PAK. J. FOOD SCI., 21(1-4), 2011: 1-6
ISSN: 2226-5899

Safety Issues in Fresh Fruits and Vegetables- A Review

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Abstract

Fruit and vegetables can be contaminated with a range of microbial and chemical contaminants. They are eaten raw, as well as food of animal origin, have long been known to serve as vehicles for transmission of infectious microorganisms in developing countries. However, the number of confirmed cases of illness associated with consumption of fresh fruit and vegetables in industrialized countries has been relatively low compared to the number due to foods of animal origin. The rapid growth of international trade in fresh produce has also resulted in outbreaks due to imported food. This is due to the trade and consumption of unsafe food. Safety issues are not only restricted to on-farm practices but also includes postharvest management practices regarding quality and safety concern. It is evident that postharvest losses in fresh commodities vary from 25 to 40 percent. These losses lead towards poor quality and ultimately unsafe food availability to end consumer. Postharvest considerations like pro-cooling, hot water treatment, irradiation, ozonation, temperature and humidity control during storage and transportation (cool chain management) significantly affects the quality retention. Prevention of contamination is the most efficient way to ensure food safety and prevent food borne illness. Thus, every effort should be made to protect food from primary sources of contamination. Biological, chemical and physical hazards may therefore vary significantly from one type of production to another. Establishment of GAP, GMP, GHP and HACCP programme including SPS measures could be effective tool to cover all aspects of growing, harvesting, packing, transportation, processing, distribution of fresh fruits and vegetables. Assistance from and collaboration between academic institutes, public health authorities, food control agencies, trade organizations and private sector in developing HACCP and GLOBALGAP system for fresh fruits and vegetables industry is necessary sustainable production and to minimize potential hazards to health due to the contamination as well to improve the export potential.

Key words: Safety issues, Contamination, Fruits and vegetable, Quality standards

Food safety aspects in fruit and vegetables

Fresh fruits and vegetables are highly perishable commodities that can easily spoil or deteriorate during produce handling along the supply chain from the producer to the final retailer. Fresh produce spoilage and often result in rapid decay and thus in product loss for human consumption. Post-harvest product losses due to spoilage can be as high as 50 per cent, and even higher for some commodities in developing countries. Accordingly, reduction of these losses, particularly if they can be avoided economically, would be of great significance for producers and consumers alike.

All fruits and vegetables are living parts of plants containing 65 to 95 per cent water. They continue their life metabolisms after harvest and thus change their characteristics depending on product handling, storage and treatment, all of which have a decisive impact on the life of the product. The nature of the produce strongly influences its vulnerability to different types of deterioration. Pakistan is blessed with favourable climatic conditions for a variety of fruits and vegetables production. Pakistan produces annually 13.67 M.T fruits and vegetables with a share of 6.65 million ton fruits and 7.02 million ton of vegetables (GOP, 2010). Out of these produce 25-30% is wasted each year.

Food borne illnesses and outbreaks associated with fresh produce

According to the FDA, in 1996 produce was associated with about 4.3% of all outbreaks (produce and non-produce) in the U.S. and 45.5% of illnesses; in 2007, produce was associated with 4.2% of outbreaks and 12.5% of illnesses (Vierk, 2008). The Center for Science in the Public Interest (CSPI) recently published a report on produce outbreaks from 1990 – 2005 (Smith DeWaal and Bhuiya, 2008). They indicated that between 1990 and 2005, there were 713 outbreaks and 34,049 cases of illness linked to produce in their database and with an average of 48 individuals afflicted per outbreak. According to CSPI, produce outbreaks accounted for 13% of foodborne illness outbreaks and 21% of illnesses in their database (Smith DeWaal and Bhuiya 2008). Clearly, outbreaks and illnesses linked to produce continue to be a major health and safety concern.

Safety hazards for fresh fruits and vegetables

Fresh fruits and vegetables can become contaminated by biological hazards, such as pathogenic organisms including bacteria, viruses and parasites, chemical hazards, and physical hazards. These hazards have all caused illness or injury in fresh produce. Biological hazards, particularly pathogenic bacteria, are the greatest concern because the risk that they pose may be amplified by growth prior to consumption and have been responsible for most of the produce associated outbreaks in the U.S. This chapter focuses primarily on the reduction of these hazards through the implementation of good agricultural practices (GAPs).

The production chain for fresh fruits and vegetables has several links: production, harvesting, post-harvest

treatments, packaging, transport and storage, each with its own contamination hazards and, depending on size of operations, of production and of processing systems in use. Safety assurance programs identify these hazards throughout the entire produce production and handling chain.

Factors thought to influence the occurrence and epidemiology of these deteriorations include the quality of irrigation water, and other agronomic practices such as the inappropriate use of manures and biosolids, pesticides, fungicides etc. Besides this inappropriate post-harvest handling from harvesting till consumption followed by packing, storage and transportation throughout the food supply chain plays an important role in physical, biological and chemical contamination of produce.

Measures to avoid such contamination have been encouraged. The general level of hygiene in handling fruit and vegetables is also a major problem contributing to cross-contamination from animal products as well as direct contamination from the food handler. Prevention of contamination is the most efficient way to ensure food safety and prevent foodborne illness. Thus, every effort should be made to protect food from primary sources of contamination.

However, this is not always possible and raw foodstuffs, particularly fruit and vegetables grown close to the soil, may be contaminated with various pathogens. In such cases, efforts should be made to establish critical control points to reduce contamination to safe levels, for example, by applying the Hazard Analysis and Critical Control Point (HACCP) system (CAC, 1997).

Physical hazards

Physical hazards are foreign material in product that can cause injury. The high moisture content and soft texture of fruit, vegetables and root crops make them susceptible to mechanical injury, which can occur at any stage from production to retail marketing. The splited or damaged portion of fruit or vegetable serves as a suitable medium for microbial growth. Micro-organisms start to multiply there causing rotting and microbial infestation. A small scratch or cut on produce surface result in increase respiration rate, heat production and fasten the ethylene production. The fruit or vegetable ultimately end in senescence. The quality deterioration occur which leave the produce completely unsafe for human consumption.

Physically, contamination can result from contact with the soil; manure; improperly composted manure; irrigation water; fecal material from wild and domestic animals; farm, pack-inghouse, and terminal market workers; contaminated equipment in fi elds, packing-house, and distribution system; wash, rinse, and fl ume water; ice; cooling equipment and transportation vehicles; cross - contamination from other foods; and improper storage, packaging, display, and preparation (Bihn and Gravani 2006 ; FDA, USDA, and CDC 1998). Water of inadequate quality has the potential to be a direct source of contamination and a vehicle for spreading localized contamination in the field, facility, or transportation environments. The most common microbial pathogens in water are Escherichia coli (E. coli), Salmonella, Vibrio cholerae, Shigella. During the last decade (1991-2002) 35,000 water born outbreaks were reported in United States (Craun, 2006). A recent outbreak of food born pathogen *listeria monocytogenes* was reported during October, 2011, in 26 states of America. It was spread through fresh cantaloupes consumption. A total of 133 persons were infected out of which 38 were died and one miscarriage was reported. The Food and Drug Administration (FDA), in conjunction with the Centers for Disease Control and Prevention (CDC) and state health departments, began to investigate a multi-state outbreak of listeriosis. Early in the investigation, multiple samples of cantaloupes from the respective farms were collected, for laboratory culturing to identify the presence of *Listeria monocytogenes*. Of the 39 environmental samples collected, 13 were confirmed positive for *Listeria monocytogenes* (Anon, 2011)

FDA identified the factors as those that were contributed to the introduction, spread, and growth of Listeria monocytogenes in the cantaloupes that include no pre-cooling step to remove field heat from the cantaloupes before cold storage. Improper transport and packing facility i.e., truck was not well sanitized to eradicate pathogen spores contamination from the field and the packing facility floor was constructed in a manner that made it difficult to clean. The packing equipment was not easily cleaned and sanitized; washing and drying equipment used for cantaloupe packing was previously used for postharvest handling of another raw agricultural commodity.

Chemical hazards

Chemical hazards may include heavy metals, pesticides residues, contaminants; fungicides etc., amongst all these pesticide residues are rankesd as most most important safety issue (Kader and Roll, 2004). In some countries, in addition to washing with water, chemical disinfectants are used to decontaminate the surface of fruits and vegetables. It has been stated that 2.5 million tonnes of pesticides are sprayed on crops every year in the world and the reported amount continues to increase with the passage of time (FAO, 2002). The same tendency can be observed in Pakistan. Organophosphate pesticides (OPPs) include a broad range of compounds with herbicides, fungicides, insecticides and others. They are applied worldwide in agriculture sector as well as in house-hold gardens. Consequently, more than 1,000 active ingredients have been included in approximately 35,000 formulations of pesticides used in agriculture sector now-a-days. Recent records disclose that major share of severe pesticide toxicity in human beings is contributed by OPPs (Ecobichon, 2001). It has been described that 37,000 cancer cases are linked with pesticide consumption in developing countries each year (WHO, 1990). Approximately, three million people are the victims of pesticide poisoning and 200,000 die each year around the world. A majority of affected people belongs to the developing countries (FAO, 2002). It is also assumed that in the developing countries like Pakistan, the occurrence of pesticide poisoning may

even be greater than estimated. This may be due to under-reporting, lack of data and misdiagnosis (Tariq, 2005).

Fruit and vegetables can be contaminated with toxic chemicals from a variety of sources. Some of these are used intentionally, such as pesticides. While adherence to GAP regarding their application and pre-harvest interval should assure the safety of produce, exposure to pesticides may pose unacceptable health risks. Industrial pollution from the environment can also result in the deposition of contaminants on the surface of produce, particularly in farm land near certain industrial sites and motorways. USDA's most recent report of produce testing reveals widespread pesticide contamination on popular fruits and vegetables (FDA, 2008). USDA found one or more pesticides on 70.3% of samples tested. The agency found a mixture of between 5 and 13 different pesticide residues tainting one of every 10 samples (10.4%) of fruit or vegetable analyzed. The Centers for Disease Control and Prevention has detected pesticides in blood and urine samples from 95.6 percent of more than 5,000 Americans tested in the agency's national biomonitoring program (CDC 2009). WTO regulations for application of pesticides, insecticides and weedicides and Codex Alimentarious Commission's SPS measures for pesticide limits should be followed to reduce the risk of contamination. Risk of chemical intake could be reduced by proper washing and chlorination of contaminated surfaces of fruits and vegetables. Time of harvesting and application of pesticide or fungicide should be considered to reduce the risk of high level of chemical residue remain in the fruit or vegetable that make it unfit for consumption.

Biological hazards

Biological hazards in fresh produce come from micro-organisms such as bacteria, fungi (yeasts and moulds), protozoans, viruses and helminthes (worms), which can also be termed microbes. Micro-organisms capable of causing human disease may be found in raw produce. Sometimes they are part of the fruit or vegetable microflora as incidental contaminants from the soil and surroundings. In other instances, they are introduced into or on food by poor handling practices in agricultural production or post-harvest processes.

Some of the higher profile outbreaks have been caused by E. coli O157:H7 – contaminated leafy vegetables, in addition to outbreaks caused by Salmonella - contaminated tomatoes, cantaloupe, and other produce items. Investigations of some of these outbreaks have led some to conclude that contamination occurred probably in the fi eld, i.e., preharvest contamination.

Bacterial hazards

Bacteria pose a common food safety risk due to their omnipresence in our environment. Pathogenic bacteria potentially contaminate fruit and vegetables in all stages of the production chain.

Viral hazards

Viruses are very small organisms that are unable to reproduce and multiply outside a living cell and that cannot therefore grow on or inside food as bacteria do. However, raw fruit and vegetables may become contaminated by viral particles with exposure to contaminated water, soil, dust or surfaces. The virus could then infect the consumer of the product if it is consumed raw. Viruses can pose serious health hazards in very low concentrations. Consequently, prevention of product contamination is essential during the production process.

Parasitic hazards

Parasites are organisms that derive nourishment and protection from other living organisms known as hosts. Parasites are of different types and range in size from tiny, single-celled organisms (protozoa**)** to larger multi-cellular worms (e.g. helminths). They may be transmitted from animals to humans, from humans to humans, or from humans to animals. Several parasites have emerged as significant causes of food and waterborne disease.

There has been a continued rise in reported outbreaks of foodborne illness associated with the consumption of fresh fruits and vegetables. Bacteria, viruses and parasites on fruits and vegetables have been linked with illness. In Canada, 18 outbreaks were documented from 1981 to 2000, with approximately 2000 people affected and 18 deaths. Alfalfa sprouts, cantaloupe, lettuce, raspberries and parsley are included amongst the implicated vehicles. The very nature of produce that makes it healthy - fresh and consumed raw - is what makes fresh produce a high-risk food for microbial contamination. Without the microbiological kill step provided by cooking, produce is vulnerable to contamination from the farm-to-fork. Pathogens can contaminate at any point along the food chain, at the farm, packing shed, processing plant, transportation vehicle, retail store or food service operation, and the home. By understanding where potential problems exist, it is possible to develop strategies to reduce risks of contamination (Tauxe *et al*., 1997). Raw produce can become contaminated with pathogenic and non-pathogenic microorganisms at a number of different stages, by several means, from production through to consumption. Laboratory studies have found that fresh produce can support the growth of organisms such as Salmonella. Shigella and Escherichia coli 0157: H7. Consequently, methods of growing, handling, processing, packaging and distribution of fresh produce have received increased attention in terms of identifying and minimizing microbiological hazards. The produce industry has now focused on developing and implementing programs aimed at reducing foodborne disease and illness.

In 1998, the U.S. Food and Drug Administration (FDA) issued its "Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables." The practices outlined in this and other industry documents are collectively known as Good Agricultural Practices or GAPs. GAPs provide general food safety guidance on critical production steps where food safety might be

compromised during the growing, harvesting, transportation, cooling, packing and storage of fresh produce.

HACCP-based programs to overcome the safety risks in fresh produce

HACCP is a system of food safety control based on a systematic approach to the identification and assessment of hazards associated with food operations and the definition of means for their control. This approach focuses on prevention and control and is advocated for every stage in the food chain, from primary producers through to the final consumer (Anon, 1998). The application of the HACCP system consists of a logical sequence of twelve steps encompassing seven basic principles, which can be implemented in any food industry. Recently, HACCP-based programs have been extended to the on-farm environment as a way to reduce risks associated with commodities before they enter the processing environment. However, there is still little known about the mechanisms whereby produce becomes contaminated, so HACCP purists argue that it is almost impossible to define true critical control points in fresh fruit and vegetable production (CODEX, 1996). The FDA, the United Fresh Fruit and Vegetable Association and the International Fresh-cut Produce Association suggest that because critical control points are unachievable, a true HACCP system is too rigid for on the farm. A HACCP-based program that incorporates the principles of carrying out a risk assessment and establishing points of control where good agricultural practices (GAPs) are applied has been shown to work in reducing risks on the farm (Powell *et al*., 2002). Some have suggested that actions controlled by human behaviour - such as hand washing, or the application of agricultural chemicals - be considered as CCPs. Others, however, have noted the difficulty in monitoring human behaviour versus monitoring pasteurization temperatures or other mechanically monitored activities. Nevertheless, reliance on well-developed and consistently performed standard operating procedures (SOPs) and GAPs can simplify the HACCP-based plan.

Hygienic conditions required for fresh fruits and vegetables

Agricultural inputs should not contain microbial or chemical contaminants (as defined under the Recommended International Code of Practice – General Principles of Food Hygiene (CAC/RCP 1-1969, Rev 3 (1997) at levels that may adversely affect the safety of fresh fruits and vegetables and taking into consideration the WHO guidelines on the safe use of wastewater and excreta in agriculture and aquaculture appropriate.

Water safe from physical, chemical or biological hazards

Growers should identify the sources of water used on the farm (municipality, re-used irrigation water, well, open canal, reservoir, rivers, lakes, farm ponds etc.). They should assess its microbial and chemical quality, and its suitability for intended use, and identify corrective actions to prevent or minimize contamination (e.g. from livestock, sewage treatment, human habitation).

Where necessary, growers should have the water they use tested for microbial and chemical contaminants. The frequency of testing will depend on the water source and the risks of environmental contamination including intermittent or temporary contamination (e.g. heavy rain, flooding, etc.). If the water source is found to be contaminated corrective actions should be taken to ensure that the water is suitable for its intended use.

