples from houses of diarrhoeal patients, and hospital. In addition, stool samples from all the six hospitalised diarrhoeal patients were also collected. All the samples were brought to the laboratory in ice box and processed for isolation, and identification of enteropathogenic bacteria namely, *E. coli, Salmonella, Shigella, B. cereus, Staphylococcus aureus, Vibrio cholerae and Aeromonas*. Media used included eosine methylene-blue agar (Hi-Media), brilliant green agar (Hi-Media), *Salmonella shigella* agar (Hi-Media), *Bacillus cereus* agar with antibiotic suppleiment (Hi-Media), Baird-Parker agar (Hi-Media) with 10% fresh egg-yolk, thiosulphate citrate bile salt sucrose agar (Hi-Media), and xylose lysine desoxycholate agar (Hi-Media), respectively. The coliform count of orange juice, sugarcane juice, and water samples, and the aerobic plate counts for microbes in juices were also determined using MacConkey broth purple (Hi-Media), and plate count agar (Hi-Media) media,

* *Corresponding Author : [1]Present address : Department of Veterinary Microbiology, College of Veterinary Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar 263 145, India.*

TABLE 1: THE RESULTS OF ISOLATION OF ENTEROTOXIGENIC MICROORGANISMS ALONG WITH THEIR ANTIBIOGRAM FROM DIARRHOEAL PATIENTS AND DRINKS.

Type of samples (No of samples examined)	Range of SPC/ml sample	Range of coliform count/100 ml sample	Enteropathogens isolated (No of isolates)	Enterotoxigenicity results (mean values) RLILT (index)	VPFT (mm)	MFPT (index)	Antibiotic resistance pattern
Stool from patients (6)	NT	NT	*E. coli* UT (6)	1.8	20	2.2	CO,CN,DO,ST,TT,S
Orange juice (2)	$2x10^6$-$5.5x10^6$	$4x10^5$-$3x10^6$	*E. coli* UT (1)	1.8	20	2.2	CO,CN,DO,ST,TT,S
			E. coli UT (1)	0.9	10	1.28	ST, TT,
			Klebsiella pneumoniae sub sp *aerogenes* (2)	1.2	15	1.40	AM, TT, NF, CO,
Sugarcane juice (2)	$1.2x10^6$-$2.0x10^6$	$2.1x10^6$-$2.3x10^6$	*E. coli* UT (1)	1.0	12	1.30	ST,TT,
			E. coli 0:134 (1)	0.7	9	1.24	CN, ST, TT, NF
			K. pneumoniae sub sp *aerogenes* (2)	1.1	13	1.28	AM, TT, NF, CO,
Ice (1)	$3.6x10^2$	$1.8x10^1$	Nil	NT	NT	NT	NT
Water (10)	$2.2x10^2$-$1.8x10^2$	0	Nil	NT	NT	NT	NT

SPC : arrobic standard plate, NT : Not aealysed or tested, UT: remained untyped, RLILT : Rabbit ileal loop test (minimum positive index is 0.5), VPFT: rabbit skin test for vasopermeability factor (minumum zone of blueing to be considered positive is 7mm), MFPT: Mouse foot pad test (minimum index for positive test is 1.24), CO: cotrimoxazole, CN: carbenicillin; DO: Doxycycline, ST : Streptomycin, TT : Tetrycycline, S : Compound sulphonamides, NF : Nitrofuradentoin, AM : Ampicillin.

respectively (ICMSF 1975; Cruickshank et al. 1975). Serotyping of *E. coli* isolates was performed by standard tube agglutination method of Sojka (1965).

The enterotoxigenicity of the bacterial isolates was assessed in cell-free culture supernatant with rabbit ileal loop test (De and Chatterjee 1953), rabbit skin test (Evans et al.1973), and mouse foot pad test (Singh et al. 1992). The antibiotic sensitivity of the bacterial isolates was determined with standard disc method (WHO 1961), using standard 10 mcg discs of streptomycin, and gentamicin; 30 mcg discs of neomycin, carbenicillin, nalidixic acid, tetracycline, doxycycline and kanamycin; 25 mcg discs of cotrimoxazole; 300 mcg discs of nitrofuradentoin; 50 mcg discs of compound sulphonamides; and 300 I.U. discs of polymyxin B sulphate.

Escherichia coli strains were isolated from 4 juice, and 6 stool samples. However, they were not all alike in their enterotoxigenicity, and antibiogram (Table 1). Out of 10 isolates of *E. coli*, 6 isolates from stool samples, and one isolate from orange juice were having similar drug resistance pattern, equivocal response in bioassay models for enterotoxigenicity tests, and remained untyped. It could be concluded that they were alike due to similarity in their biochemical characteristics, antibiogram, and enterotoxigenicity potential. Hence, orange juice could be responsible for the diarrhoeal outbreak.

Escherichia coli strains were also isolated from an other orange juice, and two sugarcane juice samples. Out of these 3 isolates of *E. coli*, two had similar biochemical characteristics, and antibiogram, but with dissimilar enterotoxigenicity (Table 1). These also remained untyped. A weakly enterotoxigenic *E. coli* 0:134, isolated from a sugarcane juice sample, was resistant to four drugs, and was biotypically unique in fermenting both sucrose, and adonitol. Rest of the isolates of *E. coli* could not ferment both of these sugars.

Enterotoxigenic *K. pneumoniae* sub sp. *aerogenes* was isolated from sugarcane juice, and orange juice samples (Table 1). All the four isolates of *Klebsiella* were resistant to ampicillin, tetracycline, nitrofuradentoin and co-trimoxazole.

In recent years, *Klebsiella* has been shown to produce enterotoxin, which is serologically similar to *E. coli* (Singh and Kulshreshtha 1992), and is also a good recipient of R factors (Orskov 1984). These two characteristics together may play a substantial role in emergence of multiple drug resistant epidemic strains. The *Klebsiella* is a well known opportunistic pathogen, and nosocomial outbreaks have frequently been reported (Holmes and Gross 1990). Orange juice containing enterotoxigenic, and multiple drug resistant *Klebsiella* strain might have played an important role to cause the disease in hospitalised individuals.

who consumed the orange juice as a healthy drink. However, further investigations are necessary to prove this point.

It is evident from the results (Table 1) that microbiological qualities of all the juice samples were unsatisfactory with 1.2 x 10^6 to 5.5 x 10^6 SPC/ml, and 4 x 10^5 to 3 x 10^6 coliform count/ 100 ml. It showed a chance of taking infective (10^{-6}-10^{10} organisms) dose of *E. coli* (Jay 1987), through a glass of sugarcane juice or orange juice. But, diarrhoeal illness is usually not common and in the present case, it may be due to varying pathogenic potential of coliforms, particularly the enterotoxigenic *E. coli*.

Thanks are due to the Chief Medical Officer, Bareilly; Medical Officer, Faridpur; technical and supporting staff of foodborne infections, and intoxications laboratory of the institute; Head, Division of Veterinary Public Health; and Director for facilities, assistance, and help.

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Received 26 August 1993; revised 19 May 1995; accepted 12 July 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 507-509

Potential of Improving Protein Quality of Wheat *Chapati* by Addition of *Bathu* (*Chenopodiu album*), Carrot (*Daucus Carota*) and Radish (*Raphanus sativus*) Leaves

GEETA LUTHRA* AND BALWINDER SADANA

Department of Foods and Nutrition,
Punjab Agricultural University, Ludhiana-141 004, India.

Sag was prepared using *bathu*, carrot, radish, and spinach leaves. The supplementary effect of these *sags* on the protein quality of wheat *chapati* was evaluated by protein efficiency ratio, and chemical score methods. *Sags* supplemented with wheat *chapati* in the ratio of (1:4) improved the protein efficiency ratio of wheat *chapati*. Among the experimental diets, protein efficiency ratio, plasma proteins, total liver weight, and liver nitrogen were maximum, when wheat *chapati* was supplemented with *bathu sag*. The chemical scores of lysine, and tryptophan in case of wheat *chapati* improved from 37 to 57 and 81-87, respectively, when supplemented with these *sags*.

Keywords : Wheat *chapati*, Protein quality, Chemical score, Liver nitrogen, Plasma protein, Protein efficiency ratio, *Bathu*, Carrot leaves, Radish leaves.