Personnel health, hygiene and sanitary facilities

Agricultural workers who have direct contact with fresh fruits and vegetables should maintain a high degree of personal cleanliness and, where appropriate, wear suitable protective clothing and footwear. Cuts and wounds should be covered by suitable waterproof dressings when personnel are permitted to continue working. Poor employee hygiene has been responsible for over 40% of source identified produce-related outbreaks (Bean and Griffin, 1990). Personnel should wash their hands when handling fresh fruits and vegetables or other material that comes in contact with them. Specific hygienic and maintenance requirements should be identified for each piece of equipment that is used and the type of fruit or vegetable associated with it.

Prevention of cross-contamination

During the primary production and post-harvest activities, effective measures should be taken to prevent cross contamination of fresh fruits and vegetables from agricultural inputs or personnel who come directly or indirectly into contact with fresh fruits and vegetables. To prevent the potential of cross-contaminating fresh fruits and vegetables, growers and agricultural workers should not use harvesting containers for carrying materials (e.g. lunches, tools, fuel, etc.) other than harvested fruits and vegetables. Equipment and containers coming into contact with fresh fruits and vegetables should be made of materials that are non-toxic. They should be designed and constructed to ensure that, when necessary, they can be cleaned, disinfected and maintained to avoid the contamination of fresh fruit and vegetables. Care must be taken when packing fresh fruits and vegetables in the field to avoid contaminating containers or bins by exposure to, manure or animal/human faeces.

Storage and transport from the field to the packing facility

Fresh fruits and vegetables should be stored and transported under conditions which will minimize the potential for microbial, chemical or physical contamination. Storage facilities and vehicles for transporting the harvested crops should be built in a manner to minimize damage to fresh fruits and vegetables and to avoid access by pests. They should be made of non-toxic materials that permit easy and thorough cleaning. They should be constructed in a manner to reduce the opportunity for potential contamination from physical objects such as glass, wood, plastic, etc.

Fresh fruits and vegetables unfit for human consumption should be segregated before storage or transport. Those which cannot be made safe by further processing should be disposed of properly to avoid contamination of fresh fruits and vegetables or agricultural inputs.

Cleaning, maintenance and sanitation

Premises and harvesting equipment should be kept in an appropriate state of repair and condition to facilitate cleaning and disinfection. Equipment should function as intended to prevent contamination of fresh fruits and vegetables. Harvesting equipment and re-usable containers that come in contact with fresh fruits and vegetables should be cleaned, and, where appropriate, disinfected on a regular basis. Equipment and re-usable containers used for fresh fruits and vegetables that are not washed prior to packing should be cleaned and disinfected as necessary.

Pre-cooling and cold storage

Potable water should be used in cooling systems where water or ice is in direct contact with fresh fruits and vegetables (e.g. hydro cooling, ice cooling). The water quality in these systems should be controlled and maintained. Forced-air cooling is the use of rapid movement of refrigerated air over fresh fruits and vegetables in cold rooms. Air cooling systems should be appropriately designed and maintained to avoid contaminating fresh produce.

Disinfestation and preserving techniques

Various disinfectants and sanitizing methods for reducing chemical, physical and biological contamination on raw fruits and vegetables are in practice like washing, chlorination, ozonation, ionizing radiations, use of organic acids etc. These techniques vary greatly with respect to differences in surface characteristics of fruits and vegetables, type and physiological state of microbial cells, and environmental stress conditions interact to influence the activity of disinfectants and sanitizers. Washing fruits and vegetables in potable water removes a portion of microbial cells. In some instances, vigorous washing can be as effective as treatment with water containing 200ppm chlorine, which generally reduces populations by 10-100-fold. Heavily contaminated fruits and vegetables should be subjected to a double wash treatment. Chlorine dioxide is useful in controlling populations of microorganisms in wash-water but varies in efficacy in killing microorganisms on the surface of fruits and vegetables. Organic acids (e.g. acetic, lactic, citric and peroxyacetic acids) have good potential as disinfectants for fruits and vegetables, but conditions under which they are most effective have not been defined. Ozonation of wash-water reduces numbers of microorganisms, thus resulting in reduced numbers on the surfaces of fruits and vegetables (Larry, 1998).

Conclusion

It is has been found that it is not enough simply to provide producers with a manual of food safety guidelines and expect full implementation and documentation. Evaluation of on-farm food safety programs found that simple manuals were not effective in overcoming the barriers to implementing the on-farm food safety program. Workable food safety programs must provide individual support for growers. A food safety coordinator can provide the one-on-one support that is needed and evaluation of such programs has indicated that this one-on-one support is one effective tool to overcoming these barriers.

Good on-farm food safety programs have a mechanism to keep records of risk-reduction practices. The documentation provides a quick reference of specific practices for interested buyers and, or also, for regulators in case of an outbreak without an in-depth investigation. Documenting when equipment sanitation occurs, what chlorine levels are in wash water or when an employee is sick, demonstrates that food safety is a priority. The documentation medium does not matter, whether it is a checklist that is posted on the wall, a computer spreadsheet or a notebook, as long as it is accessible, complete and is kept up-to-date. The personnels associated with growing and harvesting, packing and transportation should be aware of GAPs, good hygienic practices, GMPs, good hygienic practices and their role and responsibility in protecting fresh fruits and vegetables from contamination or deterioration. Agricultural workers should have the necessary knowledge and skills to enable them to carry out all the post-harvest activities and to handle fresh fruits and vegetables hygienically.

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PAK. J. FOOD SCI., 21(1-4), 2011: 7-12
ISSN: 2226-5899

Assessment of the antioxidant activity and total phenolic contents of sunflower hybrids

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Abstract

The aim of this study was to assess the potential of methanolic extract of different sunflower hybrids (FH-37, FH-106, FH-237, FH-259, 331 and FH-369) as a natural antioxidants. Free radical scavenging capacity, antioxidant activity and total phenolic contents were determined using DPPH, beta carotene and Folin-Ciocalteu assays respectively. These seeds were divided into two groups one used as such while other after dehulling. FH-369 and FH-331 had the highest free radical scavenging capacity, antioxidant activity and total phenolic contents. It was also observed that seeds with hull or shell had less phenolic contents and also lower antioxidant activity. There was a direct correlation between total phenol and antioxidant activity (R^2 = 0.9954, p 0.0001) which indicates that polyphenols are the main antioxidants. The present study shows that all these sunflower hybrids especially FH-369 and FH-331 are strong radical scavengers and can be considered as good sources of natural antioxidants for medicinal and commercial uses.

Key words: Sunflower seeds; antioxidants; total phenol; DPPH; beta carotene

Introduction

Several free radicals having different reactivities are formed through lipid oxidation during different physiological functions. These free radicals cause oxidative stress when over produced in pathological conditions (Dorman *et al*., 2003). This oxidative stress is involved for the onset of large number of diseases such as autoimmune diseases, inflammation, cardiovascular-neurological diseases, cancer and aging (Klaunig and Kamendulis, 2004; Kregel and Zhang, 2006; Wang and Maldonado, 2006). An adequate intake of natural antioxidants could protect the onset of oxidative damage in cells (Riso et *al.,* 2005; Ozsoy *et al.,* 2008). The term antioxidant refers to compounds which can scavenge free radical, inhibit lipid peroxidation and to chelating agent (Lee *et al.,* 2003). Phenolic compounds overcome this definition as they possess a wide spectrum of biological effects including antioxidant and free radical scavenging (Pellati *et al.,* 2004; Abdel-Aal *et al.,* 2006). Phenolics are compounds possessing aromatic rings with hydroxyl groups. When are in diet, these compounds provide health benefits associated with reduced risk of chronic disease (Liu, 2007).

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crop grown in the world. (Stefansson, 2007). A tiny sunflower seed is a package of healthy unsaturated fats, protein, fiber and other important nutrients like vitamin E, selenium, copper, zinc, folate, iron and phytochemicals. After palm, soy and rapeseed oil, sunflower oil was ranked fourth with a worldwide production of about 10.6 million metric tons during 2006. (FAO-STAT, 2008). The sunflower is an annual plant originated from Americas and was put in the family Asteraceae by scientist. Its production was started in Europe early in the 16th century (Pope *et al.,* 2001).

Many studies about sunflower phenolic compounds have been reported. (Pedrosa *et al.,* 2000). Sunflower polyphenols can be used as effective antioxidants for sunflower (De Leonardis *et al*., 2003). Sunflower polyphenols such as caffeic, chlorogenic and ferulic acids exert a high antioxidative potential, which might be beneficial both from a technofunctional and biofunctional point of view (Moure *et al.,* 2001; Maier *et al.,* 2009).

Most of sunflower hybrids have been previously analyzed in numerous studies concerning their chemical composition. Nevertheless, the literature data on their antioxidant activities are scarce and little is known about phenolic content and antioxidant activity. It seems that there is a significant relationship between the presence of total phenol and antioxidant activity in sunflower hybrids. The present project was undertaken to evaluate some of the hybrids produced by Ayub Agricultural Research Institute Faisalabad. In this research the antioxidant activity and total phenol contents of FH-37, FH-106, FH-237, FH-259, 331, FH-369 was investigated.

Materials and Methods

Chemicals

All chemicals and reagents were analytical grade or purest quality. Folin-Ciocalteu reagent**,** gallic acid**,** anhydrous sodium carbonate**,** 1,1-diphenyl-2-picrylhdrazyl, methanol**,** β-carotene, chloroform, linoleic

acid and tween 80 were purchased from Sigma company Sigma, Merck, Aldrich and Fluka

Apparatus

An IRMECO UV visible spectrophotometer (Model U2020) double-beam spectrophotometer equipped with a 1.0 cm path length glass cell was used.

Materials

For the investigation seeds of FH-37, FH-106, FH-237, FH-259, 331 and FH-369 were used. Half of these seeds were manually dehulled while others used as such. All these hybrids were newly developed by Oil Seed Research Institute, Ayub Agricultural Research Institute, Faisalabad.

Determination of Total Phenolic and Antioxidant Activities

Preparation of the Extracts

Approximately 10 g of seed material was crushed. From 5.0 g of this material the antioxidant components were isolated by the use of methanol. Each sample was extracted three times with 200 mL of the solvent. First, the extraction was carried out overnight by shaking, and then it was repeated two times with 200 mL of solvent. The combined extracts were vacuum evaporated to remove the solvent then weighed to determine the extraction yield, and stored at -20 °C until use.

Determination of Free Radical Scavenging Capacity via DPPH Assay

Stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was used to determine the antioxidant activity. The modified method of Hatano *et al., (*1988) described by Mathaus (2002) was used. 0.5 mL of the DPPH solution was diluted in 4.5 mL of methanol, and 0.1 mL of a methanolic solution of the extract was added. The concentration of DPPH solution was about 50 mg/100 mL. The mixture was shaken vigorously and was placed in dark allowed to stand for 45 min. The decrease in absorbance was measured at 515 nm against a blank (without extract) with a spectrophotometer. The experiment was carried out in duplicate.

Determination of Antioxidant Activity via Beta-carotene Bleaching Assay

Antioxidant activity using a *β*-carotene/linoleic acid system was also carried out according to the modified method Taga *et al*. (1984) described by Mathaus (2002). 40 mg of linoleic acid and 400 mg of Tween 20 were transferred into a flask, and 1 mL of a solution of *β*-carotene (3.34 mg/mL) in chloroform was added. This chloroform was removed by using rotary evaporation at 40 ^{0}C. Then to the residue 100 mL distilled water was added slowly and the solution was vigorously shaken to form a stable emulsion. Antioxidant solution (0.2ml) was added to an aliquot of 5 mL of emulsion. Blank was also prepared consisting of the emulsion without beta-carotene. The tubes were placed in a water bath at 40 ^{0}C. The bleaching rate of the *β*-carotene solution was determined by measurement of the difference between the initial reading in spectral absorbance at 470 nm at time 0 min and after 60 min.

Measurement of Total Phenolics

Total phenolics were determined colorimetrcially using Folin- Ciocalteau reagent (Taga *et al.,* 1984). Extract **(**150mg) was dissolved in methanol (10 mL) and 2 mL of this solution was filled up with 0.3% HCl to 5 mL. A 100-µL aliquot of the resulting solution was added to 2 mL of 2% Na2CO3 and after 2 min 100 µL of Folin-Ciocalteau reagent was added. After a further 30 min the absorbance was measured at 750 nm using a spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the absorbance of a known concentration of gallic acid standard and the results were expressed as milligrams gallic acid equivalents (GAE) per 100 gram extract.

Statistical Analyses

The data was statistically analyzed by using one-way ANOVA and a Tukey posthoc multi test was used (Steel *et al.,* 1997).The values are reported as mean ± SD.

Results and Discussion

Uncontrolled oxidation has sword of dual action, on one hand it deteriorate the quality of food stuff and secondly ingestion of such items results in health hazards. Antioxidants can be used to prevent auto-oxidation. Natural antioxidants are gaining importance to reduce the oxidative damage to food stuff and others.

Total Phenolic Contents

Phenolics and polyphenolic compounds contribute directly to antioxidative action and they constitute the main class of natural antioxidants present in plants (Awika *et al*., 2003); therefore it is necessary to calculate total phenolic content in plant species. TPC was determined following a Follin–Ciocalteu method and results were expressed as gallic acid equivalents.

The statistical analysis showed significant differences in the total phenolic content of whole and dehulled seeds of different sunflower hybrids. The results given in Table I also indicate that the total phenolic contents ranged from 2185.76 to 2965.90 mg/100g and 3162.02 to 3614.34 mg/100g between the whole sunflower seeds and dehulled seeds, respectively among different sunflower hybrids. The dehulled seeds exhibited higher content of total phenolics than the respective whole seeds of different sunflower hybrids. The highest phenolics content (2965.90±0.44 mg/100g) was found in the whole seed sample of FH-369 followed by FH-331 (2865.14±0.36 mg/100g). It is also obvious from the results (Table I) that significantly the highest phenolic contents (3614.34±0.23 mg/100g) was found in the

dehulled seeds of FH-369 followed by FH-331 (3433.56±0.30 mg/100g).

Various scientists have worked about contents of phenolic compounds in sunflower seeds (Pedrosa *et al.,* 2000). Comparison with the data obtained in the present study is hardly possible because of differing analytical methodologies, the development of novel sophisticated techniques and differences in the sample material and origin. Fiska *et al.,* (2006) determined the total phenolics contents in sunflower seeds and found to be 2700 mg/100 g on dry weight basis. Our results are corresponding as determined by Weisz *et al*. (2009) by summarizing individual amounts of all constituents and ranged from 2938.8 mg/100 g to 4175.9 mg/100 g dry matter (DM) for the dehulled kernels. They also noticed the TPCs of the sunflower kernels were found to be higher than those determined in the shells.

Free Radical Scavenging Capacity with DPPH Radical Scavenging Method

Relatively stable DPPH radical has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and thus these are used to evaluate the antioxidant activity of these compounds (Jao and Ko, 2002). DPPH is stable and commercially available organic nitrogen radicals involved in various oxidative reactions in vivo (Huang, 2005). DPPH free radical scavenging activity is one of those indicators which are important in determining antioxidant potential of selected bioactive molecules or extract containing them.

Our results for free radical scavenging activity are statistically significantly different among different sunflower hybrids. According to Table II the free radical scavenging activity ranged from 55.39 to 66.18% and 65.56 to 72.34 % between the whole sunflower seeds and dehulled seeds, respectively among different sunflower hybrids. The highest free radical scavenging activity (66.18±1.45%) was found in the whole seed sample of FH-369 followed by FH-331(65.35±0.17%) and significantly the highest free radical scavenging activity (72.34±2.28%) was found in the dehulled seeds of FH-369, followed by FH-331(70.86±2.79).

Several studies applying the DPPH assay for determining the free radical scavenging activity of oilseeds such as the sunflower's have found high antioxidant capacity values for the extracts of these seeds (Suja *et al*. 2005; Shahidi *et al*. 2007). Our results for the DPPH assay are somewhat similar to the findings of such studies. Free radical scavenging activity of the aqueous extract was found to be 58.8% in striped sunflower seeds via DPPH assay. (Giada and Mancini-Filho, 2008).

Antioxidant Activity VIA Beta-Carotene Bleaching Method

In the present study antioxidant activity of all the samples was observed in linoleic acid system (Table III). β-Carotene bleaching assay has a high specificity for lypophilic compounds. Similarly like TPC and free radical scavenging activity the results showed significant differences in the antioxidant activity of whole seeds and dehulled seed of different sunflower hybrids. Antioxidant activity via beta carotene bleaching assay ranged from 50.38 to 59.95% and 58.71 to 63.95 % between the whole sunflower seeds and dehulled seeds, respectively among different sunflower hybrids.