Wheat has been a staple food of large segment of world population (Swaminathan 1968). About 80-90% of wheat consumption in India is in the form of *chapatis* (Austin and Ram 1971). Wheat contains about 14% proteins (Pant 1970), but is deficient in lysine, an essential amino acid (Sidhu et al. 1970). Leafy vegetables such as beet, knol khol, turnip, mustard, *raya* and spinach, contain 23 to 32% proteins on dry matter basis, and are well balanced with respect to essential amino acids, viz., isoleucine, leucine, and lysine (Sawhney and Kawatra 1986; Sehgal 1975). The high supplementary value of mustard, chinese cabbage, beet, and knol khol leaves to wheat *chapati* has been reported (Jagtinder 1985; Sawhney and Kawatra 1986). Fenugreek supplemented diets were shown to result in better calcium, and nitrogen retention, which was more than adequate to meet the daily requirement of young women, as compared to basal diet (Mann et al. 1991). *Bathu*, carrot, and radish leaves contain 21 to 47% proteins on dry matter basis, and are rich, and inexpensive sources of iron, calcium, β-carotene, and ascorbic acid (Gopalan et al. 1989). The supplementary value of these greens to cereals has not been studied so far. The present investigation was, therefore, undertaken to study the supplementary value of these leaves to wheat *chapati*.

Bathu, radish leaves, spinach, and wheat flour were purchased from local market. Carrot leaves were collected from the field of Doraha village in Ludhiana district.

Preparation of sample : Three different recipes were developed, using these leaves, and spinach *sag* was used as a standard. These recipes, i.e., a) *bathu sag* (100%), b) carrot, and spinach *sag* (2:1) and c) radish, and spinach *sag* (2:1), as well as with spinach *sag*, were prepared using standard methods (Luthra et al. 1993). Radish and carrot leaves were used in combination with spinach leaves, due to lower acceptability of 100% leaves by penelists. The ingredients used were green leaves 200 g; onion and tomato 50 g each; garlic, ginger, and green chillies 10 g each; maize flour 20 g; ghee 25 g; and seasonings according to taste. Standard *sag* recipes, and wheat *chapatis* were cooked in bulk, dried at 55°C to 6.5% final moisture in cabinet drier, and ground to fine flour in a Willey mill. Wheat *chapati*, and dried *sag* powders were mixed in a ratio of 4:1 for the animal experiments, and amino acid analysis.

Animal experiment : Six diets containing 10% proteins were prepared, and these included a *chapati* diet, four diets of *chapati*, and individual *sag* combinations, and a reference diet of skim milk (Table 1). Diets contained 1% vitamin (Raghuramulu et al. 1983), and 4% mineral (Raghuramulu et al. 1983) mixtures. The diets were fed *ad libitum* to weanling albino rats, caged individually (seven rats/group). The weights at the beginning of the experiment, daily record of food intake, and animal weights at weekly intervals were recorded. Protein efficiency ratio was calculated by the method of Chapman et al (1959). At the end of the experiment, the animals were anesthetised, blood was collected from aorta, centrifuged for plasma separation, and stored at-4°C. Livers were removed, cleaned, weighed, and stored in frozen state. Protein intake was calculated from food consumed (Chapman et al.

* *Corresponding Author*

TABLE 1. COMPOSITION OF TEST DIETS (g/100 g)

Ingredients	Diets					
	Skim milk	Wheat *chapati*	Wheat *chapati* + *bathu sag*	Wheat *chapati* + spinach carrot *sag*	Wheat *chapati* + spinach radish *sag*	Wheat *chapati* + spinach *sag*
Wheat *chapati* powder	-	83.30	68.50	70.00	69.00	70.00
Leaf powder	-	-	17.12	17.50	17.25	17.50
Cellulose	5.00	3.50	3.03	2.38	3.03	2.80
Groundnut oil	10.00	8.20	2.95	4.75	3.07	2.80
Skim milk	33.30	-	-	-	-	-

Each diet contained 1 and 4 g vitamin and salt mixtures, respectively, per 100 g diet. Starch was added in all the cases (except in wheat *chapati* diet group) to make up the weight of the diet to 100 g.

1959). Faeces were collected on last three days, and analysed for nitrogen by macro Kjeldhal method (AOAC 1985). Apparent protein digestibility (%) was calculated from food, and faecal nitrogen (Hawk and Oser 1965).

Chemical analysis : Wheat *chapati*, and its four combinations with *sags* (4:1, dry weight basis) were analyzed for lysine by the method of Carpenter (1960), as modified by Booth (1971). For the determination of tryptophan, the method of Concon (1975) was followed. Methionine was estimated by the colorimetric method of Horn et al (1946), while cystine was estimated in the acid hydrolysate by the method of Liddell and Saville (1959). Chemical scores were calculated using the provisional amino acid scoring pattern of FAO/WHO (1973). Plasma protein was estimated by biuret method of Robinson and Hogden (1940). Liver nitrogen, and faecal nitrogen were estimated by macro Kjeldhal method (AOAC 1985).

Statistical analysis : The analysis of variance technique (Walter 1955) was applied to test the significance of differences between various groups, and critical difference was calculated.

Supplementation of wheat *chapati* with different *sags* improved the protein efficiency ratio of wheat *chapati* from 1.04 to 1.38 (Table 2), highest being in *bathu sag* supplemented group, and lowest in mixed radish *sag* supplemented group. Garcha et al (1971) showed increases in protein efficiency ratio values of wheat flour from 0.89 to 1.22 in mustard, and 1.24 in spinach supplemented diet. Sawhney and Kawatra (1986) reported that protein efficiency ratio of wheat *chapati* improved from 1.21 to 1.65, when supplemented with beet leaves in the ratio of 4:1. However, the PER of radish *sag*

TABLE 2. PROTEIN EFFICIENCY RATIO, APPARENT DIGESTIBILITY LIVER WEIGHT, LIVER NITROGEN AND CHEMICAL SCORES OF RATS FED DIFFERENT DIETS

Parameters	Diets						F values	CD values
	Skim milk	Wheat *chapati*	Wheat *chapati* + *bathu sag*	Wheat *chapati* + spinach carrot *sag*	Wheat *chapati* + spinach radish *sag*	Wheat *chapati* + spinach *sag*		
Protein efficiency ratio (PER)	2.83 ±0.26	1.04 ±0.04	1.38 ±0.02	1.19 ±0.01	0.97 ±0.04	1.15 ±0.07	8.9	0.89
Plasma proteins*, g/100 ml	4.8	3.5	4.6	4.4	3.0	4.3	-	-
Apparent digestibility,%	88.10 ±0.95	81.55 ±0.83	82.28 ±0.99	82.14 ±0.20	81.51 ±0.33	81.55 ±0.53	18.36	2.28
Total liver weight, g	2.38 ±0.23	1.41 ±0.05	1.90 ±0.11	1.55 ±0.17	1.37 ±0.04	1.53 ±0.11	9.8	0.44
Total liver nitrogen, g	65.02 ±7.86	28.22 ±1.64	38.20 ±4.04	42.30 ±3.17	27.67 ±2.36	37.26 ±1.18	11.25	14.14
Chemical scores								
Lysine	-	37	57	40	36	43	-	-
Tryptophan	-	81	77	87	85	82	-	-
Cystine	-	91	74	74	74	75	-	-
Methionine	-	49	43	46	52	48	-	-

* Analysis of plasma protein was done from pooled samples.

fed group was found to be less than that of wheat *chapati* because of the lower lysine content of radish leaves, than that of wheat *chapati*. The difference in protein efficiency ratio values within the experimental diets were non-significant, whereas there were significant differences ($P<0.05$) in protein efficiency ratio values of all the experimental diets as compared to control (skim milk) diet. Plasma protein content was also highest in case of *bathu sag* supplemented group. Sawhney and Kawatra (1986) reported an increase in plasma protein concentration from 4.66 to 6.10 g/100 ml, when wheat *chapati* was supplemented with beet leaves *sag* in the ratio of 4:1.

Apparent protein digestibility % of wheat *chapati* improved, when supplemented with *bathu sag* in the ratio of 4:1. There were non-significant differences among experimental diets. The total liver weights of the rats fed different experimental diets ranged from 1.37±0.04 to 1.90±0.11. There were significant increases in liver weights of the rats of *bathu* group, as compared to those fed wheat *chapati* alone. The liver nitrogen was highest in case of mixed carrot *sag*. There was a significant improvement in total liver nitrogen of *bathu*, and carrot *sag* fed group, as compared to the rats fed wheat *chapati*.

With supplementation of *bathu sag*, the chemical scores of wheat *chapati* for lysine content improved from 37-57, but mixed radish *sag* did not show any improvement (Table 2). Scores for tryptophan were found to be maximum, when wheat *chapati* was supplemented with carrot-spinach *sag*. Chemical scores of cystine, and methionine in case of wheat *chapati* did not show any improvement. This may be due to higher cystine, and methionine contents of wheat *chapati*, as compared to *sag* recipes. Among all green leafy preparations, the supplementary effect of *bathu* leaves to wheat *chapati* was maximum in terms of protein quality parameters, followed by that in case of carrot leaves supplemented *chapati*.

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Received 22 July 1993; revised 16 August 1995; accepted 7 September 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 510-512

Some Physico-chemical Properties of Tanzanian Commercial *Acacia* Gums (Gum Arabic)

G.S. MHINZI* AND D.M.S. MOSHA

Department of Chemistry,
University of Dar-es-Salaaam, P.O. Box 35061, Dar-es-Salaam, Tanzania, East Africa.