The highest antioxidant activity (59.95±0.26%) was found in the whole seed sample of FH-369 followed by FH-259 (56.93±1.39%) and the highest antioxidant activity (63.95±1.06%) was found in the dehulled seeds of FH-369, followed by FH-331 (63.15±1.92%). Our results are similar to the earlier findings of some scientists. The antioxidant activity from sunflower residue was found to be near 70% by Matthaus *et al.,* (2002). And also the antioxidant activity of sunflower seeds determined by Velioglu *et al.,* (1998) was 72.9%.

Relationship between Total Phenolics and Antioxidant Activity

There is a significant relationship between accumulation of high amount of phenolic compounds and antioxidant activity (Figure 1) (R^2 = 0.9954, p=0.0001). It is clear from the results that there is a direct relationship between total phenolics contents and antioxidant activity of different sunflower hybrids. It means if seeds have more phenolics contents they also have high antioxidant activity and vice versa. Different results have been reported on the aspect of relationship between phenolic content and antioxidant activity. Some scientists found correlation between the total phenolic contents and the antioxidant activity while some found no such relationship. A parallel increase between phenol content and antioxidant activity was found during germination of Pangium edule (Andarwulan *et al.,* 1999).

Tsaliki *et al.* (1999) also observed an increase in the antioxidant activity of lupin seed. And also a linear positive relationship existed between the antioxidant activity and total phenolic acids content of the tested *Ocimum* accessions (Javanmardi *et al.,* 2003). Maillard and Berset (1995) found no correlation between antioxidant activity and phenolic contents in malts and he said other compounds are also responsible for the antioxidant activity. Also no relationship between antioxidant activity and phenolic composition was found in citrus residues (Bocco *et al.,* 1998), fruit berry, fruit wines (Heinonen and Lehtohen *et al.,* 1998) or in plant extracts (Kahkonen *et al.,* 1999).

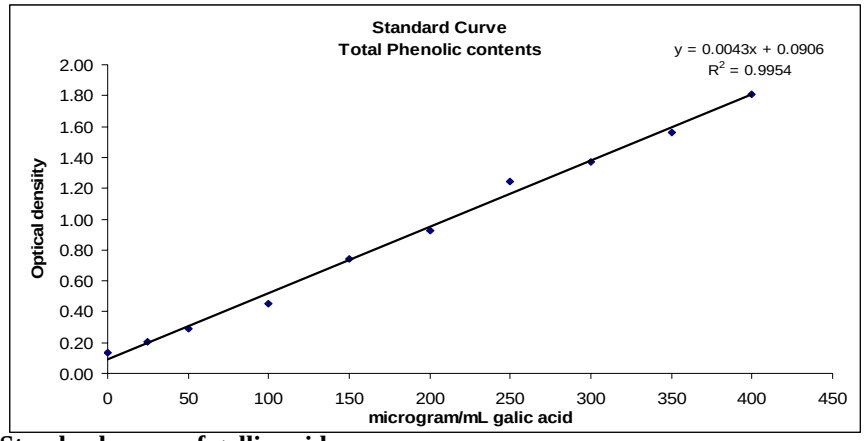

Fig. I Standard curve of gallic acid

Table I Means value of total phenolic contents in whole and dehulled sunflower seeds on dry bases

Hybrids	TPC (mg/100g)	
	WS	DS
FH-37	2233.12±0.23d	3162.26±0.21c
FH-106	2571.67±0.35 b	3428.34±0.36b
FH-237	2404.31±0.29c	3225.82±0.63c
FH-259	2185.76±0.33d	3162.02±0.42c
FH-331	2865.14±0.36a	3433.56±0.30b
FH-369	2965.90±0.44a	3614.34±0.23a

The values shown are means ± SD of three replicates, calculated on a dry weight basis.
Any two means not sharing same letter differ significantly from each other

Table II *DPPH Radical Scavenging capacity of different sunflower seed hybrids*

Hybrids	Free radical scavenging capacity *(%)*	
	WS	DS
FH-37	58.33±1.44c	65.56±0.50d
FH-106	55.39±0.75c	69.79±1.04bc
FH-237	60.33±1.21bc	68.58±2.98c
FH-259	59.49±1.35c	66.47±0.93d
FH-331	65.35±0.17a	70.86±2.79b
FH-369	66.18±1.45a	72.34±2.28a

Values are mean ± SD of two replicates
Any two means not sharing same letter differ significantly from each other

Table III *Beta carotene bleaching assay of different sunflower seed hybrids*

Hybrids	Antioxidant activity *(%)*	
	WS	DS
FH-37	52.38±0.76cd	58.71±1.92d
FH-106	51.12±1.54d	60.15±0.71bcd
FH-237	50.38±2.68d	61.36±1.34b
FH-259	56.93±3.78abc	59.06±0.15d
FH-331	55.94±1.39bc	63.15±1.92a
FH-369	59.95±0.26ab	63.95±1.06a

Values are mean ± SD
Any two means not sharing same letter differ significantly from each other

Conclusion

As it is clear from the results all the studied hybrids possessed antioxidant activity and total phenolics while FH-369 and FH-331 showed the highest results. Dehulled seeds possessed higher contents of antioxidant activity and total phenol contents as compared with whole seeds. Also there was a strict linear relationship between total phenolics and antioxidant activity for the studied hybrids. As mentioned earlier, sunflower hybrids have major medicinal effects and used traditionally. Therefore the potency of these extracts could provide a chemical basis for health benefits claimed. It needs further studies to assess their potential components as effective natural remedies.

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PAK. J. FOOD SCI., 21(1-4), 2011:13-21
ISSN: 2226-5899

Isolation and simultaneous detection of flavonoids in the methanolic and ethanolic extracts of *Coriandrum sativum* L. seeds by RP-HPLC

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Abstract

Reversed-phase high performance liquid chromatography (RP-HPLC) method with UV/VIS detection was established for the separation and identification of flavonoids in the methanolic and ethanolic extracts of coriander (*Coriandrum sativum* L.) seeds. Separation was achieved using a column RP-C_{18} VARIAN Pursuit XPs with dimensions 250 x 4.6mm using a mobile phase of formic acid and acetonitrile gradient. Rutin, quercetin, chlorogenic acid and caffeic acid were identified and quantified in the multi-component methanolic and ethanolic extracts by comparing with the respective standards using the method of RP-HPLC. The methanolic extract produced sharp peaks in comparison to the ethanolic extract. Chlorogenic acid was predominant in methanolic extract followed by rutin, caffeic acid and quercetin while rutin was predominant in ethanolic extract followed by chlorogenic acid, caffeic acid and quercetin. Though similar compounds were identified in both the extracts, the concentration and purity of the compounds were superior in the methanolic extract as compared with that of the ethanolic extract. Besides, chromatograms of ethanolic extracts had peak tailing which indicates that the bio-active principles are extracted better in methanol.

Key words: RP-HPLC, *Coriandrum sativum* L., flavonoids, rutin, quercetin, chlorogenic acid, caffeic acid.

Introduction

Coriander (*Coriandrum sativum* L.) is a culinary plant from the family Umbelliferae, which is extensively cultivated in India, Russia, central Europe, Asia and Middle East. The dried fruits are extensively employed as a condiment, especially for flavoring sauces, meat products, bakery and confectionery items (Ravi et al., 2007). Coriander seeds contain an essential oil (up to 1%) and the monoterpenoid, linalool, is the main component. Coriander seed is a popular spice and finely ground seed is a major ingredient of curry powder. The seeds are mainly responsible for the medicinal use of coriander and have been used as a drug for indigestion, against worms, rheumatism and pain in the joints (Wichtl, 1994). In the folk medicine, the seeds of coriander are used as an aromatic, carminative, stomachic, antispasmodic and against gastrointestinal complaints such as dyspepsia, flatulence and gastralgia. The seeds are also used as an ingredient in the laxative preparations to prevent stomach griping (Nadakarni, 1976; Jain, 1991).

The volatile compounds are major components of spice and aromatic plants. They are isolated by steam distillation and received the name essential oils which exhibit diverse biological properties, including antioxidant activity (Lee and Shibamoto, 2001). Polyphenolic phytochemicals are ubiquitous in the plant kingdom. These important aromatic secondary metabolites of plants are consumed in significant amounts in daily life. The composition of polyphenolic phytochemicals is influenced by maturity, cultivar (Lee and Jaworski, 1987), cultural practices, geographic origin, climatic and storage conditions and processing procedures (Spanos and Wrolstad, 1990). Some phytochemicals are known as neutraceuticals, which provide health benefits because of their biological activities (Dillard and German, 2000). Research on phytochemicals has been driven in recent years by their beneficial health effects, including antioxidant, anticarcinogenic, and antimutagenic activities (Huang et al., 1992) and their ability to reduce the risk of coronary heart disease (Hertog et al., 1993).

Many of today's synthetic drugs originate from the plant kingdom. Herbal drugs are proved as effective as synthetic drugs with lesser side effects (Balasubramanian et al., 2005). Coriander is one of a few savory plants, a potential source of phenolic compounds having biological activities. In Morocco, coriander has been documented as a traditional treatment for diabetes, indigestion, flatulence, insomnia, renal disorders and loss of appetite, and is a diuretic and all parts of the plant are edible, but the fresh leaves and the dried seeds are the most common parts used in cooking (Aissaoui et al., 2008). Due to the complexity of the natural mixtures of phenolic compounds of various plants it is rather difficult to elucidate their structure and assess the antioxidant and biological potentials. Indeed, the determination of individual flavonoid glycosides form plant extracts could prove to be a difficult task.

The aim of this work was to analyze and identify some of the phenolic compounds present in coriander (*Coriandrum sativum* L.) seeds by using RP-HPLC, a high-resolution chromatographic technique widely used for the simultaneous extraction and identification of phenolic compounds.

Materials and Methods

Apparatus

The chromatography separation was performed using a Shimadzu LC-20AD with a quaternary pump system. Auto injector or auto sampler (SIL-20ACHT) was used for 20 µl of sample injection. Separation was carried out at ambient temperature with a column (VARIAN Pursuit XPs- C_{18}) dimension 250 x 4.6mm, S/N 436, protected by a guard column. The detector signal was recorded with a UV/VIS detector (SPD-20A)

Reagents

Formic acid, acetonitrile, methanol, ethanol, water and all the other reagents were of HPLC grade.

Plant material

Coriander seeds were purchased from local grocery, cleaned to be free from extraneous materials, shade dried and ground to a coarse powder using electric blender.

Preparation of sample extracts

The extraction was carried out by mixing 50g of seed powders in 250ml of methanol and ethanol separately. Both the solutions were stirred regularly for 15 days. The extracts were filtered and dried using flash evaporator. The residues after evaporation were weighed and made up to 10ml with methanol and ethanol respectively. Sample cleanup was done to remove the impurities using a C18 Sep-Pak cartridge and 20 µl aliquots were analyzed by HPLC.

Preparation of standard solutions

Rutin and quercetin: Standard stock solutions of two flavonoids, rutin and quercetin were prepared in methanol and ethanol separately, at concentrations of 100ppm (10 mg of the standards were dissolved in 25ml methanol and ethanol in separate volumetric flasks, sonicated and volume made up to 25 ml with respective solvents to give 400ppm, 2.5ml of stock solution was taken and made up to 10ml with methanol and ethanol respectively to give concentrations of 100ppm).

Caffeic acid and chlorogenic acid: Standard solutions were prepared by dissolving 10mg of the standards in methanol and ethanol separately in 25ml volumetric flasks, sonicated and volume was made up to 25 ml with respective solvents to give concentration of 400ppm.

Procedure

RP-HPLC with C_{18} columns is the most popular technique for the analysis of polyphenols of the different foods. A UV-vis multiwavelength detector (SPD-20A) was used because all phenolic compounds show intense absorption in the UV region of the spectrum. This method used for the separation of caffeic acid and chlorogenic acid (320nm), rutin and quercetin (370nm) included mobile phase 0.5% formic acid: Acetonitrile (ACN)(70:30) at a flow rate 0.9ml/min; column (VARIAN Pursuit XPs- C_{18} dimension 250 x 4.6mm) at 40^{o}C temperature. The polyphenols identification was based on the comparison of their retention times with those of the standard solutions (Sigma-Aldrich).

Results and Discussion

Free radicals and other reactive species are thought to play an important role in many human diseases. The plants have been regarded as having considerable health benefits, due to their main antioxidant compounds viz. phenolics which effectively neutralize or scavenge the free radicals. Flavonoids, a group of phenolics almost universal pigments of plants, are important parts of the human diet and considered as active principles of many medical plants. There are a few reports on flavonoid constituents of *Coriandrum sativum* L. The literature points out that some activities can be especially related to these flavonoids i.e., antioxidant, anti-inflammatory, anticarcinogenic, antimutagenic antiulcerative, antihepatotoxic and antiangiogenesis for quercetin, antiplatelet and vasodilatatory activities for luteolin (Halliwell and Whiteman 2004).

Phytochemicals, especially phenolics in plant foods, are suggested to be the major bioactive compounds responsible for their health benefits, which can be attributed to the antioxidant and metal chelating abilities of phenolic compounds. Phenolics have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and other free radicals produced by lipid peroxidation (Calgorotto *et al*., 2007). In order to study the therapeutic effects of various phytochemical compounds present in herbs and spices, it is necessary to extract them from the source prior to the analysis. Extraction of phenolic compounds in plant materials is influenced by their chemical nature, the extraction method employed, sample particle size, storage time and conditions, as well as presence of interfering substances. Phenolic extracts of plant materials are always a mixture of different classes of phenolics that are soluble in the solvent system used. Additional steps may be required for the removal of unwanted phenolics and non-phenolic substances such as waxes, fats, terpenes and chlorophylls (Naczk and Shahidi, 2004).

Due to the complexity of the natural mixtures of phenolic compounds of various plants, it is rather difficult to elucidate their structure and assess the antioxidant and biological potentials. Indeed, the determination of individual flavonoid glycosides form plant extract could prove to be a difficult task. Hence, it was aimed in this work to isolate and identify some of the phenolic compounds present in the methanolic extract of coriander (*Coriandrum sativum*) seeds by using RP-HPLC which is a high-resolution chromatographic technique widely used for the simultaneous extraction and identification of some of the phenolic compounds.

In the course of optimization of the methods for separation and analysis of the flavonoid aglycones caffeic acid, chlorogenic acid, quercetin and rutin the in seeds of

Coriandrum sativum L. through reversed-phase high performance liquid chromatography (RP-HPLC) with UV detection, different combinations of isocratic and gradient techniques and good resolution of the flavonoids was achieved. The flavonoids were identified by comparison with the chromatogram of the standard flavonoid compounds obtained under similar conditions. This method gave a quick analysis of the flavonoids present in the methanolic and ethanolic extracts of *Coriandrum sativum* L. seeds.

The retention time (RT) for the standards viz. methanolic caffeic acid, chlorogenic acid, quercetin and rutin were 4.033, 3.605, 11.638 and 3.711, (Fig 1,2,4 and 5) respectively while the retention time (RT) for the same standards prepared in ethanol were 4.048, 3.754, 11.637 and 2.602, (Fig 7,8,10 and 11) respectively and compounds in the methanolic (Fig. 3 and 6) and ethanolic (Fig. 9 and 12) extracts were identified by comparing their retention times with those of the standards.

The experimental data pertaining to retention times, area under standard peaks as well as sample peaks of various polyphenols in the methanolic and ethanolic extracts are presented in Table 1 and 2 respectively. All the four flavonoids were in higher concentration in the methanolic extract than those in the ethanolic extract (Table 1 and 2) indicating better extractability of the flavonoids in methanol. Methanolic extract had highest amount of chlorogenic acid while ethanolic extract had highest amount of rutin. The methanolic extract produced sharp peaks in comparison to the ethanolic extract. Chlorogenic acid was predominant in methanolic extract followed by rutin, caffeic acid and quercetin while rutin was predominant in ethanolic extract followed by chlorogenic acid, caffeic acid and quercetin. Though similar compounds were identified in both the extracts, the concentration and purity of the compounds were superior in the methanolic extract as compared with that of the ethanolic extract. Besides, chromatograms of ethanolic extracts had peak tailing which indicates that the bio-active principles are extracted better in methanol which could be due to variable nature and solubility of the compounds present in respective extracts.