The properties of Tanzanian commercial *Acacia* gums were compared with commercial *Acacia* gum samples from Sudan. The solubility, and average insoluble gel contents (cold water insoluble gel, and hot water insoluble gel) of Tanzanian export grade *Acacia*È gums were similar to those of Sudanese *Acacia* gums, but were not similar to Sudanese *tahl* gum as had been previously asserted. However, the ash, alkaline earth metal contents were lower than those of Sudanese gum arabic. The average viscosity of Tanzanian export grade *Acacia* gums was approximately one half that of Sudanese gum arabic at concentration of 100 g/l.

Keywords : Commercial *Acacia* gums, Solubility, Viscosity, Alkaline earth metals.

At least 900 species of the genus *Acacia* have been identified (Glicksman 1983), out of which only a few produce gum of commercial importance. Commercial *Acacia* gum (gum arabic) from Sudan is derived mainly from *Acacia senegal. Acacia seyal* is known to produce a somewhat lower quality gum, which commands a lower price (Adamson and Bell 1974). The properties of *Aracia* gums vary widely depending on the species, geographical location, and age of plant (Phillips et al. 1980). Gum arabic is more soluble in water, as compared to other tree exudate gums (e.g. karaya gum), and it is possible to prepare solutions of up to 55% concentration (Glicksman 1983). Tanzania is known to have the capacity of producing more than 2000 tonnes of *Acacia* gums per annum (Annual Trade Reports 1921-86). However, the current output has declined to below 500 tonnes per annum. The precise reason for this decline is not clear. Potentiometric titrimetry (Mhinzi and Mosha 1993) has shown that the bulk of the commercial product is derived mainly from *Acacia drepanolobium.* It has been suggested (Adamson and Bell 1974) that Tanzanian commercial gum arabic is less soluble, and is similar to *tahl* gum. The present communication reports the physico-chemical properties of Tanzanian and Sudanese commercial *Acacia* gums.

Origin of samples : The Tanzanian commercial gum arabic samples were bought directly from traders in the gum producing regions (Dodoma, Singida and Tabora). The Sudanese cleaned, and sifted grade samples were supplied by Messrs. Arthur Branwell & Co. Ltd., London.

Sample preparation : The samples were milled to pass through a 2 mm sieve, spread out in trays for seven days to allow them to equilibrate with atmospheric moisture, and then stored in the air-tight containers.

Experimental procedure : Moisture content, insoluble matter, and ash were determined by standard methods (British pharmacopoeia 1980). Cold water insoluble gel content was determined as the insoluble fraction, when a gum sample was stirred in cold deionized water for two hours, whilst hot water insoluble gel was the fraction, which was insoluble in hot deionized water at 92–95°C. Calcium, and magnesium contents were determined by an atomic absorption spectrophotometer, whereas viscosity was determined at 30*C, using Ubbleholde suspended level capillary viscometers. The optical density was determined, using a Griffin Model 40 colorimeter.

In general, the solubility of Tanzanian export grade (grade-1) gum arabic is comparable to Sudanese gum arabic (Table 1). The average cold water insoluble gel, and hot water insoluble gel for Tanzanian export grade *Acacia* gums are very similar to those for Sudanese gum arabic. The insoluble gel fraction is very small, as compared to *Albizia zygia* gum, which contains 80% cold water soluble fraction, and 20% insoluble gel (Ashton et al. 1975) or *Khaya grandifoliola* gum, which contains a cold water insoluble gel of about 70% (Aslam et al. 1978a). Some tree exudate gums (e.g., gum karaya, gum tragacanth) are water-swellable, rather than water-soluble, and absorb water to form viscous dispersions at low concentrations (Glicksman 1983). The present study shows that the solubility of Tanzanian commercial gum arabic is very similar to that of Sudanese gum arabic, and superior to *tahl* gum. Thus, the average insoluble gel content of Tanzanian export grade *Acacia* gums (*vide supra*) is about 20% (one fifth) that of Sudanese *tahl* grade

* *Corresponding Author*

TABLE 1. SOME PHYSICO-CHEMICAL PROPERTIES OF TANZANIAN AND SUDANESE *ACACIA* GUMS

Sample	Grade	Moisture, % (w/w)	Ash, % (w/w)	Insoluble matter, % (w/w)	CWIG, %(w/w)	HWIG, % (w/w)	Calcium, g/100 g	Magnesium, g/100 g	Optical density	Viscosity (centipoise), 100 g/l	150 g/l
Tanzanian											
T1	1	14.3	1.9	0.1	0.2	0.2	0.72	0.09	0.12	3.38	8.48
T2	1	14.4	2.0	0.1	0.2	0.2	0.55	0.10	0.16	3.86	9.16
T3	1	14.4	1.4	0.1	0.2	0.2	-	-	0.14	2.56	7.62
T4	1	14.2	1.8	0.1	0.2	0.1	-	-	0.10	3.44	7.86
T5	1	13.8	1.9	0.2	0.4	0.4	0.51	0.13	0.06	4.07	9.85
T6	1	13.9	1.5	0.2	0.4	0.3	0.52	0.07	0.05	3.48	8.48
T7	2	12.6	2.5	1.2	0.8	0.6	-	-	0.28	3.80	9.29
T8	2	13.6	2.2	0.6	2.0	1.9	-	-	0.19	6.70	15.66
Sudanese											
S1	Cleaned	13.8	3.7	0.6	0.2	0.1	0.75	0.25	0.06	7.30	14.62
S2	Cleaned and sifted	14.1	3.3	0.2	0.2	0.1	-	-	0.08	6.80	14.00
S3	*Tahl*	14.9	5.3	-	1.1	1.0	1.25	0.17	0.41	9.00	20.57

CWIG Cold water insoluble gel. HWIG = Hot water insoluble gel.

gum (Table 1). The precise reason for the formation of varying amounts of insoluble gel by gums from *Acacia* is still unclear. However, the gel fraction has been shown (Anderson and Dea 1968) to possess a higher molecular weight than the soluble fraction.

The average viscosity of Tanzanian commercial gum arabic is lower than that of Sudanese gum arabic at the same concentration (Fig. 1). The ability of forming less viscous solutions even at high concentrations makes gum arabic an excellent stabilizing, and emulsifying agent, when incorporated with large amounts of insoluble materials (Glicksman 1983). The viscosity of Tanzanian grade 1 gum arabic is reproducible, and does not vary greatly from batch to batch (Table 1), as has been observed in gum *ghatti* (Jefferies et al. 1977). It has been suggested that the low viscosity of *Acacia* gums may be attributed to the complex, highly branched nature of the *Acacia* gum molecules, which are closely packed rather than linear (Glicksman 1983).

In agreement with previous reports (Mhinzi and Mosha 1993; Artaud et al. 1977), the results in Table 1 show a higher proportion of calcium, as compared to magnesium, for each sample. However, the average alkaline earth metal content in Tanzanian commercial gum arabic is lower than that in Sudanese gum arabic. These results also show lower levels of calcium, and magnesium in Tanzanian commercial gum arabic, as compared to previously reported data for *Acacia* gums (Artaud et al. 1977). The addition of calcium ions is known to enhance the viscosity of aqueous solutions of *Khaya grandifoliola* gum (Aslam et al. 1978a,b).

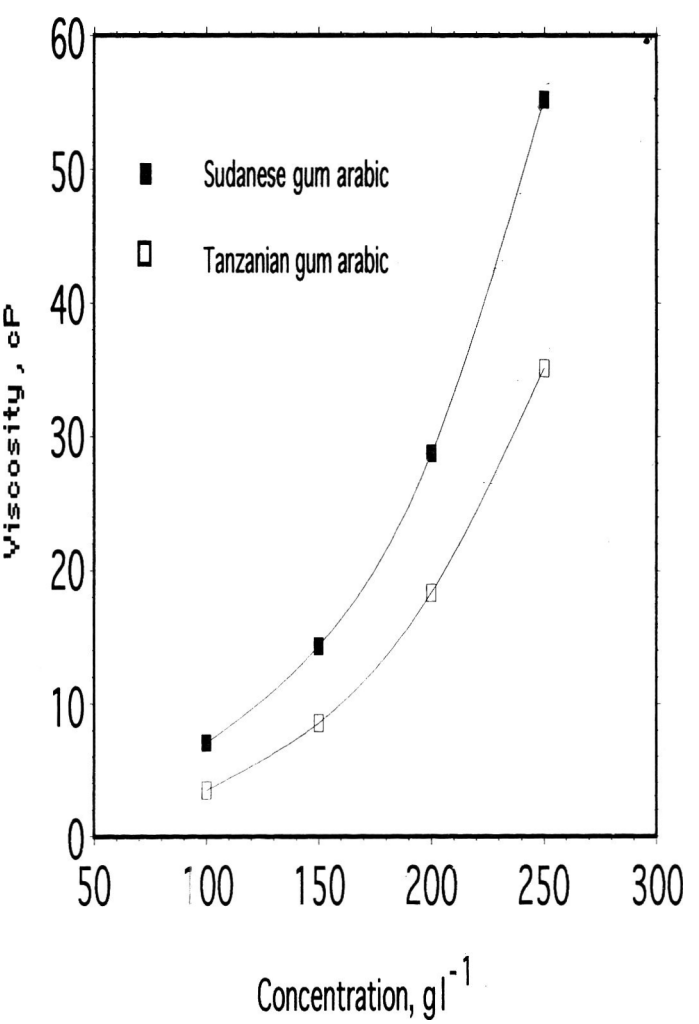

Fig. 1: The viscosity of Tanzanian and Sudanese commercial Acacia gums at 30°C.

However, this has not been conclusively established for *Acacia* gums.

In conclusion, the results presented in this communication show that some properties of Tanzanian export grade commercial gum arabic are comparable to those of Sudanese gum arabic and can compete in various industrial applications.

The authors thank NORAD for financial support and the British Council for provision of a Landrover for sample collection.

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Received 15 September 1994; revised 1 July 1995; accepted 12 September 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 513-515

Vitamin C Contents of Processed Vegetables

S.B. TAPADIA, A.B. ARYA* AND P. ROHINI DEVI

Department of Foods and Nutrition, College of Home Science,
Marathwada Agricultural University, Parbhani - 431 402, India.

Vitamin C contents of four green leafy vegetables, viz., *ambat chuka (Rumex vesicarius)*, coriander leaves *(Coriandrum sativum)*, fenugreek leaves *(Trigonella-foenumgraecum)*, and spinach *(Spinacia oleracea)* as well as three non-leafy vegetables, viz., brinjal *(Solanum melongena)*, clusterbean *(Cyamopsis tetragonoloba)* and *tinda (Citrullus vulgaris)* were estimated, after subjecting to different processing techniques, such as cutting, washing, washing after and before cutting and cooking with and without the use of the lid. Maximum vitamin C content was recorded in coriander leaves, followed by fenugreek leaves, spinach, *ambat chuka*, clusterbean, *tinda* and brinjal. Vitamin C content was significantly high in the vegetables processed without stalk, except for fenugreek leaves, which showed more vitamin C, when used with stalk. Cooking without lid, and washing after cutting showed significant losses of vitamin C.

Keywords : Vitamin C, Leafy vegetables, Non-leafy vegetables, Cutting, Washing, Cooking.

Vegetables are often subjected to different processings at household or industrial processing levels. Losses of vitamins and minerals during processing can occur either due to oxidation or by dissolving into water (Vail et al. 1978). Water soluble vitamin C gets destroyed easily during washing, and cooking (Bord Bury and Singh 1986; Khader 1988). The present study aims at determining the losses of vitamin C during household processings of vegetables.

Commonly consumed and locally available four green leafy vegetables, viz., *ambat chuka (Rumex vesicarius)*, coriander leaves *(Coriandrum salivum)*, fenugreek leaves *(Trigonella foenumgraecum)* and spinach *(Spinacia oleracea)*, in addition to three other vegetables, viz., brinjal *(Solanum melongena)*, cluster bean *(Cyamposis tetragonoloba)* and *tinda (Citrullus vulgaris)* were selected for the study.

Two variations in processing of each vegetable were used, based on its parts. Leafy vegetables and brinjal with stalk, cluster bean with rib and *tinda* with peel were classified under variation I (W_1). Leafy vegetables and brinjal without stalk, cluster bean without rib and *tinda* without peel were placed under variation II (W_2).

The details of the processing techniques as used in the study are as follows :

a) *Vegetable as such (P_1) :* All the vegetables were used without application of any processing technique except in case of brinjal and *tinda*, which were cut into pieces of 5 cm (length size).

b) *Cutting (P_2) :* Vegetables were cut into pieces of 2.5 x 2.5cm size, using stainless steel knife.

c) *Washing (P_3) :* The selected sample was washed in 10 ml tap water.

d) *Washing after cutting (P_4) :* Vegetables were first cut, and then washed in 10 ml tap water.

e) *Washing before cutting (P_5) :* Vegetables were first washed in 10 ml tap water, and then cut.

f) *Cooking with the use of the lid (P_6) :* Leafy vegetables were cooked with the use of lid in 10 ml water for 2 min and other vegetables for 3-8 min.

g) *Cooking without the use of the lid (P_7) :* Vegetables were cooked as above, but without using the lid.

The sample size used for application of processing techniques was 2 g. Same quantity was used for estimation of vitamin C by 2-6 dichloro-indophenol method (Raghuramlu et al. 1983) in triplicate. The data were statistically analyzed by employing analysis of variance (Panse and Sukhatme 1976).

Among leafy vegetables, coriander leaves showed relatively high vitamin C content, followed by fenugreek leaves, spinach and *ambat chuka*, while cluster bean contained maximum vitamin C, followed by *tinda* and brinjal.

The selected destalked leafy vegetables showed significantly higher vitamin C, as against vegetables with stalk, except for fenugreek leaves. In case of other vegetables, significantly greater values of vitamin C were recorded for brinjal with stalk, cluster bean with rib and *tinda* with peel, when compared with brinjal without stalk, clusterbean without rib and *tinda* without peel (Table 1). Significantly higher vitamin C in destalked leafy vegetables (i.e., only leaves) could be suggestive of higher concentrations of vitamin C in leaves. While the reduction in vitamin C of other destalked vegetables may be due to oxidation of vitamin C during peeling, cutting and removing the stalk.

* Corresponding Author

TABLE 1. VITAMIN C CONTENT OF SELECTED VEGETABLES (mg/100 g) DURING PROCESSING

Vegetables		Variations		Processing techniques						
		W_1	W_2	P_1	P_2	P_3	P_4	P_5	P_6	P_7
Leafy vegetables										
Ambat chuka	(V_1)	12.9	20.6	28.8	21.4	24.5	10.9	17.4	10.6	4.3
Coriander leaves	(V_2)	77.9	88.6	100.3	89.7	91.8	86.4	88.0	64.1	62.9
Fenugreek leaves	(V_3)	70.3	60.4	91.5	84.4	87.7	48.2	82.1	36.1	27.9
Spinach	(V_4)	19.3	25.4	34.4	22.4	30.9	15.9	24.8	17.1	11.2
Other vegetables										
Brinjal	(V_5)	10.6	6.7	15.6	11.2	13.1	6.6	10.4	3.0	0.6
Cluster bean	(V_6)	24.9	18.8	36.2	26.7	32.2	16.7	21.7	10.1	8.8
Tinda	(V_7)	10.5	10.1	18.9	14.6	17.0	7.5	9.8	3.7	0.9

	Source	SE ±	CD at 5%
Leafy vegetables	V	0.18	0.52
	W	0.13	0.37
	V x W	0.26	0.73
	P	0.24	0.69
	V x P	0.49	1.38
Other vegetables	V	0.16	0.46
	W	0.13	0.38
	V x W	0.23	0.65
	P	0.25	0.70
	V x P	0.43	1.22

The losses of vitamin C due to peeling, washing and cooking were also reported by Oteng et al (1987).

Maximum retention of vitamin C was observed, when vegetables were washed, followed by cutting operation. Loss of vitamin C was higher, when the vegetables were cooked without using the lid. Cooking without the use of the lid resulted in maximum loss of vitamin C in all the vegetables, followed by the processing, involving washing after cutting and just cutting. This might be because of the increased activity of ascorbic acid oxidizing enzymes due to heating, which leads to destruction of ascorbic acid and leaching of the vitamin C in washing water. Similar results were obtained by Padma et al (1982), Bord Bury and Singh (1986), Khader (1988) and Oteng et al (1987).

The processing techniques, when coupled with leafy vegetable variation, showed that variation W_1 had significantly low vitamin C content in case of all the processing techniques, when compared with variation W_2 (Table 2). However, cooking without the use of lid had no significant effect on vitamin C, when combined with variations. In other vegetables, it was found that the coupling of variation W_2 with all processing techniques, the content of vitamin C was significantly less, as compared to that in case of variation W_1. This might be because of discarded outer skin, stalk or rib of the vegetables. The results are in line with those of Vail et al (1978).

The interactive effects between vegetable variations and processing techniques indicated that the vitamin C was lowered significantly in destalked *ambat chuka*, coriander leaves and spinach, when subjected to processings other than those in vegetables as such, except in case of fenugreek leaves with stalk. All other vegetables failed to retain vitamin C significantly, when subjected to processing techniques. The loss of vitamin C in the following decreasing order: cooking > cutting and washing > cutting > washing.

It can be concluded that maximum loss of vitamin C occurred in the process of cooking, followed by the combined process of washing and cutting.