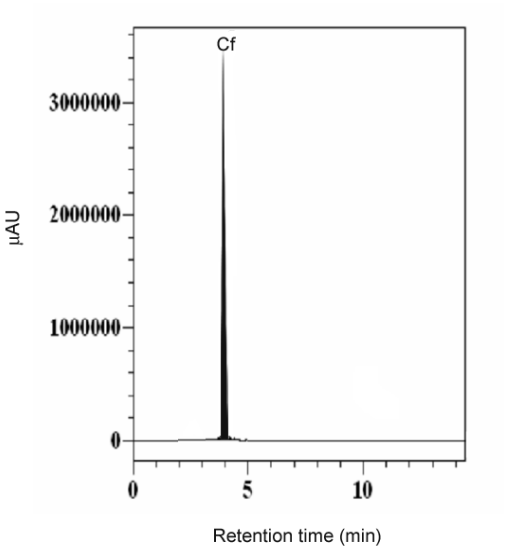

Fig.1 HPLC chromatogram of standard caffeic acid in methanol

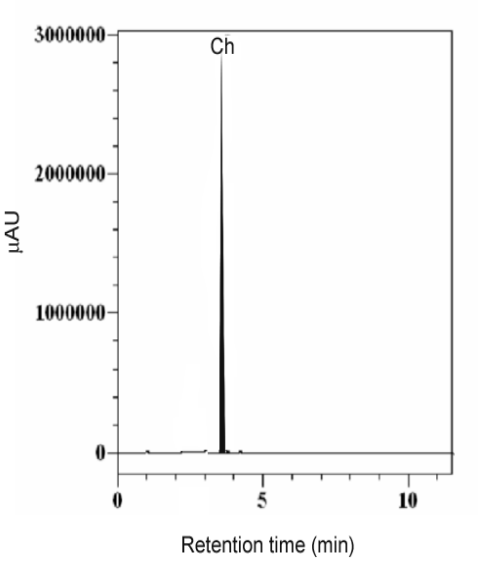

Fig.2 HPLC chromatogram of standard chlorogenic acid in methanol

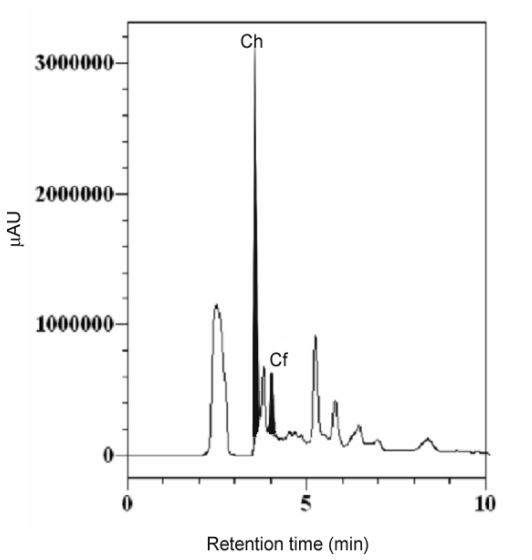

Fig.3 HPLC chromatogram of methanolic extract

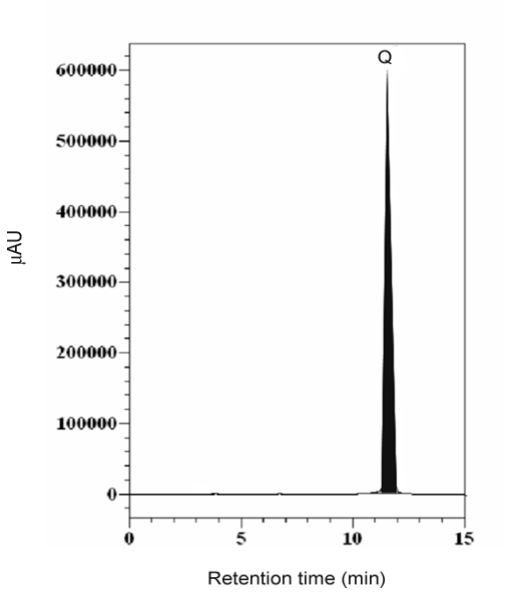

Fig.4 HPLC chromatogram of standard quercetin in methanol

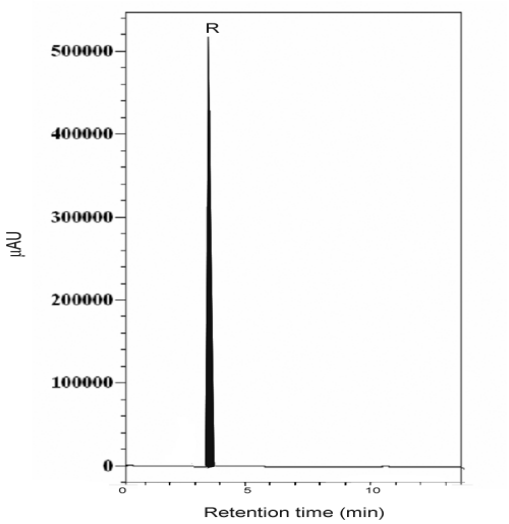

Fig.5 HPLC chromatogram of standard rutin in methanol

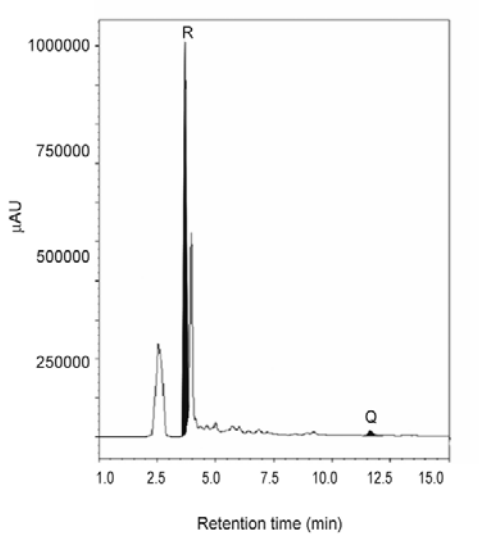

Fig.6 HPLC chromatogram of methanolic extract (Detection at 370nm, peaks : R-rutin, Q-quercetin)

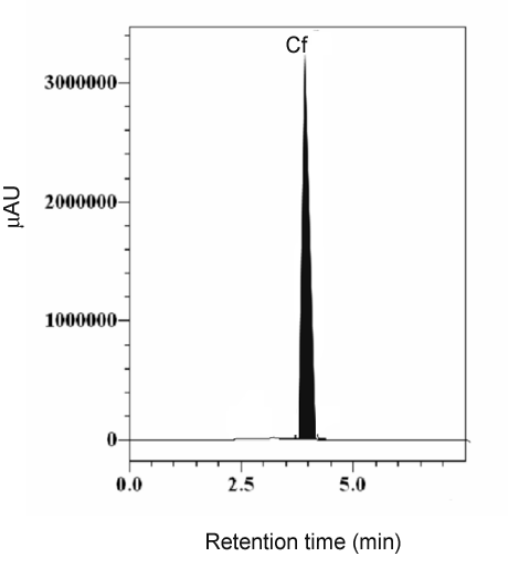

Fig.7 HPLC chromatogram of standard standard caffeic acid in ethanol

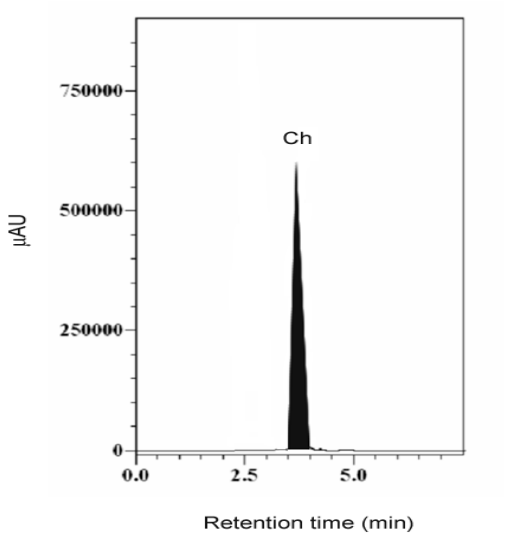

Fig.8 HPLC chromatogram of chlorogenic acid in ethanol

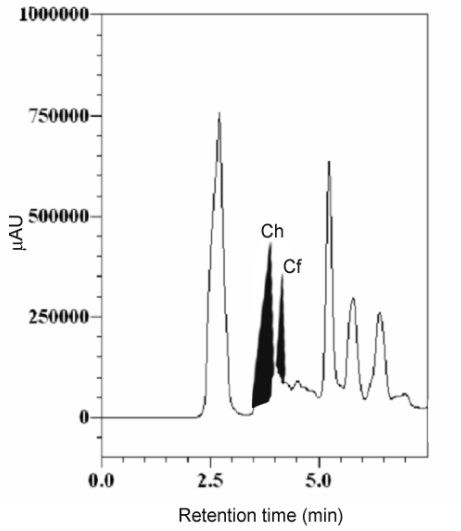

Fig.9 HPLC chromatogram of ethanolic extract (Detection at 320nm, peaks : Ch-chlorogenic acid, Cf-caffeic acid)

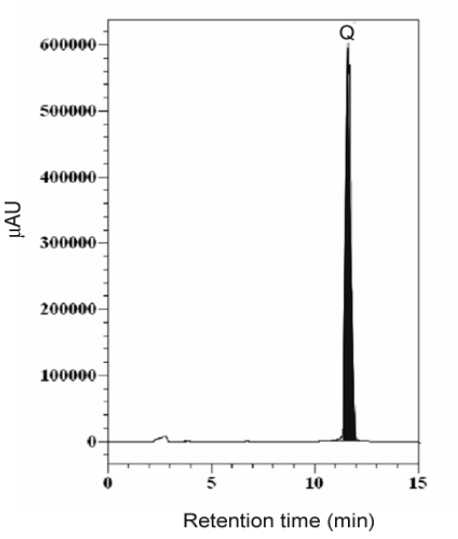

Fig.10 HPLC chromatogram of standard quercetin in ethanol

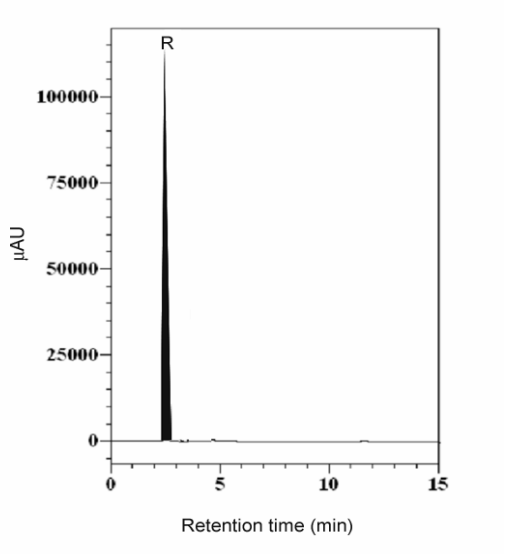

Fig.11 HPLC chromatogram of standard rutin in ethanol

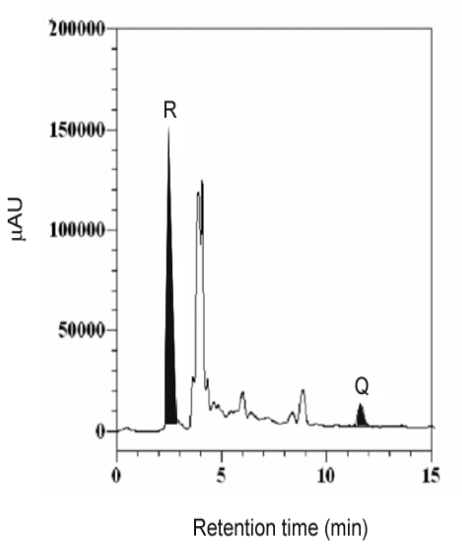

Fig.12 HPLC chromatogram of ethanolic extract (Detection at 370nm, peaks : R-rutin, Q-quercetin)

Table 1

Retention time, peak area and concentration of the compounds in the methanolic extract of coriander seeds

S. No	Compounds	Retention time	Peak area of the standard	Peak area of the compound in the sample	Concentration (%)
1	Caffeic acid	4.033	30271994	2537878	1.89
2	Chlorogenic acid	3.605	11466523	14420915	28.01
3	Quercetin	11.638	9280414	182395	0.11
4	Rutin	3.711	2307114	4960987	11.48

Table 2

Retention time, peak area and concentration of the compounds in the ethanolic extract of coriander seeds

S. No	Compound	Retention time	Peak area of the standard	Peak area of the compound in the sample	Concentration (%)
1	Caffeic acid	4.048	35611226	912467	0.074
2	Chlorogenic acid	3.754	1688610	449472	0.45
3	Quercetin	11.637	9059782	223896	0.07
4	Rutin	2.602	1788621	856806	1.30

Conclusion

In the prepared multi-component extracts of coriander seeds, good amounts of rutin, quercetin, chlorogenic acid and caffeic acid were identified. The analysis revealed rutin to be predominant in both the extracts followed by quercetin, chlorogenic acid and caffeic acid. Though similar flavonoids were identified in both the extracts, the concentration and purity of all the compounds were superior in the methanolic extract as compared with that of the ethanolic extract. The chromatograms of extract prepared in ethanol showed additional peaks indicating the presence of some unidentified compounds released during the extraction in ethanol and identification of such compounds warrants further investigation.

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PAK. J. FOOD SCI., 21(1-4), 2011:22-32
ISSN: 2226-5899

Garlic (*Allium Sativum* L.) as an antimicrobial and antioxidant agent in beef sausages

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Abstract

Spices are the building blocks of flavor in food. Their primary functions are to provide aroma, texture and color to food. In addition they also act as preservative, and provide nutritional, and health benefits. Garlic is known for their antioxidant and antimicrobial properties. Antioxidants are the compounds that inhibit or delay the oxidation reactions in foods. There is more emphasis on natural antioxidant sources these days as compared to the synthetic ones because of their health benefits. In sausages preparation at different levels they not only decrease lipid oxidation but also inhibit the microbial activity. It has been revealed that the antioxidant and antimicrobial activity of garlic is a concentration dependent phenomenon as their concentration increases in the sausages the spoilage that took place due to microbial activity or due to the lipid oxidation tends to decrease. Their addition also effect on the taste, flavour, texture, water activity, pH and colour of the sausages.

Keywords: Garlic, Sausages, Antimicrobial, Antioxidant

Introduction

Antioxidants are the compounds which delay or inhibit the oxidation of other molecules by stopping the initiation of oxidizing chain reactions. The two basic types of antioxidants are synthetic and natural. Synthetic antioxidants include Butylated Hydoxyanisole (BHA) and Butylated Hydoxytoluene (BHT) which are being restricted these days due to their carcinogenicity. Natural antioxidants are of plant origin and they include vitamins, phenolic compounds and flavonoids (Hudson, 1990; El-Ghorab *et al*., 2007).

Antioxidants have been found to slow and inhibit lipid oxidation. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are synthetic antioxidants that have been used in processing. However, BHA has been shown to cause lesion formations in rats while BHT may cause internal and external haemorrhaging at high doses (McCarthy *et al*., 2001). BHT/BHA have been shown to convert certain ingested materials into toxic substances and carcinogens (Farag *et al*., 1989). Due to these recent concerns related to health risks, the focus has shifted to the use of natural antioxidants (Sanchez-Escalante *et al*., 2003).

Antimicrobials agents are used in food for two main reasons (1) to control natural spoilage processes (2) to prevent/control growth of micro-organisms for food safety. Natural antimicrobials are derived from animal, plant and microbial sources. However, methods and mechanisms of action, as well as the toxicological and sensory effects of natural antimicrobials, are not completely understood (Ponce *et al*., 2008). Even without a more comprehensive understanding of how natural antimicrobial substances work, there is a growing effort to develop new effective methods that rely primarily on their use to enhance food safety (Chen *et al*., 2008).

Health benefits of spices

The word "spice" came from the Latin word "species," meaning specific kind. The name reflects the fact that all plant parts have been cultivated for their aromatic, fragrant, pungent, or any other desirable properties including the seed, kernel, leaf, bark, stem, rhizome, root, bulb, flower and fruit. For people throughout the world, spices stimulate the appetite, add flavor and texture to food, and create visual appeal in meals. Spices have been savored and sought around the world from the earliest times because of their diverse functions. Their primary functions are to flavor food and to provide aroma, texture, and color. Spices also provide secondary effects, such as preservative, nutritional, and health functions. They are used in cooked and semi-cooked foods, sauces, dressings and soups, and some of the vegetable spices are consumed raw (Kakar and Iwao, 1974; Revankar and Sen, 1987). As early as 1500 BC, Egyptians used spices as preservatives. This research is focused on spices which are commonly used in Pakistan i.e. garlic.