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TABLE 2. EFFECT OF INTERACTION BETWEEN VEGETABLES, VEGETABLE VARIATIONS AND PROCESSING TECHNIQUES ON VITAMIN C CONTENT (mg/100 g)

Variations	Processing techniques							
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	Mean
				Leafy vegetables				
W_1	61.9	53.3	56.3	35.7	51.8	31.0	26.2	42.2
W_2	65.7	55.6	61.1	42.0	54.3	32.6	26.9	48.8
V_1W_1	23.1	15.3	18.8	7.1	13.1	8.5	4.6	13.0
V_1W_2	34.5	27.6	30.3	14.6	21.7	11.7	3.9	20.6
V_2W_1	96.6	84.4	86.8	80.6	84.4	59.3	53.7	78.0
V_2W_2	104.0	94.9	96.8	92.1	91.9	72.1	63.9	88.0
V_3W_1	97.2	90.3	93.1	41.8	89.3	43.1	38.1	70.4
V_3W_2	85.8	78.4	82.2	54.5	75.8	29.1	17.5	60.3
V_4W_1	30.5	23.1	26.6	13.1	20.2	13.1	8.5	19.3
V_4W_2	38.4	21.7	35.2	18.8	29.4	21.0	13.9	25.4
				Other vegetables				
W_1	25.6	18.0	22.3	15.1	15.0	6.2	4.7	15.3
W_2	21.6	16.0	19.1	9.4	12.8	4.5	3.0	11.9
V_5W_1	18.1	13.1	15.3	9.2	12.4	4.6	1.2	10.6
V_5W_2	13.1	9.2	10.9	3.9	8.3	1.4	0.0	6.7
V_6W_1	41.9	31.8	37.1	18.8	22.4	13.8	9.4	24.1
V_6W_2	30.0	21.9	27.3	14.6	20.9	9.4	7.1	18.8
V_7W_1	21.6	15.5	18.8	9.5	11.7	4.6	1.1	10.5
V_7W_2	16.1	13.1	15.6	5.5	7.8	2.6	0.8	10.1

	Source	SE ±	CD at 5%
Leafy vegetables	P x W	0.35	0.95
	V x P x W	0.70	1.95
Other vegetables	P x W	0.35	0.99
	V x P x W	0.62	1.72

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Received 19 April 1994; revised 3 July 1995; accepted 12 September 1995

J. Food Sci. Technol., 1995, Vol. 32, No. 6, 516-518

Microbiological and Biochemical Changes during the Production of *Sekete* - A Fermented Beverage Made from Maize

G.O. ADEGOKE*, R.N. NWAIGWE AND G.B. OGUNTIMEIN

Department of Food Technology, University of Ibadan, Ibadan. Nigeria.

At the early stages of *sekete* fermentation, *Leuconostoc mesenteroides*, *Streptococcus* spp., *Pediococcus cerevisiae* and *Saccharomyces* spp. were dominant. *Lactobacillus delbruecki*, *Bacillus subtilis* and *Saccharomyces* spp. were isolated after 24 h fermentation. *Lact. brevis* and *Propionibacterium* spp. dominated the fermentation of *sekete* after 48 h. During the fermentation process, the pH of the grains decreased from 5.1 to 4.3 and the titratable acidity increased from 0.1% (0 h) to 0.4% (48 h). Iron, phosphorus, potassium, sodium and calcium increased initially and then decreased as fermentation progressed. Total sugars decreased from 0.3 to 0.1 mg/g, while the alcohol content of *sekete* increased from 0 to 4.0% after 48 h fermentation.

Keywords : Fermentation, *Sekete*, Maize, Microbiological and chemical changes.

In Nigeria, fermented maize products are used widely for breakfast, and as weaning foods for babies (Akinrele 1970). Traditionally, *sekete*, a fermented beverage from maize is produced by steeping maize in water, followed by draining and air-drying in a broken clay pot lined with banana leaves. The grains are moistened for 3 to 5 days for germination and the malted grains are ground in mortar. The coarse particles obtained are mixed with water (1:2) in a clay pot, which is later brought to boil. The liquid so obtained is cooled, filtered, reheated for about 15 min, and then bottled as *sekete* (Sawyerr 1988). With many indigenous fermented foods from the tropics being products of great antiquity (Stanton 1985), and with the paucity of data on *sekete*, the changes that take place during fermentation of this alcoholic cereal beverage are described.

Maize grains were obtained from the Institute of Agricultural Research and Training, Ibadan, Nigeria. Production of *sekete* was carried out in the laboratory as per the process flow chart shown in Fig. 1. For the identification of organisms isolated during fermentation of *sekete*, the following phenotypic tests were done, viz., Gram staining, growth characteristics on culture media, motility, catalase and carbohydrate fermentation tests (ICMSF 1978). Thereafter, bacteria were identified according to the methods recommended by Krieg and Holt (1984), while yeasts were identified using the methods of Davenport (1987). During the fermentation of *sekete*, proteins, fat crude fibre, ash, alcohol, dry matter, mineral and moisture contents were determined by using AOAC (1975)

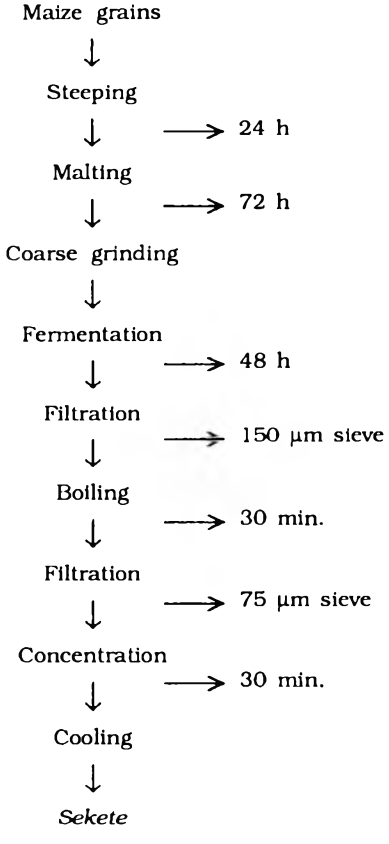

Fig. 1. Process flow chart for the laboratory production of *sekete*

methods. Total sugars were determined as described by Dubois et al (1956), and pH of the fermenting beverage was measured every 12 h.

While *B. subtilis*, *Lact. delbrueckii* and *Saccharomyces* spp. were isolated after 24 h fermentation, *Leuc. mesenteroides*, *Ped. cerevisiae* and *Streptococcus* spp. were isolated at the beginning of fermentation (Table 1). Thereafter, *Lact. brevis* and *Propionibacterium* spp. dominated the

* *Corresponding Author*

TABLE 1. MICROBIAL SUCCESSION DURING FERMENTATION OF *Sekete*

Fermentation period, h	Aerobic bacteria	Yeasts
0	*L. mesenteroides* *P. cerevisiae* *Streptococcus* spp.	*Saccharomyces* spp.
24	*Lact. delbrueckii* *B. subtilis*	*Saccharomyces* spp.
48	*Lact. brevis* *Propionibacterium* spp.	

fermentation of *sekete*. The presence of lactic acid bacteria during fermentation of *sekete* is not a specific case, as these bacteria are reported to be present in other foods also. For example, *Lact. brevis* and *Lact. fermentum* have been found during the fermentation of sauerkraut, pickles and sour dough bread made from rye (Spicher 1960). Yeasts and lactic acid bacteria have been reported in *dosa* fermentation (Soni et al. 1986). Prior to malting, *Aspergillus niger*, *A. flavus* and *Rhizopus* spp. were isolated from the maize grains used for the production of *sekete*. Microbial populations increased during the fermentation of *sekete* (Fig. 2). While the presence of *A. flavus* is not desirable because of its association with the production of toxic metabolites, nonetheless, *A. flavus*, *A. niger* and *Rhizopus* spp. have also been isolated during fermentation of Oriental *bungkil* (Rahayu et al. 1986). During fermentation of *sekete*, there were changes in the pH and titrable acidity levels (Fig. 2). These changes were probably due to the high level of acid produced during the fermentation process.