Spices have a profound influence on the course of human civilization. They permeate our lives from birth to death. In everyday life, spices succor us, cure us, relax us, and excite us. Ancient peoples such as the Egyptian, the Arab and the Roman made extensive uses of spices, not only to add flavor to foods and beverages, but as medicines, disinfectants, incenses, stimulants and even as aphrodisiac agents. In Europe, Middle East and Asia they were used to preserve meat, bread and vegetables. No wonder they were sought after in the same manner as

gold and precious metals. There are many forms in which spices are available e.g. fresh, dried and frozen; whole, ground, crushed, pureed, as pastes, extracts, or infusions (Raghavan, 2007).

The nonvolatile and volatile flavor components of spices, also referred to as oleoresins, are produced by grinding or crushing the spices, extracting with a solvent, and then removing the solvent. Oleoresins have the full flavor, aroma, and pungency of fresh or dried spices because they contain the high boiling volatiles and non-volatiles, including resins and gums that are native to spices. The nonvolatile components create the heat and or pungency of black pepper, mustard, ginger, and chile peppers. These components can be acid-amides, such as capsaicin in red pepper or piperine in black pepper, isothiocyanates in mustard, carbonyls such as gingerol in ginger, and thioethers such as the diallyl sulfides in garlic or onion (Raghavan, 2007).

The taste of a spice such as sweet, spicy, sour, or salty, is due to many different chemical components such as esters, phenols, acids, alcohols, chlorides, alkaloids, or sugars. Sweetness is due to esters and sugars; sourness to organic acids (citric, malic, acetic, or lactic); saltiness to cations, chlorides, and citrates; astringency to phenols and tannins; bitterness to alkaloids (caffeine and glycosides); and pungency to the acid-amides, carbonyls, thio ethers, and isothiocyanates (Raghavan, 2007).

Spices can be used in foods as antioxidants. They help fight the toxins created by our modern world. Heat, radiation, UV light, tobacco smoke, and alcohol initiate the formation and growth of the free radicals in the human body. Free radicals damage the human cells and limit their ability to fight off cancer, aging, and memory loss. Many spices have components that act as antioxidants and that protect cells from free radicals. The chemical components responsible for antioxidant activity in ginger are gingerol and shogoal (Raghavan, 2007).

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Spices include leaves (bay, mint, rosemary, coriander, laurel, oregano), flowers (clove), bulbs (garlic, onion), fruits (cumin, red chilli, black pepper), stems (coriander, cinnamon), rhizomes (ginger) and other plant parts (Shelef, 1983). The importance of spices can be found not only in their flavouring, but also in their medicinal, preservative and antioxidant properties (Darmadji *et al*., 1993). Being plants, the natural foodstuffs, spices appeal to consumers who tend to question the safety of synthetic additives (Farag *et al*., 1989).

Spices in meat and meat products

Fresh meat and meat products can be easily contaminated with microorganisms and, if these are not properly handled and preserved, they support growth of spoilage and pathogenic bacteria, leading to loss of their quality and constitute potential health problems (Vemozy *et al*., 2002). Refrigeration storage is usually the most common preservative method of fresh meat and meat products. In order to extend refrigerated storage time, antimicrobial and antioxidant additives especially those of synthetic origin, are added to beef products. However, consumers increasingly demand use of natural products as alternative preservatives in foods, as the safety of synthetic additives has been questioned in last years (Abdel-Hamied *et al*., 2009). The addition of antioxidants to food products earns increasing popularity as a powerful means for extending the shelf-life of products and for decreasing the nutritional losses by preventing or slowing the oxidation process (Tsuda *et al*., 1994).

The palatability of foods is mainly affected by sensorial factors including appearance, taste, color, and flavor, in addition to internal factors such as physical properties or nutrients. In particular, the flavors of meat and meat products constitute one of the most important considerations in the food industry. However, oxidative products produced during storage and resulting in undesirable rancidity could be developed by microbial spoilage or lipid oxidation, and these products have been shown to deteriorate the quality and safety of meat and meat products. Studies associated with natural or synthetic antioxidants for the inhibition of microbial spoilage and lipid oxidation have been extensively conducted. Synthetic antioxidants, such as butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), and tert-butyl hydroquinone (TBHQ) can significantly delay lipid oxidation however they are limited to their use as additive agents in food systems because they might contain many factors hazardous to health (Branen, 1975). Thus antioxidants and antibiotics derived from natural resources are perceived by consumers as being better and safer than synthetics.

Gill (1986) defined meat spoilage as “any single symptom or group of symptoms of overt microbial activity, manifested by changes in meat odor, flavor, or appearance.” In addition, several scientists have recognized that microbial growth is the most important factor in controlling the spoilage of meat (Jay, 1996) and bacterial numbers of 107 and 108 per cm2 cause noticeable changes in off-odor and slime, respectively. Meat spoilage is typically a surface phenomenon (Gill, 1986).

The discoloration of meat from red to brown during storage results from the oxidation of oxymyoglobin (OxyMb) to metmyoglobin (Mitsumoto *et al*., 1993). In meat, lipid oxidation occurs primarily in membrane phospholipids (PLs). It has been demonstrated that OxyMb and cell membrane PLs oxidations are closely interrelated in muscle foods (Kanner and Harel, 1985). On the other hand, microbial contamination in meat is an important factor associated with meat quality. It has been found that bacterial contamination, such as Salmonella typhimurium, E. coli O157:H7 and Listeria

monocytogenes, impacted meat safety (Cutter, 2000). Based on these observations, it appears that the oxidations of OxyMb and lipid as well as microbial contamination are serious concerns for meat producers and consumers.

Meat and meat products pigment and lipid oxidation are interrelated (Yin & Faustman, 1993). Genot *et al.,* (1991) concluded that O_2 can initiate lipid peroxidation, leading to the formation of prooxidant substances capable of reacting with oxymyoglobin (OMb) and resulting in metmyogobin (MMb) formation. Anton *et al.,* (1993), postulated that OMb could be oxidized not only by lipid-oxy radicals but by other pro-oxidant radicals generated by O_2. Several investigators reported that the susceptibility of myoglobin to autoxidation is the main factor in explaining colour stability in meat and meat products (Renerre *et al.*, 1992).

Ground beef is a popular food item that is used in a variety of dishes. Once the animal has been slaughtered, the meat is fabricated into wholesale or retail cuts. Trim and other cuts of meat are then further processed and ground. This increases the surface area of the meat which allows the increased adherence and growth of the bacteria. The color of ground beef is affected not only by microbial growth, but also by chemical reactions such as lipid oxidation. (Nawar, 1996).

Sausages; Nutritional Quality

Sausages are acceptable to consumers today because they are convenient, economical, have good variety and nutritional value and contain important amounts of high quality protein. Sausages are also good sources of various essential minerals such as iron, zinc, folic acid, vitamins B6 and B12 and fat for energy (Pearson & Tauber, 1984). According to Ranken (2000) the primary economic purpose of sausages was original but the sausage products take little time to prepare with some being ready-to-serve or simply warmed before serving. For these reasons sausage is favoured by both working men and women.

Sausage is one of the popular foodstuffs among meat products (Barbut, 2001). However during storage, quality attributes of the product deteriorate due to lipid oxidation and microbial growth. Lipids oxidation is responsible for reduction in nutritional quality as well as changes in flavor (Aguirrezabal *et al.*, 2000), while microbial contamination can precipitate major public health hazards and economic loss in terms of food poisoning and meat spoilage. Thus, application of suitable agents possessing both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf-life and preventing economic loss (Yin & Cheng, 2003). Raw materials that can be used during the sausage making from cattle include, boneless primal cuts such as chucks, plates (flat rib), flanks and boneless bull meat, cheek meat, head meat, hearts, tripe, livers, tongues and weasand meat (muscle around oesophagus). Relevant meat sources from sheep are boneless primal cuts, hearts, cheek meat and tongues (Wilson, 1960). Sausages, in addition to their meat components, have additional sources of organisms in the seasonings and other formulation ingredients/additives such as spices that are added during production (Jay, 2000). Intestines for meat stuffing are collected directly after slaughtering and dressing the carcass and are immediately and thoroughly cleaned. The intestines of the digestive tract of cattle and sheep are used as sausage casings during the making of sausages (Ockerman and Hansen, 1988).

Garlic as antimicrobial and antioxidant agent

With the rise in bacterial resistance to antibiotics, there is considerable interest in the development of other classes of antimicrobials for the control of infection. Garlic (Allium sativum) has been used as a medicine since ancient times and has long been known to have antibacterial, antifungal and antiviral properties. More recently garlic extract has been shown to be an effective agent for controlling methicillin-resistant Staphylococcus aureus. (Cutler and Wilson, 2004). The main antimicrobial constituent of garlic has been identified as the oxygenated sulphur compound, thio-2-propene-1-sulfinic acid Sallyl ester, which is usually referred to as allicin. Allicin is not present in raw garlic. It is formed rapidly by the action of the enzyme, allinase (alliin lyase) on S-allyl-L-cysteine-sulphoxide (alliin) when the garlic is crushed. Allicin reacts very rapidly with free thiol groups, via thiol-disulphide exchange and, therefore, it is thought that its main mechanism of antimicrobial action is through interaction with thiol-containing enzymes, including cysteine proteases and alcohol dehydrogenases. Because these enzymes tend to be essential for bacterial nutrition and metabolism it has been suggested that development of resistance to allicin arises 1000-fold less easily than it does to certain antibiotics. Indeed, allicin and garlic extract have been shown to have a wide spectrum of antibacterial activity, including effects on Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Clostridium, Mycobacterium and Helicobacter species.(Bakri and Douglas, 2005).

Garlic (Allium sativum) is bulbous perennial vegetable spice belongs to family Alliaceae. It is locally known as “Lehsun” in Pakistan. Garlic is an earth wonder the name of which has been lived by Turks without forgetting during long history and extensive geography of them (Akcicek, 2006). Garlic the land of which is said as Middle and West Asia steps has a place among eldest crop plants. This plant, which is of great medical importance takes place inside many foods especially, meat ones due to its sharp odour, appetizer property and bitter taste and gives flavour to them (Kutevin and Turkey, 1987). 100g garlic has 63.8 g of water, 28.2 g of carbohydrate, 5.3 g of protein, 0.2 g of

oil, 11 g cellulose and contains 140 calories (Baytop, 1999; Kutevin and Turkey, 1987). Garlic can be consumed as fresh and has also its pills, capsules and extracts. (Ayaz and Alpsoy, 2007). Garlic is a plant, which kills bacteria, fungus, parasites and lowers glycaemia and cholesterol and has liver protector property and includes antitumor agents. Garlic, with >200 chemical substances in its body, has the capacity of protecting human body against many illnesses. Although, it is said that garlic should be consumed as fresh for it can be effective, some researchers argue that in some situations its cooked and waited extracts and oils can provide better protection against free radicals and infections than fresh garlic. Garlic cloves include a mixture of mono and polysulphides smelling very heavy (Baytop, 1999; Ayaz and Alpsoy, 2007).

Garlic is commonly used as a spice in different recipes. They form an important part of curry, curry powders and many savory spice mixtures. Garlic is not only used as spice and culinary purposes but they also have medicinal uses. It act as antimicrobial as well as antioxidant agent. Garlic is a good source of antioxidants having good anti-mutagenic and anti-inflammatory properties. The antioxidant properties of garlic outperform commonly used chemical antioxidants (BHA and BHT). Garlic also have high total phenolic content showing high antioxidant activity. These properties make Garlic good free radical scavengers (Kikuzaki and Nakatani, 1993; Schulick, 1993; Thippeswamy and Naidu, 2005).

Garlic (Allium sativum L., Alliaceae) has been playing one of the most important dietary and medicinal roles in human beings for centuries. It has been cultivated since ancient times, used as a spice and flavouring and, due to its potential benefits in preventive and curative medicine, has been used in many cultures (Rivlin, 2001). Even today, the medical use of garlic is widespread and growing. Epidemiological, clinical, and preclinical studies have shown close relation between dietary habits, including garlic intake, and the occurrence of disease. Furthermore, garlic was investigated extensively for health benefits, which has resulted in more than 1000 publications over the past decade. It is considered to be one of the best disease-preventive foods, based on its potential and varied effects (Amagase, 2006). A wide array of therapeutic effects, such as hypolipidaemic, antiatherosclerotic, hypoglycaemic, anticoagulant, antihypertensive, antimicrobial, antidote (for heavy metal poisoning) and hepatoprotective, has been reported (Rivlin, 2001).

Garlic (Allium sativum L.) is a common food spice, used widely in many parts of the world. For many centuries various species of genus Allium have been used as vegetables and spices, and as folk medicines for the curing of various diseases. The powerful and unusual flavors of many of these plants and their possible medical applications have attracted the attention of plant physiologists and chemists (Akgul, 1993). It has been cultivated for centuries all over the world on account of its culinary and medicinal properties (Sharma & Prasad, 2001). Many of the spices and herbs used today were known to the people of the ancient cultures throughout the world, and they were valued for their preservative and medicinal powers as well as their flavor and odor qualities (Zaika, 1988). Most of its prophylactic and therapeutic effects are ascribed to specific oil- and water-soluble organosulfur compounds, which are responsible for the typical odor and flavor of garlic (Sivam, 2001). Garlic is mainly consumed as a condiment in various prepared foods such as mayonnaise and tomato sauce, salad dressing, meat sausage and pickled products. Works have been conducted on the antimicrobial effects and drying of garlic (Sharma & Prasad, 2001).

Garlic is also cultivated for its medicinal properties and this aspect is steadily increasing in the world. It has benefits in lowering total plasma cholesterol, reducing blood pressure and decreasing platelet aggregation (Sterling and Eagling, 2001). Garlic products have become popular in recent years and a variety of culinary and pharmaceutical preparations are now available in market (Velisek *et al*., 1997). The constituents of garlic are divided into two main groups, sulfur-containing compounds and non-sulfur-containing compounds. Most of the medicinal effects of garlic are referable to a sulfur compound known as allicin (Schulz, 1998). The intact garlic clove does not contain allicin but rather its precursor, the non-protein amino acid alliin. Alliin is converted to allicin, pyruvate and ammonia by the enzyme allinase, when the bulb is cut or crushed (Rabinkov *et al*., 1994). The amount of allicin in fresh garlic is highly variable. It has been known that allicin content, which released from garlic samples from various regions, is very variable (Schulz, 1998). Furthermore agronomic parameters also cause variations in phytomedical levels (Amrita *et al*., 2009). According to Beek pharmacopoeia (2007), the minimum allicin content to ensure pharmaceutical and economical viability of garlic powder products should be 4.5 mg/g. Hence it is important to standardize garlic, i.e. breeding a garlic clone with suitable content of allicin and agronomical traits which are needed for the large-scale culture and drug production. Garlic is a sterile species and reproduces only by vegetative propagation. A series of different ecotypes have been established over time in various areas of cultivation. Considerable morphological and biochemical variations between and within ecotypes are displayed. These differences were described with the objective of selection the best quality of active substance (Avato *et al*., 1998).

Garlic was analysed for moisture, protein, oil, energy, fiber, ash, water-soluble extract, pH, acidity, DMS and essential oil contents. Crude protein, crude oil, crude fiber, crude energy, DMS and essential oil contents

were found to be 17.3%, 0.34%, 2.17%, 410.7kcal/100 g, 1779lcg/kg and 0.14, respectively. (Haydar *et al*., 2005). Garlic include a wide range of primary and secondary non-sulfur biomolecules, such are steroidal glycosides, essential oil, anthocyanins, lectins, prostaglandins, fructan, pectin, adenosine, vitamins B1, B2, B6, C and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids and essential amino acids (Biljana *et al*., 2008). It is generally considered that health-related functions are mostly attributed to the fresh garlic content, rich in c-glutamylcysteine and many other sulfur-containing compounds in it, giving a characteristic flavour formed during storage and processing (Banerjee et al., 2003).

The ratio of volatiles to non-volatiles varies among spices causing flavor similarities and differences within a genus and even within a variety. Within the genus *Allium*, for example, there are differences in flavor among garlic, onions, chives, shallots, and leeks, which differ in this ratio. They vary depending upon the species of spice, its source, environmental growing and harvesting conditions, and storage and preparation methods. Even the distillation techniques can give rise to varying components through loss of high boiling volatiles, with some components not being extracted or with some undergoing changes. Non-volatiles in a spice also vary with variety, origins, environmental growth conditions, stage of maturity, and postharvest conditions (Peter, 2001). Garlic is one of the most commonly used ingredients as a flavor enhancement for sausage. In addition to flavoring the foods, garlic is appreciated for its medicinal properties. Garlic has a wide spectrum of actions; not only antibacterial, antiviral, antifungal and antiprotozoal, but also has beneficial effects on the cardiovascular and immune systems (Harris *et al*., 2001).

Garlic extracts have also been shown to have antioxidant activity in different in vitro models. The antioxidant activity of Allium plants has mainly been attributed to a variety of sulphur-containing compounds and their precursors (Nuutila *et al.*, 2003). These compounds have been also reported as responsible for their in vitro antibacterial activity (Tsao and Yin, 2001). Garlic, as an anti-bacterial agent, is effective against many more gram negative and gram positive bacteria like Helicobacter pylori, E. coli, Lactobacillus casei and that this effect is sourced from allicin inside it (Celli *et al*., 1996; Lemar *et al*., 2005).

The antibacterial effect garlic apparently results from interaction of sulphur compounds, like allicin, with sulphur (thiol) groups of microbial enzymes (such as trypsin and other proteases), leading to an inhibition of microbial growth. Many bacterial strains, both gram-positive and gram-negative, can be inhibited with garlic, and some strains were inhibited much more strongly by allicin or garlic extract compared to antibiotics (Bakri and Douglas, 2005). The bacterial strain Staphylococcus aureus causes pus-producing infections, such as boils, as well as pneumonia and urinary tract infections. Cultures of this strain (as well as Salmonella enteritidis, the bacterium responsible for salmonella food poisoning, and several fungi) are effectively inhibited by garlic and onion oil or extracts (Benkeblia, 2004). Other microbes inhibited by garlic include Bacillus subtilis, a gram-positive bacterium found in soil, Escherichia coli, a common toxin-producing, food-borne bacterium, and Saccharomyces cerevisiae, a yeast species (Lai and Roy, 2004). Remarkably, mouthwash containing garlic significantly reduced total salivary bacteria, including Porphyromonas gingivalis, the bacterium causing gingivitis (Bakri and Douglas, 2005).

The application of natural ingredients containing antioxidants and antibiotics may prove useful as an antioxidant and antimicrobial source in meat and meat products without any quality defects. In particular, the *Allium* family of plants contains a number of sulfur and phenolic compounds (Lanzotti 2006), which has excellent antioxidant and antimicrobial activity. Garlic (*Allium sativum*) and onion (*Allium cepa*), both from the *Allium* family, are two of the most commonly utilized ingredients as flavor enhancers for several foods. They have been shown to possess antimicrobial (Benkeblia 2004) and antioxidant effects in many previous studies (Iqbal and Bhanger 2007), and contain a variety of functions, including antibacterial, antiviral, antifungal, antiprotozoal, and anticancer effects as the result of a number of sulfur, phenolic, and selenium compounds (Tang and Cronin, 2007). Garlic contains approximately 3 times more sulfur-containing compounds than onion (Lawson 1996), whereas, onion contains higher amounts of flavonol compounds than garlic (Lanzotti 2006).

Garlic showed effective antioxidant activity in vivo and in vitro (Jackson *et al*., 2002). Garlic includes to >200 components such as volatile oils (allicin, alliin and ajoene) consisting of sulphur, enzymes (alliinase, peroxidase and miracynase), carbohydrates (sucrose, glucose), minerals (germanium, selenium, zinc), amino acids like cysteine, glutamine, isoleucine and methionine, bioflavonoids like quercetin and cyanidin and allistatin I and allistatin II, C, E and A vitamins and niacin, B_2 vitamins and beta carotene (Gulsen Goncagul and Erol Ayaz, 2010).

The antioxidant activity and total phenolic content of garlic etanolic extract was determined by Charanjit and Harish, (2002), by using β carotene and folin Ciocalteau reagent method respectively. They found that the ethanolic extract of garlic shows 62.1% antioxidant activity and the total phenolic content were 145.0 ± 5.9.

The antioxidant activities of the methanol extracts of garlic was determined by two methods, inhibition of lipid peroxidation induced by tert-butyl hydroperoxide in isolated rat hepatocytes and scavenging activity against diphenylpicrylhydrazyl radical. The total phenolics and the main flavonoids of the hydrolysed

garlic sample were analysed. The antioxidant activities obtained by the two methods were compared. Both methods gave similar antioxidant activities for pure compounds and Allium extracts. However, the radical scavenging method had many benefits compared to the lipid peroxidation method, being easier, cheaper, more specific and reproducible. The radical scavenging activities also correlated positively with the total phenolics of the extracts. Quercetin and kaempferol were found to be the most abundant flavonoids in the hydrolysed sample. The total phenolic contents levels were detected for garlic (75–700 GAE mg kg-1). Garlic is very high in antioxidants, its activity being about 1300 Trolox equivalents/100 g. (Anna *et al.*, 2003)

The antimicrobial activity of garlic and garlic-derived organosulfur compounds was widely investigated against both food spoilage bacteria and food-borne pathogens (Leuschner and Ielsch, 2003; Naidu, 2000; Unal *et al*., 2001). Sulphur and polyphenols present in garlic respond to the antibacterial, antifungal and antioxidant activity (Benkeblia, 2004).

Garlic is a plant, which kills bacteria, fungus, parasites and lowers glycaemia and cholesterol and has liver protector property and includes antitumor agents. Garlic, with >200 chemical substances in its body, has the capacity of protecting human body against many illnesses. Garlic consumed as fresh provides protection against free radicals and infections (Ayaz and Alpsoy, 2007).

In vivo antioxidant effects of several organosulfur compounds derived from garlic and onion have been studied (Yeh and Liu, 2001). In these studies, two lipophilic organosulfur compounds, diallyl sulfide (DAS) and diallyl disulfide (DADS) and two hydrophilic organosulfur compounds, s-ethyl cysteine (SEC) and n-acetyl cysteine (NAC), protected against lipid-related oxidations by activating associated antioxidant enzymes. On the other hand, antimicrobial activities of DAS and DADS against several human concerned medical pathogenic bacteria and fungi have been studied in our laboratory (Tsao and Yin, 2001a; 2001b) and others (O'Gara, Hill, and Maslin, 2000). These authors found that DAS and DADS effectively inhibited the growth of Helicobacter pylori, Escherichia coli, methicillin-resistant Staphylococcus aureus, Pseudomas aeruginosa, Klebsiella pneumoniae, Candida spp. and Aspergillus spp.

Ethanolic extract of garlic at GC-MS was performed. The major compounds of GC-MS analysis areallicin (5.37%), palmitic acid (4.52%), 3-deoxy-dmannoic lactone (3.86%), thymine (3.50%) and hexanedioic acid bis (2 ethylhexyl) ester (1.87%) (Shobana *et al*., 2009). Cavallito and Baily (1944) reported that the principle antimicrobial component of garlic is allicin (diallyl disulfide and diallyl trisulfide).

High performance thin layer Chromatography (HPTLC) analyses revealed allicin was the major compound present in different concentration of each extract, in accordance with previous study (Canizares *et al*., 2004). Allicin concentrations vary between aqueous and ethanolic extracts of *sativum* (14.64% and 15.61%) and *ophioscordon* (5% and 9%). These observations when correlated with antibacterial nature of sub-varieties of garlic extract revealed that allicin concentration increases the antibacterial property of garlic. Allicin present in the ethanolic extract of *sativum* inhibited almost all the organism with a significant zone of inhibition (Shobana *et al*., 2009) . The other compounds present in the extracts may also be responsible for the antibacterial nature. This is accordance with Wills (1956), who studied the effect of allicin solutions on the growth of both gram-positive and gram-negative bacteria.

Suree and Pana (2005), determined the inhibtry action of crude ethanolic extracts and essential oils of 14 spices including cardamom, cinnamon, clove, coriander, cumin, garlic, ginger, holy basil, kaffir lime leaves and peels, lemongrass, mace, nutmeg, black and white pepper, and turmeric were examined for their antibacterial activity against 20 serotypes of *Salmonella* and 5 species of other enterobacteria using disk diffusion method as preliminary screening. Garlic oil possessed moderate antibacterial activity in this study. *E. coli* was more sensitive to garlic extracts than *E. aerogenes* which is in agreement with previous observations. The major antimicrobial compound in garlic is allicin. Garlic extracts have been found to possess antibacterial property against several bacteria including *S.* Typhimurium, *S.* Typhi, *E. coli*, *Bacillus cereus*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*.

Priscila *et al*.,(2007) studied the antibacterial activity of methnolic extract of garlic, the Minimal Inhibitory Concentration (MIC) were carried out for fifteen *Salmonella* Typhimurium, *S. aureus*, *Enterococcus* sp and *E. coli* strains plus one ATCC strain and the found that Garlic extracts showed high antimicrobial action against Gram negative strains.

Antibacterial activity of ethanolic extrac of garlic extract has been evaluated against *Klebsiella pneumoniae, Staphylococcus aureus, Morganella morgani, Candida albicans, Escherichia coli* and *Proteus vulgaris.* Garlic extract showed excellent antibacterial activity against *P. vulgaris* and *Morganella morgani.*Garlic extract showed antibacterial activity and the activity was a linear function of concentration (Melvin *et al*., 2009).

Ethanolic extract of garlic was highly effective against all the bacterial species that was taken for the study whereas *Escherichia coli*, *S.typhi* and *S.flexineri* were sensitive to ethanolic extract of ophioscordon (Al-Delaimy and Ali, 1970). They also reported that 4% (w/v) fresh garlic in extract inhibited the growth of S. aureus, E. coli, S. typhi and S. dysenteriae. Garlic is rich in anionic components such as nitrates, chlorides and sulfates as well as other water soluble components common in most

plants which may be responsible for its antibacterial activity (Astal, 2004).

Antibacterial activity of two varieties of garlic (ophioscordon and sativum) against enteric pathogens such as *Escherichia coli, Proteus m irabilis, Salmonella typhi, Shigella flexineri and Enterobacter aerogenes*. Aqueous extract of both the garlic varieties inhibited the growth of enteric pathogens at the concentrations of 200,300,400 and 500mg. However *Enterobacter aerogenes* was not susceptible to the aqueous extract of both the garlic varieties. Ethanolic extract of sativum was found to be highly effective against all the bacteria tested. The major compounds of GC-MS analysis include allicin (5.37%), palmitic acid (4.52%), 3-deoxy-dmannoic lactone (3.86%), thymine (3.50%) and hexanedioic acid bis (2-ethylhexyl) ester (1.87%). The principle antimicrobial component of garlic is allicin (diallyl disulfide and diallyl trisulfide). Allicin is the major compound in the GC-MS analysis. However high levels of palmitic acid (4.52%) in garlic extract also posses antibacterial activity. The phytochemical constituents are varied between the garlic varieties, which could be the reason for the variation in their antimicrobial properties (Shobana *et al*., 2009).

Park *et al*., (2008), studied the antimicrobial and antioxidant effect of garlic and onion in fresh pork belly and lion during refrigerated storage and they found that the by their addition there is considerable decrease in the microbial activity and lipid oxidation. The peroxide value is also much lower than the control once. The result shows that there is no significant change in changes in proximate compositions but the pH values of belly and loin were range from6.13 to 6.27 and 5.76 to 5.93, respectively.the color of lion increased with increase in storage time but the colour of belly increased in first 7 days then after it decreased. There is significant differences in per oxide values were observed in the bellies containing sodium ascorbate (1.09 meq/kg) and garlic powder (1.19 meq/kg) as compared to that of the controls (1.84 meq/kg), whereas POVs of loins with sodium ascorbate (3.62 meq/kg) and onion powder (3.52 meq/kg) were lower than that of control (5.00 meq/kg; $P < 0.05$). The different effects of garlic and onion on POVs of different meat cuts might be due to not only the differences in the chemical composition between belly and loin cut, but also the different interaction of antioxidant activity with the addition of garlic and onion. Microbial counts in the belly and loin with different ingredients are determined. Approximately 14 d were required to reach total bacterial counts of 7 log CFU/g in both the belly and loin, and these were maintained until the end of storage.Total plate counts (TPCs) were lower in both the belly and loin cuts containing garlic and onion powder than the control.

Chopped garlic added to raw meatballs which is a traditional food product eaten raw in Turkey has a slowing-down effect on microbiological growth in ground meat depending on the garlic concentration (Aydin *et al*., 2007). The antioxidant and antimicrobial protection of garlic derived compounds, diallyl sulfide, diallyl disulfide, s-ethyl cysteine, n-acetyl cysteine were investigated against discoloration, lipid oxidation and microbial contamination in ground beef. By the addition of garlic-derived organosulfur compounds significantly delayed both oxymyoglobin and lipid oxidations. The antioxidant protection from these organosulfur compounds was dose-dependent and showed significantly greater antioxidant activity than a-tocopherol. The presence of diallyl sulfide and diallyl disulfide in ground beef significantly reduced total plate count and inhibited the growth of five inoculated pathogenic bacteria, Salmonella typhimurium, Escherichia coli, Listeria monocytogenes, Staphyllococcus aureus and Campylobacter jejuni. The result demonstrates that the application of these garlic derived compounds in meat or other food systems could enhance color, lipid and microbial safety (Mei-chin Yin and Wen-shen Cheng, 2003).

Sallam *et al.,* (2004) determined the antioxidant and antimicrobial activity of garlic in chicken sausages. They prepared sausages from ground chicken by using the different formulation to contain either fresh garlic (20, 30 or 50 g/kg), garlic powder (6, 9 or 15 g/kg), garlic oil (0.06, 0.09 or 0.15 g/kg) and BHA (0.1 g/kg). They found that the moisture, protein and fat contents (g/100 g) in the control sausage were 71.3 ± 1.23, 16.4 ± 0.57 and 7.98 ± 0.31 respectively. Addition of the different garlic forms did not cause any significant changes in these contents, pH values, which tended to increase with storage time. However, after 21 days of storage no significant difference was detected between pH of fresh garlic-formulated sausage 7.03 and any of the other sausage formulations, which were ranged from 6.85 to 6.90. The initial TBA value was 0.140, and after 21 days of storage, it ranged from 0.151 to 0.175 in FG-formulation, 0.162 to 0.187 in GP-formulation, 0.198 to 0.214 in GO-formulation and averaged 0.191 in BHA-formulated samples. These values were significantly lower than that of control samples (0.278). The initial POV was 6.32, however after 21 days of storage, it ranged from 4.92 to 6.22 in FG-formulated samples, 5.68 to 6.91 in GP-formulations, and 7.74 to 8.88 in GO-formulations, while it was 7.21 in BHA-formulated samples. These values were significantly lower than that of the control (15.61). The POV of the FG-formulated sample (50 g/kg) was also significantly lower than that of BHA-formulated samples. Initial aerobic plate count (APC) in the sausage was 4.41 log10 CFU/g and during the first 10 days of storage the count in all sausage formulations remained below 7 log10 CFU/g which is the MPL (Maximal Permissible Limit) for APC recommended by ICMSF (1986). At storage day 21, sausage samples formulated with either FG (30 g/kg) or GP (9 g/kg) maintained lower APC (6.42 and 6.94 log10

CFU/g, respectively) than the MPL, while APC in each of control, BHA- and GO-formulated samples exceeded the limit of 7 log10 CFU/ g by 2.46, 2.23 and 1.12–1.64 logs, respectively. After 21 days storage, APC of both FG- and GP formulated sausage were significantly lower than that of either the control or BHA-formulated samples. Moreover, FG-formulated samples showed significantly lower APC than those of the GO formulations. However, addition of garlic oil or BHA resulted in no significant difference in APC when compared with control samples. This study concluded that fresh garlic, garlic powder and garlic oil provide antioxidant and antimicrobial benefits to raw chicken sausage during cold storage (3°C) and the effects are concentration dependent. Among the garlic forms studied, fresh garlic at a concentration of 50 g/kg sausage demonstrated the most potent effect, but such a high concentration may not be acceptable by many people because of its strong flavor.

Conclusion

The overall evaluation of this study concludes that garlic have a good antioxidant. It showed appreciable amounts of antioxidant compounds showing good inhibition properties against the free radicals. They exhibited good antioxidant and antimicrobial activity in beef sausages. So this study concludes that garlic have good antioxidant and antimicrobial potential and these spices can be used to produce novel natural antioxidants as well as flavoring agents that can be used in various food products.