Total sugar content of the fermenting mass increased within the first hours, but it decreased towards the end of fermentation (Fig. 3). This decrease could be attributed to the utilization of

TABLE 2. BIOCHEMICAL CHANGES DURING THE FERMENTATION OF *Sekete*

Parameter	Fermenting maize		Fermented mass	*Sekete*
	0 h	24 h	48 h	
Dry matter,%	30.9*	30.9	30.9	20.1
Proteins, %	0.4	0.4	0.9	1.1
Ash, %	1.4	1.4	3.6	3.6
Calcium, ppm	400.0	100.0	0.0	0.0
Sodium, ppm	400.0	400.0	400.0	500.0
Potassium, ppm	500.0	500.0	500.0	300.0
Iron, ppm	60.0	90.0	60.0	60.0
Phosphorus, ppm	360.0	450.0	0.0	0.0

* Mean of 5 replicates

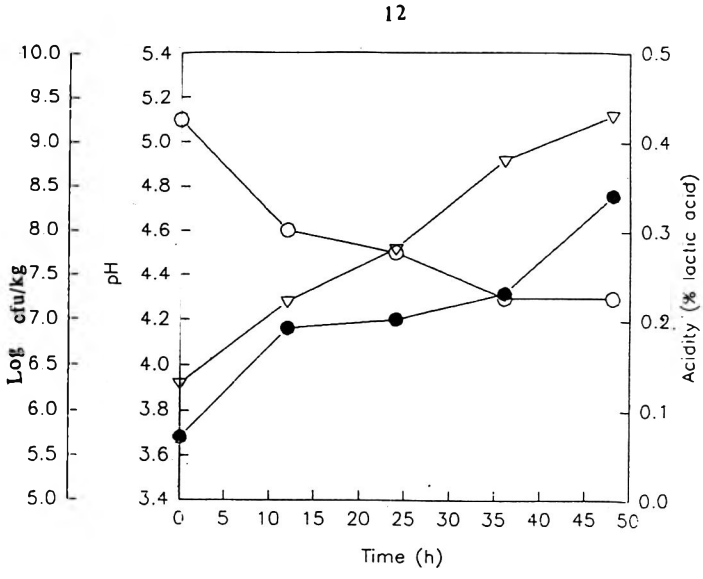

Fig. 2 Acidity (▽), pH (○) and growth (●) profiles during fermentation of *sekete*.

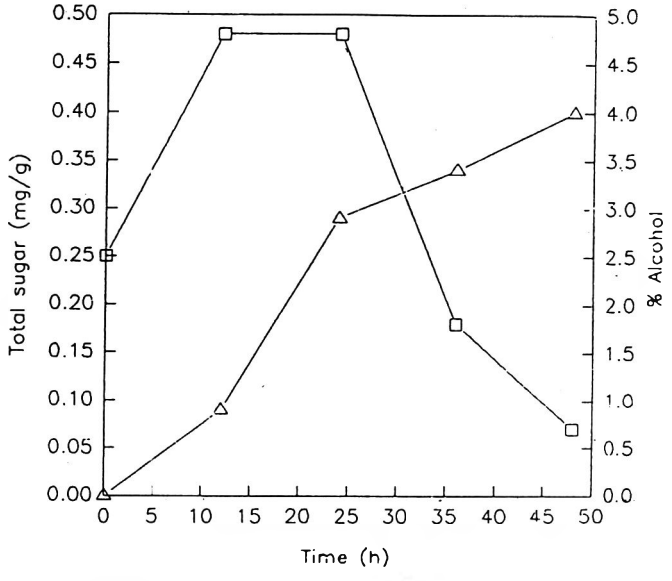

Fig. 3 Alcohol (△) and total sugar (□) profiles during fermentation of *sekete*.

the sugars by the fermenting microflora of *sekete*, as microorganisms are known to depend on reducing sugars for their metabolic processes. Increase in the protein content of *sekete* during production (Table 2) might be due to synthesis of enzymes and other proteins by the fermenting microflora of *sekete*. However, decreases in the mineral content of *sekete* during the fermentation process (Table 2), could be attributed to the utilization of these minerals for cell synthesis by the fermenting microflora.

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Received 19 April 1994, revised 19 July 1995; accepted 9 September 1995

Control of Fish Quality. J.J. Connell. 4th edition. 1995. Fishing News Books. Blackwell Science Ltd., Osney Head, Oxford OX 2 OEL. U.K. pp. 192, Price UK £ 25/-

Quality of fishery products, unlike other food items, is difficult to define because of such diverse variations in fish species including crustacea and molluscs, their habitats and sensitivities to environmental conditions. Fish, from catch to consumption, is also prone to contamination by a variety of microorganisms, which affect their quality during handling and storage. Fishery products over the past few decades have become a major item of international trade. The items include fresh, frozen, cooked, dried items marketed whole, as fillets, or parts, breaded, minced, salted, pickled or smoked. Therefore, increasing attempts are being made to define quality standards of fishery products with respect to freshness, microbial safety and environmental pollution. The present book is an attempt to present in a non-technical language, a balanced view of the different aspects of quality of fish and fishery products and the measures, which can be taken to regulate them. The book is generally aimed at conventional products such as, frozen and fresh fish, while special products such as, minces, fish oils, protein concentrates etc. are outside the scope of the book.

The first and second chapters deal with the quality terminology such as, quality assurance and quality control. This is followed by description of intrinsic qualities with respect to species, size, sex, composition, parasites, natural toxicity, and environmental contamination. Chapters 3 and 4 discuss the various ways in which quality deterioration takes place in fish products. Suggestions for proper icing, freezing, smoking, canning, salting, drying etc., have also been provided. Chapter 5, although deals with the important topic of food safety, is only a three page text with two tables, indicating incidence and severity of risks from different products and causes. Microbial aspects of fish quality including incidence of different pathogens and their control are surveyed in Chapter 6. In addition, some passing mention to nutritive value, additives, yields, and packaging and labelling are also given. Methods of quality evaluation are briefly covered in Chapter 7 such as, chemical, microbiological and instrumental methods. Some information is provided on quality assurance, HACCP and regulatory inspections in chapter 8. Brief coverage of official inspection requirements in certain countries (Chapter 8), and standards (Chapter 9) are likely to be interesting to the industry, particularly the 'model purchase specification' and 'scoring system for assessing freshness' of some fish species (Appendix I). Chapter 10 is on 'Code of Practice'. The fish exporter may also be interested in the United States standards for grades of whole or dressed fish (Appendix 4), and the recommended microbiological limits for seafoods (Appendix 5).

The book can serve as a handbook to fish processors, exporters and regulatory inspectors concerned with the quality, and safety aspects of major fishery products. Researchers, specialists and students interested in specific details may have to look for other sources for in-depth informations. Many of these sources are given at the end of the book.

V. VENUGOPAL
BHABHA ATOMIC RESEARCH CENTRE
BOMBAY - 400 085

Meat and Meat Products - Technology, Chemistry and Microbiology. Volume-3, Food Products Series, by Alan H. Varnam and Jane P. Sutherland; Published by Chapman & Hall, 2-6 Boundary Row, London 8E18HN, U.K. Printed in Great Britain by St. Edmundsbury Press, Bury St. Edmunds, Suffolk. 1995 pp, 430, Price £ 24.99/-

This book is in a paper back, and has been printed on a permanent acid-free paper. It consists of 430 pages, divided into nine chapters, further reading and index. It covers all the three basic disciplines-Technology, Chemistry and Microbiology, without whose knowledge it is very difficult to understand and appreciate the science of Meat and Meat Products. A special feature of this book is "Exercises" at the end of each chapter, which will help the reader to transform from an acquirer of knowledge to a user. Most of these exercises are based on real situations and are brain storming.

Chapter 1, which is 'Introduction' deals with importance of meat in human diet and nutrition, animal and birds exported for meat production; composition and quality attributes of meat; structure and functions of muscle; lipid, water, colour and flavour of meat as well as pathogens, which can be transmitted through meat. Chapter 2 deals with "Conversion of Muscle into Meat" covering various aspects, starting with pre-slaughter treatments;

slaughter techniques; electrical stimulation; processing of meat; relationship between technology and post-mortem changes; biochemical changes during maturation, spoilage, grading, quality control and quality assurance. Uncooked, comminuted and reformed meat products have been discussed in chapter 3. It covers the selection of ingredients for various types of comminuted and reformed products; variation in processing; chemical and physical changes during comminution and structure of comminuted meat products, their spoilage and role of preservatives.

Chapters 4, 5, 6 and 7 cover various cured, cooked type meat products. Chapter 7, however, exclusively deals with fermented sausages. Chapter 4 discusses various curing techniques; cured and smoked meats; chemistry of curing; risk of nitrosamine formation; effect of sodium chloride in meat structure; microbial spoilage as well as public health hazards associated with cured meats. The cooked meat and meat products have been described in chapter 5. It also discusses the industrial scale cooking and post-cooking handling of meat and meat products. The details for cooked cured meat products, which form a major chunk of cured meats have been given in Chapter 6. It discusses technology, chemistry and microbiology of cooked cured meat products. Under technology, different types of cooked cured meats e.g., ham, bacon, poultry, tongues etc. have been discussed. It also contains manufacture processes; quality assurance and control; nature of cured meat pigments; role of nitrate in inhibiting the development of warmed-over flavour as well as public health and spoilage aspects of these products. The nature of various types of fermented sausages, their manufacturing technology, role of lactic acid bacteria during fermentation; quality assurance and control; chemical changes occurring during fermentation; their nature of flavour and aroma as well as the spoilage, and microbiological hazards associated with fermented sausages form a part of chapter 7.