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PAK. J. FOOD SCI., 21(1-4), 2011:33-44
ISSN: 2226-5899

Development of texturized vegetable protein using indigenous sources

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Abstract

The mandate of present study was the preparation of texturized vegetable protein using indigenous sources (chick pea) as raw material and to evaluate the texturized vegetable protein based patties. As for as the effect of temperature on physical attributes is concerned it showed a linear relationship between temperature and expansion ratio with values ranging 1.85, 1.88, 1.89 and 1.92 for four treatments T_1, T_2, T_3 and T_4 respectively. T_4 was most suitable value of temperature for maximum expansion. But for bulk density results were declining with increase in temperature having values ranging 0.63g.L, 0.61g/L, 0.59g/L and 0.57g/L for four treatments T_1, T_2, T_3 and T_4 respectively. The results for fat content and ash content of texturized vegetable protein were non significant. Moisture content was decreased by increasing barrel temperature of extruder. The protein of final product showed results ranging 22.39, 22.360, 22.34 and 22.33 for four treatments respectively. The color, flavor, taste, texture and overall acceptability of patties showed increasing trend with rise in temperature. Due to all attributes close to texturized vegetable protein made of soybean, the product was approved by sensory panel for use as color, texture and taste fulfilled the desired characteristics.

Keywords: Texturized vegetable protein,

Introduction

Texturized vegetable protein (TVP) has prime importance in food industry as well as from the health point of view. TVP is cholesterol free and used worldwide due to quality protein of plant source. Its utilization is also related with religious, cultural and economic issues especially it is popular in vegetarian. Also worldwide relief agencies use this product besides its utilization in child school nutrition programmes. It is easy to handle and larger shelf life than real meat and also have health benefits (Riaz, 2000).

Textured vegetable protein (TVP) are generally, those fabricated vegetable products that can be used to replace meat completely in a food serving and those textured vegetable protein entities that can be eaten in combination with meat as extenders. These textured plant protein resemble meat in chewness and flavor (Siddique, 2000). Animal protein, particularly meat is expensive and on worldwide basis in short supply (Birch *et al.*, 1986). Hence, there is a need to make the animal protein replacer. This can be done by utilizing concentrated plant proteins and their processing. Although most vegetable proteins are of inferior quality to animal protein but legumes are good source of protein (Siddique, 2000).

Today, due to increasing consumer demand for healthy diets and concerns about rising meat prices, it is perception that plant protein based food materials will get prime importance as meat alternatives worldwide. This is the reason that various types of plant protein based meat products are now seen in the market. There are three categories of consumers. One who limits certain animal products because of religious dietary restrictions, the second group consists of those looking for a healthier alternative to meat. The third group consists of people who are looking for cheaper protein sources. Development of texturized vegetable protein is landmark of modern technology. This can help the mankind to combat with the challenges of the modern era. This is because; problems are increasing by the day. The major problems which are likely to happen include economic, cultural, religious and social problems and TVP can help in this regard (Liu, 1997).

The texturization of plant proteins has been a major development in the food industry. Processes, like extrusion, have been developed to impart a fibrous structure to amorphous plant proteins. In this production process proteins are effectively denatured during moist thermal process of extrusion. Denaturation of protein lowers solubility, renders it digestible and destroys biological activity of enzymes and toxic proteins (Smith, 1975).

Depending upon chemical composition of proteins and properties of individual constituent's legumes especially soybean is valuable for production of texturized vegetable proteins mainly by extrusion process. Texturizing is done using high temperature, pressure and shear forces on proteineous and non-proteineous consituents, maintaining limited excess of water in extruder.(Ledward and Mitchell, 1988).

Legume grains occupy an important place in human nutrition, especially in low-income groups of people in developing countries. Legumes are prepared for consumption in many ways, such as whole legumes called grains or dehusked and split legumes, known as dhals. They are generally good sources of slow release carbohydrates and are rich in proteins (18–25%) and

Soya bean is unique in containing about 35–43% proteins. They are also good sources of minerals and vitamins. It has been reported that germinated legumes are rich in vitamin C and in some cases there is an increase in the riboflavin as well as niacin contents upon germination. They are also the cheapest sources of supplementary proteins in vegetarians diets (Swaminathan, 1988).

Dehulled chickpea splits as chana dhal contains approximately 20.8% protein, 5.6% fat, 2.7% minerals, 1.2% fiber and 59.8% carbohydrate (Gopalan *et al*., 1995).The Chickpea splits are used in vast variety of forms. They may be ground to flour (besan), cooked into thick or thin gruels or combined with cereals in diverse way to make traditional foods (khichdi, dhokla, puran poli) and used in the preparation of sweet meats (Achaya, 1984). Soybeans vary widely in nutrient content based on the specific variety and growing conditions, but typically they contain 35 to 40% protein, 15 to 20% fat, 30% carbohydrates, 10 to 13% moisture, and around 5% minerals and ash (Riaz, 2000).

Pakistan being an under developed country is facing so many problems in different fields of life and food is the major sector. Due to economic recession prevailing worldwide and affecting Pakistan as well the prices of commodities are very high, especially animal meat is not in the approach of common citizen. In this regard there is need to develop alternative ways and means of alleviating this economic decline in food sector and present study is carried out to develop an alternative of animal meat utilizing indigenous sources. As this meat alternative, so called texturized vegetable protein, is being produced in developed countries from soybean but in Pakistan due to less production of soybean, it is not economical to use soybean so chickpea is seconded due to high protein content. The mandate of present study was the preparation of texturized vegetable protein using indigenous sources as raw material and to prepare and evaluate the texturized vegetable protein based patties.

Materials and Methods

Procurement and cleaning of raw material

Raw material (chickpea) was purchased from Ayub Agriculture Research Centre Faisalabad and cleaned manually in order to remove dust particles and stones, damaged seeds, seeds of other crops and other impurities such as weeds and metals. After cleaning, grains were dehulled and again cleaned manually. Dehulled and cleaned grains of chickpea were milled to get flour of size 15-20 mesh size for further process of extrusion (Riaz, 2000)

Extrusion Process

The texturized vegetable protein prepared from chickpea source used in this study was made on a single screw extruder (Extru-Tech, Inc.Model # KN, Sabetha, Ks 66534). The extrusion process was carried out as directed by Sakatal *et al*., (1999). The extruder was fitted with a 2 start screw and a 2-hole die with 4 mm apertures. The ingredients were fed in the form of chickpea flour of 15 to 20 mesh size. Preconditioning was done in preconditioner of extruder at temperature 40 $^{\circ}$C and 10 to 25 % moisture content provided by steam. The extruder was operated at barrel temperatures between “140”, “145”, “150” and “155”°C for four different treatments T_1, T_2, T_3 and T_4 respectively. Due to high temperature treatment of barrel a laminate was formed along the barrel of extruder and moved towards the die of extruder under high pressure and emerged out of die in the form of a round shaped cylindrical extrudates of texturized vegetable protein. As the extrudates emerged from the die, it was cut into 10-30mm long pieces with a rotating knife. The samples of texturized vegetable protein were dried at 30-35°C for 30-45 min in a drier and stored in plastic bags at room temperature for further analysis.

Physical analysis

The expansion ratio of texturized vegetable protein was determined by measuring diameter of extrudates at several different locations along the strand of extrudates (Rayas *et al.,* 1998). The bulk density was calculated by dividing the weight of extrudates by its volume presented by method of Okaka and Potter (1979). Water solubility index was determined from the amount of dried solids recovered by evaporating the supernatant, and was expressed as gram dried solids per gram of sample (Anderson *et al.,* 1969). The Water absorption of texturized vegetable protein was measured by the centrifugation method of Vani and Zayas (1995). The water and oil absorption capacities were expressed as grams of water or oil bound per gram of the sample on a dry basis. The Hardness of the texturized vegetable proteins was determined by using TA.XT.PLUS. (Texture Analyser Stable Micro-Systems UK) (Veronica *et al*. 2006). Method of Krishna and Ranjhan (1981) with slight modification was used to estimate the Calorific Value (C .V) of the texturized vegetable protein by using Parr Oxygen Calorimeter. The pH of texturized vegetable protein was determined by pH meter model (WTW Series pH 720) by the method of Rhee *et al*. (2004).

Chemical assays of texturized vegetable protein

All the samples were analyzed for the moisture, ash, fat, protein and fiber contents according to their respective method Nos. 44- 15A, 08-01, 30-10, 46-10 and 32-10, given in AACC (2000).

Preparation of patties

The patties were prepared by the method as directed by Crowe *et al.,* (2001). Texturized vegetable

protein was ground and hydrated. Then coarse ground beef was blended with the appropriate amount of hydrated TVP. The mixture of ground beef and TVP was then ground by passing through a meat grinder fitted with a 0.32-cm die plate, and ground mixture was held at 4°C until used to make patties. TVP based ground beef patties were formed by placing 48 g 50% TVP ground beef into a cylindrical mold and hand patting to a uniform thickness of 1.3 cm. The patties were held in refrigerator until cooked. Then the patties were cooked at 185°C for 3.5 min, flipped, and allowed to cook for an additional 2.5 min to an internal temperature of 70°C. The patties were allowed to cool to room temperature and then samples were taken for sensory evaluation.

Sensory evaluation of patties

The samples of TVP based patties were subjected to sensory evaluations conducted by a panel of judges from the staff and postgraduate students of National Institute of Food Science And Technology (NIFSAT). A 9- point hedonic scale (from 1=extremely disliked to 9= extremely liked) was used to determine the preference in color, flavor, taste, texture and overall acceptability according to the procedure described by Stone and Sidel (1998).

Statistical analysis

Data obtained was analyzed statistically as described by Steel et al., (1997).The data were interpreted by analysis of variance (ANOVA) using M-Stat C software package as described by Steel et al. (1997). ANOVA tested the significance of the differences between samples at 5% level of significance. LSD test was used to determine the level of significance that existed between the mean values.

Results and Discussion

Physical characteristics of texturized vegetable protein

Physical tests and observations of extrudates actually describe some of their quality characteristics and its evaluation with respect to the consumer point of view. These parameters are of great importance as it determines the over all acceptability of the final product as these parameters are responsible to attract one's eye towards product by enhancing its physical appearance and an appealing outlook besides the organoleptic properties. The mean values regarding the physical characteristics of texturized vegetable protein are given in Table 1

Expansion ratio (ER) of texturized vegetable protein

. It is evident from the results that treatments (temperature) have significant effect on the expansion ratio of texturized vegetable protein. The expansion ratio of texturized vegetable protein varied from 1.85 to 1.92. It is evident from the results that extrusion temperature significantly affected on the expansion ratio of the texturized vegetable protein. The highest expansion ratio (1.92) was observed for highest temperature (155°C) while lowest extrusion temperature (140°C) gave lower value of expansion ratio for texturized vegetable protein (1.85). The results also make it very obvious that with gradual increase in temperature of barrel the expansion ratio increased with same rate. This showed that the Overall expansion increased linearly with increasing temperature to 155 °C.

An increase in temperature resulted in an increase in expansion. Temperature was a dominant variable affecting macroscopic characteristics of extrudates. The different levels of temperature affected all macroscopic (expansion) properties of extrudates. A similar range of expansion ratio has also been reported for other pulses (peas, lentils) when extruded under similar conditions (Patil *et al*., 2007).

The results are also in accordance with the findings of Dogan and Karwe (2003). They observed that increase in barrel temperature show a positive linear effect on expansion ratio of the final product. Also by gradual rise in temperature there is gradual rise in expansion ration of the extrudates and this occurs to a certain level of temperature which is 168 ^{0}C. They suggested the existence of temperature plateau for expansion, between 150 and 170 ^{0}C depending on the type of food material. This phenomenon may be caused by excessive structure breakdown and starch degradation under high temperature which weakened the extrudates structure and therefore caused it to collapse. On the other hand the decreased expansion at high temperature could also occur. But at 155 ^{0}C there was gradual increase in expansion ratio of the texturized vegetable protein due to gelatinization of starch content of raw material and breakdown of protein bonding to optimum value.

The results were also in accordance with findings of Pe´rez *et al*. 2006. They observed that maximum expansion was produced with extrusion at 155 ^{0}C. This can be explained by the fact that when materials are forced through an extruder die their water content vaporizes, and the simultaneous vapour flash off expands their starch content, producing a porous, sponge-like structure in the extrudate. Extrudate degree of expansion is closely linked to the size, number and distribution of air cells within the material.

Bulk density (BD) of texturized vegetable protein

It is evident from the results that treatments (temperature) have significant effect on the bulk density of texturized vegetable protein. The bulk density of texturized vegetable protein varied from 0.63 to 0.57g/L. It is evident from the results that extrusion temperature significantly affects the bulk density of the texturized

Table 1 Mean values for expansion ratio, bulk density, calorific value, pH, water absorption capacity, oil absorption capacity, water solubility index and hardness and breaking strength of of texturized vegetable protein

	T_1 (140°C)	T_2 (145°C)	T_3 (150°C)	T_4 (155°C)
Expansion ratio	1.85c	1.88 b	1.89b	1.92a
Bulk density g/L	0.63a	0.61b	0.59 b	0.57c
Calorific value	4196 d	4199 c	4203 b	4207 a
pH	7.42a	7.43a	7.42a	7.42a
Water absorption capacity	1.38c	1.41bc	1.42ab	1.45a
Oil absorption capacity	0.12c	0.17b	0.21a	0.24a
Water solubility index	2.02c	2.05bc	2.07ab	2.10a
Hardness and breaking strength	25.6a	20b	17c	16.03c

vegetable protein. The highest bulk density (0.63g/L) was observed for lowest temperature (140°C) while highest extrusion temperature (155°C) gave lower value of bulk density of texturized vegetable protein (0.57g/L). It is also clear from results that bulk density decreases with gradually increase in temperature.

During extrusion bulk density was influenced by temperature and it decreased with increasing temperature. If expansion ratio increased it would be logical to assume that bulk density would decrease, under similar conditions but bulk density increased abruptly when temperature increased from 130 to 140 °C. This could be due to the effect of high temperatures on viscosity and starch degradation resulting in less expansion.. Bulk density and expansion are also related to starch gelatinization. (Rayas *et al.,* 1998). According to these authors an increase in gelatinization increased expansion and decreased bulk density.

There is also relationship between bulk density and expansion ratio. When temperature of barrel is increased the expansion ratio rises to certain degree of temperature while the bulk density of extrudates show the negative course of action in this regard. Bulk density also describes the degree of expansion undergone by the melt as it exits the extruder. The sectional expansion ratio considers only in the direction perpendicular to extrudates flow, while bulk density considers expansion in all directions (Altan *et al*., 2008).

Calorific value of the texturized vegetable protein (cal/gm)

Calorific value means the heat/energy generated by the product when burned/digested. Every product has its specific calories and texturized vegetable protein exhibited results regarding calorific value on evaluation by bomb calorimeter. It is evident from the results that treatments (temperature) have significant effect on the calorific value of texturized vegetable protein. The calorific value of texturized vegetable protein varied from 4196 to 4207. It is evident from results that extrusion temperature highly significantly affects the calorific value of the texturized vegetable protein. These results are in accordance with the findings Fuhrmeister and Meuser (2003). They stated that by increasing the barrel

temperature in extrusion cooking the calorific value is enhanced this may be a result of modification of carbohydrates, proteins, fats etc due to rise in temperature as there is maximum bond disruption of material being extruded when temperature is raised because maximum energy is being provided for bond disruption and rearrangement of structure of material take place which may enhance the calorific value of product.

pH of texturized vegetable protein

It is evident from the results that treatments (temperature) have non significant effect on the pH of texturized vegetable protein. The pH of texturized vegetable protein extrudates varied from 7.42 to 7.43. It is evident from the results that extrusion temperature non significantly affects the pH of the texturized vegetable protein. The highest pH (7.43) was observed for high temperature (155°C) while low extrusion temperature (140°C) gave low value for texturized vegetable protein pH (7.42). It is clear from the results that pH does not decrease much but a minute decrease is observed which is negligible and this decrease is not due to temperature change gradually because at low temperature (140°C) the value is 7.42 and at highest temperature (155°C) the value is same but at temperature 140°C the value is a little bit high 7.43 which shows that this minute change is not due to temperature rising. These results are in accordance with the findings of Riaz, (2000) he found that by increasing barrel temperature there was no significant effect on the pH of extrudates. He observed the results from soy extrudates and found that pH did not change significantly due to change in temperature but a minute change was there in extrudates of different temperatures. So it is clear from observations that pH of the extrudates of different treatments (temperatures) was same.