Frozen, dried and intermediate meats and meat products have been covered in chapter 8 and 9. Chapter 8 gives various types of frozen meat and meat products; basic principles of freezing, precautions and special requirements during freezing and frozen storage of meats. It also illustrates effect of freezing and structure of meat and chemical changes during frozen storage. The topics of chapter 9 are various types of dried and intermediate meats and meat products; formulation of intermediate moisture meats; manufacture of meat extracts and use of dried meat, and meat extracts in other foods. Microbiology, an important aspect of dried meats, intermediate moisture meats and meat extracts has also been discussed in this chapter.

The references which may be consulted for enhancing the knowledge from various chapters have been mentioned in further reading (pp 413-416). This is a feature, unique to this book. Subject index has been given on pages 417-430. This book is a valuable addition to the variety of books available on meat and meat products. It has been written in a simple and understandable script. It gives a full comprehensive understanding of meat science for under-graduate as well as post- graduate students of Meat Science and Technology, and those entering the meat industry.

AMRINDER SINGH BAWA
GURU NANAK DEV UNIVERSITY,
AMRITSAR - 143 005

Toxicological Evaluation of Certain Veterinary Drug Residues in Food. WHO Food Additives Series : 34-Prepared by the Forty third Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), IPCS, International Programme on Chemical Safety, World Health Organization, Geneva, 1995. pp 153. Price in developing countries Sw fr. 31.50/-.

The Monographs contained in this volume were prepared by the forty third joint FAO/WHO expert committee on Food Additives (JECFA), which met in Geneva, Switzerland, during 15-24 November, 1994. These monographs summarize the safety data on selected veterinary drug residues reviewed by the committee. Draft for individual drug has been prepared separately by experts of the field. The monographs on each drug are presented in a general format covering explanation of the drug, biological data viz., biochemical aspects, toxicological studies, observation on humans followed by comments, evaluation and references spread over 3 sections. The data reviewed include b-adrenceptor blocking agents-carazolol, antimicrobial agents - dihydrostreptomycin, streptomycin, spiramycin and tranquilizing agent - azaperone.

Studies on carazolol is the extension of the 38th meeting and in this report, additional information concerning to pharmacological no effect levels on humans is reported and ADI (Acceptable Daily Intake) values confirmed. Detailed studies are reported on antimicrobial drugs. Biochemical effects on different animal species are documented.

Toxicological studies have been well explained, and includes acute toxicity, short term toxicity, long term toxicity/carcinogenicity studies, and special studies on embryo toxicity/tartogenicity, genotoxicity, ototoxicity and microbiological effects in different animal species. For many of the drugs, reproductive toxicity, immune response, renal toxicity and local toxicity are also reported. Microbiological effect on dairy starter culture is reported for dihydro-streptomycin. Many tables giving details about toxicity effects are presented on each drug. MIC (Minimum inhibitory concentration) of many drugs on different bacterial species is also presented. ADI values for individual drug have been presented, giving details about calculation. For azaperone, further information has been presented with additional data, as per the recommendations of the 38th meeting.

Annexures have been included in the monographs, Annexure I presents reports and other documents resulting from previous meeting of the joint FAO/WHO expert committee on Food Additives, Annexure II details about abbreviations used in monograph. Annexure III gives the list of members present at Geneva 1994 meeting. Recommendations on these compounds in terms of ADI and MRL (Maximum residue levels) are presented in Annexure IV. This has additional recommendation for one more drug dexamethasone (Glucodorticosteriod), but the details have not been reported in this monograph. Acorrigend pertaining to monograph has also been shown in Annexure V.

The data summarised in this monograph serve as basis for ADI and MRL for the veterinary drug residues in foods. This volume and others in WHO Food additive series contain information, that is useful to those who practise and manufacture veterinary drugs, government and food regulatory personnel, toxicological laboratories and research workers.

The get up, printing and presentation of the monograph are good and the book is recommended for institutions working on biological action of drugs and chemicals, and their residues in food.

N. SHARMA
INDIAN VETERINARY RESEARCH INSTITUTE, IZATNAGAR - 243 122, U.P.

Analysing Food for Nutrition Labeling and Hazardous Contaminants - Food Science and Technology Series/65 : Edited by Ike J. Jeon and William G. Ikins : Published by Marcel Dekker Inc, 270, Madison Avenue, New York, NY 10016, 1995, pp 496, Price : $165.00

The Nutrition Labeling and Education Act of 1990 (NLEA) was signed into a law during November 8, 1990. This far reaching legislation, made the labeling of nearly all foods with 'Nutrition facts' mandatory. Accordingly, the United States Food and Drug Administration (FDA) issued regulations on nutrition lebeling during January 1993. However, a number of needs remain. Of these, the most crucial need is sensitive and validated analytical methods. This aspect has been documented in the FDA proposal, and FDA explicitly acknowledged concern over the availability of analytical methods for complex carbohydrates, sugars and protein quality. Without accurate and reliable analytical methods, compliance with the NLEA would be impossible as would enforcement. To address the needs, the book entitled 'Analysing Food for Nutrition Labeling and Hazardous Contaminants' is invaluable in covering validated analytical methods and discussions. The subject matter is covered in two parts. Part I deals with analytical methods relating to nutrition labeling of dietary components and the second part on the analysis of hazardous contaminants like mycotoxins, pesticides, toxic compounds derived from lipids, toxins formed as a result of cooking or processing of food and naturally occurring hazards in foods.

Each chapter of this book is devoted to giving the reader more insight into the merits and demerits of analytical methods, available for a particular dietary component. The validated analytical methods and other sophisticated sensitive methods available for total fat, fatty acids, cholesterol, carbohydrates and sugars, soluble, insoluble and total dietary fibres, proteins and amino acids, minerals and vitamins are discussed in-depth, and the importance of each dietary component has been explained in detail with definition, classification and structural formulae wherever required in different chapters, preceded by the first chapter on Current Regulatory Status of Nutrition Labeling by Prof. Ike J. Jeon, giving an account of the extensive renovation of the 'Food Label' since 1974. The status of new nutrition labeling is mandatory and food manufacturers must comply with the FDA requirements by May

1994. The mandatory and voluntary dietary components required for 'Nutrition Facts' are listed along with standard nutrition label formats. Serving size as defined by FDA, presentation of nutrition information, analytical methods to be specified or not, health claims are discussed appropriately.

In the second part of the book, mycotoxin analysis with an informative introduction followed by sampling, extraction, clean-up method, selection of analytical method for separation and quantification including the latest methods like GC/MS, MS/LC and biological and immunochemical methods using Certified Reference Materials are discussed in detail, with a big list of references numbering 367.

Pesticide residue analysis in food is a large research area. While the market for pesticides will continue to grow, at the same time, there is an increased concern about the effects of pesticides on health and environment, necessitating development of sensitive analytical methods. The chapter on "Pesticides" provides an overview of analytical methods for the major classes of pesticide residues, keeping in view the maximum residue limits for certain food items set by FAO/WHO Codex Alimentarius Commission. The classes of pesticides covered in this chapter are based on similarities in chemical structures, viz., benzimitazoles, carbamate insecticides, halogenated hydrocarbon pesticides, organophosphate insecticides, pyrethrin insecticides, alkyl phenolic acid herbicides, triazine herbicides and miscellaneous pesticides like ivermectin and urea herbicides. Recent progress in the analysis of lipid oxidation products, viz., malondialdehyde, 4-hydroxyalkenals, cholesterol oxides and cyclopropenoid fatty acids are reviewed in this chapter entitled "Toxic compounds derived from lipids" with an emphasis on chromatography for specific determination, and mass spectrometry for identification and confirmation.

Toxins formed during cooking or processing of foods, viz., N-nitrosamines, non-volatile N-nitroso compounds and polycyclic aromatic hydrocarbons are discussed, giving the latest information on health implications and sophisticated analytical methods for their detection, and determination.

The last chapter on "Naturally occurring health hazards in foods" describes shellfish poisons, fish poisons, toxic glycosides in food (cyanogenic glycosides), glycoalkaloids, and toxic plant phenolics (safrole, gossypol, coumarins). The sensitive analytical methods available for the determination of these compounds are reviewed.

This comprehensive book provides an up-to-date information on the various analytical procedures for all mandatory/optional dietary components and hazardous contaminants in foods, and serves as an invaluable asset to food scientists, manufacturers, regulatory agencies and libraries.

M.N. KRISHNAMURTHY
CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
MYSORE - 570 013

The Environmental Health Criteria 159 Glyphosate, by Dr. H. Mensink and Dr. P. Janssen: Published by WHO. 1994, pp 177. Price : Sw. fr. 18.90/-

The committee constituted to offer suggestions to implement as the International Programme on Clinical Safety (IPCS) has brought out another noteworthy publication as 159 Environmental Health Criteria on Glyphosate.

The first chapter of the book gives an excellent resume of all the information that has been included in the book. The second chapter gives a description of the structure of glyphosate, and its physical and chemical properties. There is also a detailed Table (1), listing the commercial formulations and another equally informative Table (2), giving the sampling, preparation and analysis of glyphosate.

The third chapter deals with the sources of human and environmental exposure, and its use as a post-emergent, systemic and non-selective herbicide. There is a paucity of data on world production of glyphosate and its occurrence in environmental biota. There is also no information available on estimates of daily human exposure through food and drinking water.

The fourth chapter on "Environmental transport, distribution and transformation" provides valuable information on the transport of glyphosate through water, absorption by soil, its biodegration and dissipation from plants. Fig. 3 of this chapter gives a simple outline of the degradation pathway of glyphosate in soil. The chapter concludes with an account on bioaccumulation, which is rather low in view of its high water solubility and ionic character.

The fifth chapter has a detailed Table, giving maximum concentrations of glyphosate in air, water and soil from various countries with appropriate references. This chapter carries very useful information on estimates of glyphosate in individuals, who are occupationally exposed during manufacture

or use. This is followed by an account on kinetics and metabolism of glyphosate in laboratory animals and humans (sixth chapter). The pharmacokinetics studies have been conducted in various species namely, rats, rabbits, hens, goats and monkeys. The absorption from the gastrointestinal tract, following oral route is about 30-36 of the dose. This compound is not metabolised, and 99% of it is cleared from the body after 168 hours.

The seventh chapter which deals with the effect of glyphosate on laboratory animals and its behaviour in some *in vitro* test systems gives a very exhaustive account of the effect of glyphosate in short term and long term feeding studies in animals. The important observations on acute toxicity studies conducted by different groups in various animal species have been focussed in Tables 10 and 11 of this chapter. Overall, glyphosate had exhibited low level of acute toxic effect in experimental animals after oral or dermal administration. The long term toxicity conducted in mice and rats showed adverse effect at relatively high dose levels. This chapter concludes with data on teratogenicity reproductive toxicity and mutagenicity. The data do not indicate glyphosate to be a mutagen, carcinogen or teratogen. Table 15 depicts the mutagenicity data from various test systems, ranging from *in vitro* bacterial mutagenicity to *in vivo* mammalian tests.

The eighth chapter gives a brief account of the effect of glyphosate on humans. The effect of one of the glyphosate formulations namely "Round Up" on human is mentioned in this chapter. The effect has been categorized as asymptomatic, mild, moderate and severe. These observations are mostly from cases of intentional and accidental exposure. Three studies in human volunteers, pertaining to irritation/sensitization suggested that this formulation namely "Round up" had no adverse effect. The levels of exposure in humans appear to be far below the doses that were used in animal experimentation to produce any toxic effect.

The ninth chapter has been categorized as (a) laboratory studies and (b) field studies. Extensive investigations have been conducted using microbes, aquatic and terrestrial organisms. This chapter carries Table 17, enumerating studies on aquatic microbes and glyphosate and Table 18, giving details of the studies related to glyphosate, and some aquatic animal kingdom species like molluscs, echinoderms, crustaceans, fishes etc. Table 19 is on similar lines as 17 and 18, except that data on glyphosate formulations have been compiled here. Tables 20 and 21 highlight important points related to chronic toxicity of glyphosate and its formulations in aquatic organisms. The last Table of this chapter (Table 22) projects some interesting information on the chronic toxicity of glyphosate and its formulations in birds. Glyphosate or its formulations are only slightly toxic to aquatic microbes or some of the marine lives and birds.

The tenth chapter of this monograph pertains to evaluation of human health hazards, and effects on the environment following exposure to glyphosate. The chapter begins with some information on short and long term studies on glyphosate in animals exposed through diet. According to the available information, occupational exposure in humans is low. No adverse effect has been reported. Figure 4 of this chapter outlines the methodology for deriving estimated environmental exposure concentration and their effects. The effects of glyphosate exposure in different organisms with emphasis on possible hazard effects accompanied by estimated exposure levels and toxic doses have been tabulated excellently in Tables 25 and 26. This chapter is followed by suggestions for future areas of research on glyphosate. The monograph concludes with an impressive list of more than three hundred references.

Dr. H. Mensink, Dr. P. Janssen, Dr. M. Gilbert and Dr. P.G. Jenlins, of the task group on environmental health criteria for glyphosate, who met at the Institute of Terrestrial Ecology, UK, deserve sincere appreciation for their significant contribution in the preparation and publication of this monograph. This book is bound to be useful for people engaged in research in pesticide chemistry/toxicology.

KALPAGAM POLASA
NATIONAL INSTITUTE OF NUTRITION
HYDERABAD - 500 007

AUTHOR INDEX
Vol. 32, 1995

Subject Index
Vol. 32, 1995

ACKNOWLEDGEMENTS
Vol. 32, 1995

I. The Editor expresses his gratitudes to the following referees for their valuable help in the evaluation of papers published in the Journal during 1995. The figures in bracket indicate the number of papers evaluated.

Abraham, K.O. (1)
Achaya, K.T. (1)
Akhilender Naidu (2)
Alur, M.D. (3)
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Appaiah, K.M. (1)
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Vijaya Rao, D. (5)

II. The Editor expresses his gratitudes to the following publishers for submitting books to the JOURNAL for reviewing during 1995. Numbers in bracket indicate the books submitted.

American Association of Cereal Chemists, Inc.,. USA	(2)
Association of Microbiologists of India, Mysore Chapter, DFRL, Mysore	(1)
Blackie Academic Professional, UK	(4)
Blackwell Scientific Publications, USA	(2)
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Eagen Press, USA	(1)
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T.R. Publications (P) Ltd., Madras	(1)
VCH Publishers, USA	(3)
Wiley Eastern Ltd., New Delhi	(1)
World Health Organisation, Switzerland	(7)

III. The Editor expresses his gratitude to the following Industrial House for advertising in the JOURNAL during 1995:

FOOD INGREDIENT SPECIALITIES PRIVATE LIMITED, MADRAS

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

Invites Nominations for Fellows of AFST (I) for the year 1995

The Association has pleasure in inviting nominations 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.
3. The title of Fellow has so far been awarded to 36 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 1996.**

Selection of Fellows

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

Privilege of a Fellow

The Fellow shall be entitled to the following rights:

1. The awardee will be entitled to add FAFST after his 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 1996.**

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

P.C.S. NAMBIAR

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 | **Seconder's name & signature**

Date ; | **Date ;**

Station : | **Station ;**

(Signatures of supporters from their personal/general knowledge)

(1)

(2)

(3)

I agree for the above nomination

(Name & Signature)

... .

Note : (1) Six 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 1996.**

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

INVITES

NOMINATIONS FOR AFST (I) AWARDS FOR 1995

Nominations for the following awards of the AFST(I) for the year 1995 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 1996.**

PROF. V. SUBRAHMANYAN INDUSTRIAL ACHIEVEMENT AWARD

The guidelines for the award are :

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

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

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

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

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

The envelope containing the nominations, along with bio-data and contributions (six copies) should be superscribed **"Nomination for Prof. V. Subrahmanyan Industrial Achievement Award - 1995."**

LALJEE GODHOO SMARAK NIDHI AWARD

The guidelines for the award are :

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

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

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

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

The envelope containing the nominations along with bio-data and contributions (six copies)should be superscribed **"Nomination for Laljee Godhoo Smarak Nidhi Award 1995"**.

BEST STUDENT AWARD

The award is to be given to a student having a distinguished academic record and undergoing the final year course in Food Science and Technology in any recognised University in India.The aim of the award is to 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 1995.

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 (six copies).

The envelope containing the nomination should be superscribed **"Nomination for Best Student Award 1995".**

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 furnish evidence of either;

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

OR

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

The application along with details of contributions and bio-data (six copies) may be sent by registered post with the envelope superscribed **"Nominations for Young Scientist Award 1995".**

BEST PAPER AWARD

This award is to be given by the AFST(I) Educational and Publication Trust to the author(s), who have contributed to the best paper to the *Journal of Food Science and Technology* published in 1995. A panel of experts, constituted by the Central Executive Committee, will scrutinize the issue and select the best paper for the award.

P.C.S. NAMBIAR

HON. EXECUTIVE SECRETARY

INDIAN FOOD INDUSTRY

A Publication of Association of Food Scientists and Technologists (India)

Contents of November - December 1995 issue

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.... to meet the ever growing firce competition
and demanding consumer....

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Phone : 0091-44-6211305 / 0091-44-6211346
Telegram : 'Foodingred' Fax : 0091-44-6264839

Published by the Secretary AFST (India) CFTRI, Mysore-570 013 and Printed at
M/s. Jwalamukhi Job Press, 44/1, K.R. Road, Bangalore-560 004, India.