Water and oil absorption of texturized vegetable protein

It is evident from the results that treatments (temperature) have highly significant effect on the water absorption capacity of texturized vegetable protein**.** The water absorption capacity of texturized vegetable protein varied from1.38 to 1.45 g/g. It is evident from results that extrusion temperature significantly affects the water absorption capacity of the texturized vegetable protein. The highest water absorption capacity (1.45g/g) was observed for highest temperature (155°C) while highest extrusion temperature (140°C) gave lower value water absorption capacity (1.38g/g). It is also clear from results that water absorption capacity increased with gradually increase in temperature. The water absorption capacity (WAC) of different cereals ranged from 1.33to 1.47 g/g. Water absorption characteristics represent the ability of a product to associate with water under conditions where water is limiting (Singh, 2001). The results are in accordance to the findings of Noguchi, 1889 they proposed that when temperature of barrel of extruder is raised there is relative increase in water absorption capacity of the texturized vegetable protein. This is because water absorption capacity of extrudates mainly depends on temperature of barrel. When temperature of barrel is raised the melt viscosity of food mix is decreased and layer formation of fiber is enhanced. In this way protein aggregation and cross linking is enhanced prevention the subsequent water absorption and facilitating the formation of more laminating structure of material being extruded and more solidification after passing through die. This enhances the water absorption capacity of extrudates on rehydration. The results of present study also matched with study of Hayashi *et al.*1992. Gujska and Khan (1990) studied the effect of temperature on WAC on high starch fractions of pinto and navy beans during extrusion. WAC increased from 3.0 (110°C) to 4.0 (132°C) for pinto beans but could not be evaluated at 150°C because the extrudates burned. In the present study water absorption increased to 150^0C but this is quite rational as water absorption capacity varies at different temperatures for different types of materials being extruded. This variation is evident from the work of Gujska and Khan (1990) who reported in same study that navy beans exhibited a maximum WAC (4.0) at 132^0C and lower values (3.83) at 150^0C.

It is evident from the results that treatments (temperature) have significant effect on the oil absorption capacity of texturized vegetable protein. The oil absorption capacity of texturized vegetable protein varied from 0.12to 0.24 g/g. It is evident from results that extrusion temperature significantly affects the oil absorption capacity of the texturized vegetable protein. The highest oil absorption capacity (0.24 g/g) was observed for highest temperature (155°C) while lowest extrusion temperature (140°C) gave lower value of oil absorption capacity of texturized vegetable protein (0.12 g/g). It is also clear from results that oil absorption capacity increases gradually relative increase in barrel temperature. It is generally observed that oil absorption during deep fat frying is essentially a quantitative water replacement process where higher initial moisture results in greater oil absorption during frying. Such a relationship has been demonstrated in extrusion formed deep fat fried tapioca chips (Nair *et al.,* 1996) and in tortilla chips (Moreira *et al.,* 1997). Gautam *et al,* (1987) using a staining procedure has demonstrated that oil uptake by a fried snack occurs primarily in areas that exhibit moisture loss as extrusion causes lowering of moisture content so oil absorption capacity is enhanced by increasing temperature. Oil absorption varied significantly, suggesting that extrusion variable temperature critically influences oil absorption.

According to Kinsella (1976) more hydrophobic proteins show superior binding of lipids, implying that non-polar amino acid side chains bind the paraffin chains of fats. The OAC of most cereals ranged from 1.05 to 1.17 g/g.

Water solubility index (WSI) of texturized vegetable protein

It is evident from the results that treatments (temperature) have non significant effect on the water solubility index of texturized vegetable protein. The water solubility index of texturized vegetable protein varied from 2.02 to 2.10. It is evident from results that extrusion temperature significantly affects the water solubility index of the texturized vegetable protein. The highest water solubility index (2.10) was observed for higher temperature (155°C) while at lowest extrusion temperature (140°C) gave lower value for water solubility index (2.02). It is also clear from results that water solubility index increases with increase in temperature. WSI increased significantly with increasing temperature, which may be related to starch depolymerization at higher temperatures, reducing molecular length of amylose and amylopectin chains. These results confirmed those of Anderson *et al*. (1969) who had extruded bean and sorghum. Also Gujska and Khan (1990) reported a significant increase in WSI with increasing extrusion temperature. However, Gujska and Khan (1990) found that WSI decreased significantly with increasing moisture in extrusion of pinto bean flour from 35.3 at 20% feed moisture to 21.1 at 30% feed moisture. Moreover, increasing the rice flour level from 0% to 25% decreased the water solubility index (WSI) of extrudate significantly. However increasing the flour level from 25% to 50%caused a slight decrease in the water solubility index (WSI) of extrudates but not significantly. (Anderson *et al*., 1969).

Hardness and Breaking strength of texturized vegetable protein

It is evident from the results that treatments (temperature) have highly significant effect on the hardness and breaking strength of texturized vegetable protein. The hardness and breaking strength of texturized vegetable protein varied from 16.03 to 25.6 N. It is evident from results that extrusion temperature highly significantly affects the hardness and breaking strength of the texturized vegetable protein. The highest hardness and breaking strength (25.6N) was observed for lowest temperature (140°C) while highest extrusion temperature (155°C) gave lower value of hardness and breaking strength of texturized vegetable protein (16.03N). It is also clear from results that hardness and breaking strength decreases with gradually increase in temperature. Peak values of graphs as shown in Figures showed the maximum force required to break the product being analyzed for textural properties so called hardness and breaking strength. These results match with the results of Chinnaswamy and Hanna, (1988) they stated that decreasing hardness and breaking strength was related to decrease in bulk density of the extrudates with increasing barrel temperature, resulting in a less material in the area being tested. A more expanded product may have a weaker structure or lower mechanical strength. Hardness and breaking strength of extrudates was significantly affected by changing the barrel temperature besides other variables. Barrel temperature had significant quadratic effects on extrudates hardness and breaking strength. Other independent variables like feed moisture also affect the hardness of the extrudates. Hardness and breaking strength decreased with decreasing feed moisture content. The hardness and breaking strength of extrudates ranged from 18 to 34 N as elaborated by Veronica *et al*, 2006. Low hardness and breaking strength which is also a favored property of extrudates was observed at high temperature. The hardness and breaking strength of expanded extrudates is a perception of the human being and is associated with expansion and cell structure of the product (Veronica *et al*., 2006).

Chemical analysis of texturized vegetable protein

The extrudates of texturized vegetable protein were evaluated for their chemical properties. The mean values for of different constitutes regarding chemical composition of texturized vegetable protein are given in Table 2.

Moisture contents of texturized vegetable protein

The moisture content of texturized vegetable protein varied from 9.92 to 9.20%. It is evident from the results that extrusion temperature significantly affected the moisture content of the texturized vegetable protein. The highest moisture content (9.92%) was observed for lowest temperature (140°C) while highest extrusion temperature (155°C) gave lower value of moisture content of texturized vegetable protein (9.92%). It is also clear from the results that moisture content decreases with gradually increase in temperature. The results regarding the moisture content were in accordance to the study of Ding *et al*, (2006). They proposed that the moisture content is affected as we change the temperature of barrel. Moisture content (the quantitative determination of total water content) of the final product determines the stability and quality of food material as moisture content of the final product affects different nutritional as well as organoleptic properties of food and most importantly it determines the texture of product.

Protein contents of texturized vegetable protein

The crude protein of texturized vegetable protein varied from 22.39 to 22.33%. It is evident from the results that extrusion temperature significantly affects the

crude protein of the texturized vegetable protein. The highest crude protein (22.39%) was observed for lowest temperature (140°C) while highest extrusion temperature (155°C) gave lower value of crude protein for texturized vegetable protein (22.33%). It is also clear from results that crude protein decreases to very minute degree with gradual increase in barrel temperature. This was due to high temperature of barrel that protein molecules were readily denatured and bonding was disrupted and rebonding occurred as high temperature facilitated the formation of new bonding and denaturing certain enzymes and pigments that create obnoxious flavor and enhance certain ant-nutritional characteristics. Due to denaturization of these enzymatic proteins there existed a very minute decrease of protein content in final product as proposed by Riaz, 2000. It showed that by increasing barrel temperature the protein content of extrudates decreased but to very minute extent. The results were in accordance to the findings of the Riaz, 2000. He observed that in general as the amount of protein in the extrudates increased, expansion ratio decreased and color of final product also changed. This is because the expansion ratio is due to starch content of raw material, more the starch content more will be the expansion ratio. But in case of protein there are different processes takes place during extrusion cooking process the most important is denaturization of protein molecules. It did not mean that protein is totally denatured but due to high temperature and pressure and shearing effect material is intermingled within the extruder and resultantly bonding of protein molecules is disrupted and new bonding occurs that leads towards formation of new product when passing through die of small aperture and which is of modified form of protein and is more desirable in view of modern needs of time (Riaz, 2000).

Ash contents of texturized vegetable protein

The ash content for texturized vegetable protein remained nearly same as the after different treatments of temperature during extrusion. It is evident from the results that treatments (temperature) have non significant effect on the ash content of texturized vegetable protein. The ash content of texturized vegetable protein varied from 3.3 to 3.29%. It is evident from results that extrusion temperature none significantly affects the ash content of the texturized vegetable protein. The highest ash content (3.3%) was observed for lowest temperature (140°C) while highest extrusion temperature (155°C) gave lower value of ash content for texturized vegetable protein (3.29%). It is clear from the results that ash content did not decrease much but a minute decrease was observed which is negligible. This shows that extrusion cooking results in considerable retention of nutrients like certain minerals such as calcium, iron and zinc. This is attributed to a high-temperature, short-time process of extrusion cooking, thereby yielding a better product. The results are in accordance with the findings of (Ding *et al.*, 2006).

Fat contents of texturized vegetable protein

It is evident from results that treatments (temperature) have non significant effect on fat contents of texturized vegetable protein. The fat content of texturized vegetable protein varied from 1.63 to 1.66%. At highest temperature (155°C) value of fat content was 1.66 % and at lowest temperature 140°C) value was 1.66% as well. It is evident from results that barrel temperature during extrusion non significantly affects the fat content of texturized vegetable protein. Bredie *et al* 2002 reported that the addition of fat during extrusion decreases to very minute degree due to degree of gelatinization of starch content of material and due to decrease of the barrel temperature caused by the lubricating effect fat. Fat also decreases conversion of starch content during extrusion by preventing severe mechanical breakdown of the starch granules by rotating screw and preventing water from being absorbed by starch. Reduced starch conversion/gelatinization ultimately results in decreased expansion. The results showed that the fat content of texturized vegetable protein remained same by varying temperature of barrel. As the temperature of barrel increased there is no significant difference in fat content of texturized vegetable protein but to a negligible degree. Fat portion of material is also utilized to lubricate the screw and barrel and imparts a characteristic flavor on extrusion cooking. This also showed that extrusion cooking results in considerable retention of fat in the extrudates. Fat level used for extrusion in present study was ideal because for production of texturized vegetable protein value 0.5 to 6.5 is recommended (Riaz, 2000).

Fiber contents of texturized vegetable protein

The crude fiber of texturized vegetable protein varied from 2.10 to 2.07%. It is evident from results that extrusion temperature none significantly affects the crude fiber of the texturized vegetable protein. These results showed that application different level of barrel temperature non significantly affected the crude fiber content of texturized vegetable protein. The highest crude fiber (2.10%) was observed for lowest temperature (140°C) while highest extrusion temperature (155°C) gave lower value of crude fiber of texturized vegetable protein (2.07%). This shows that by increasing the temperature there is no significant difference among different treatments and final product. All treatments showed almost similar results. As extrusion is a high temperature and short time cooking process which let the certain contents of material less affected and ensures the retention of these nutritional components. The earlier

Table 2 Proximate composition of texturized vegetable protein

Treatment	Moisture content %	Protein content %	Ash content %	Fat content %	Crude fiber %
T_1 (140°C)	9.92a	22.39a	3.3a	1.166a	2.10a
T_2 (145°C)	9.50b	22.360b	3.29a	1.165a	2.09ab
T_3 (150°C)	9.23c	22.34c	3.29a	1.63b	2.09bc
T_4 (155°C)	9.20d	22.33c	3.29a	1.66c	2.07c

traditional cooking processes showed that the crude fiber significantly increased by cooking treatments but these cooking methods were simple and traditional and did not involved any controlled environment like levels of temperature and keeping some other parameters constant. This increase could have been due to protein-fiber complexes formed after possible chemical modification induced by the soaking and cooking of dry seeds also there was effect of antinutritional factors (Bressani, 1993). But extrusion cooking affects less due to short time and high temperature treatment. The antinutritional factors are destroyed and certain other pigments and components that cause obnoxious flavor and taste are also evaporated through an opening during extrusion cooking the results of all treatments showed less considerable difference on treating at varying level of temperature (Riaz, 2000).

Sensory evaluation of texturized vegetable protein

The results regarding sensorial characteristics of texturized vegetable proteins based patties are presented in Table 3.

Color of texturized vegetable protein patties

Color is an important characteristic of a product with respect to its sensory attributes and it appeals consumer attraction towards itself. It is evident from the results that treatments (temperature) have significant effect on color parameter of texturized vegetable protein. The values of color of texturized vegetable protein based patties varied from 6.33 to 6.41. It is evident from results that extrusion temperature highly significantly affects the sensory evaluated color of the texturized vegetable protein. The highest color value (6.41) was observed for highest temperature (155°C) while lowest extrusion temperature (140°C) gave lower value of color for texturized vegetable protein (6.33). It is also clear from results that sensory evaluated color increases with gradual increase in temperature. The results are in accordance to the findings of Rokey (2000) who found that color changes during the extrusion process, can provide important information regarding the degree of thermal treatment. By applying different levels of temperature of barrel during extrusion cooking create a varying degree of color of final product. This is because when temperature is high, cooking is done properly and development of color occurs to optimized degrees and hence imparting color to final product which is desirable and acceptable by consumer. But when temperature is low the color development process is slow due to availability of insufficient temperature requirement. Analysis of variance indicated that the effect of temperature on moisture content was to greater extent and as moisture content is important in color development so it affected the color development in final product of extrusion (Ding *et al*, 2006). So increasing temperature the bond disruption process is also enhanced and hence a positive effect on color parameter of product was observed in texturized vegetable protein. A higher temperature would increase product internal temperature, which promotes the browning reactions in sugar content of raw material and due to these browning reactions a characteristic color was produced. But the lower temperature would decrease the heating of internal composition of the dough inside the extruder and resulted in lighter products. (Altan *et al*., 2008). The results were also similar to the study of Badrie and Mellowes (1991). They proposed that when barrel temperature is increased during extrusion cooking there is relative increase in color intensity of the extruded material. When temperature is increased there is change in color intensity due to browning reactions of sugar content of the material. In the end color intensity is enhanced. The results of present study were similar to early findings and there was an increase in color value with gradual rise of temperature which is desirable.

Flavor of texturized vegetable protein patties

It is evident from the results that treatments (temperature) have significant effect on the flavor of texturized vegetable protein. The values of flavor of texturized vegetable protein based patties varied from 5.86 to 5.99. It is evident from the results that extrusion temperature significantly affects the sensory evaluated flavor of the texturized vegetable protein. The application of gradual rise in temperature left positive affect on flavor of TVP.The highest observed value of flavor of texturized vegetable protein (5.99) was for highest temperature (155°C) while lowest extrusion temperature (140°C) gave lower value of flavor for texturized vegetable protein (5.86). It is also clear from results that sensory evaluated flavor increases with gradually increase in barrel temperature of extruder during extrusion process. These results match with the results given by Bhandari *et al*, (2001) he reported that by increasing temperature of extrusion flavor is developed in extruded product due to activation of flavor producing compounds at higher temperature. The results are in accordance to the findings of Kadlec, 2000. He proposed that flavor is enhanced by increasing barrel temperature due to presence of sugar contents in raw material and when this sugar content is heated at high temperature complex chemical reactions occur and resultantly a characteristic flavor is produced. Also when heated at high temperature certain obnoxious flavor is degraded but degree of formation of new flavor is more than this degradation. The results of present study showed that with increase in barrel temperature there was significant increase in flavor of texturized vegetable protein.

Taste of texturized vegetable protein patties

It is evident from the results that treatments (temperature) have significant effect on taste of the texturized vegetable protein. These values varied from 6.87 to 6.97. It is evident from results that extrusion temperature significantly affected the taste of the texturized vegetable protein. The highest sensory evaluated taste (6.97) was observed for highest temperature (155°C) while lowest extrusion temperature (140°C) gave lower value for of sensory evaluated taste of texturized vegetable protein (6.87). These results make it very clear that by gradual increase in temperature during extrusion process the taste of extruded material enhanced as it is evident in T_4. The sensory evaluated taste has significant difference between the extrudates obtained from different treatments. The results matched with work of Rampersad *et al*, (2003). He proposed that when temperature of processing technique is increased to a certain degree it produce a desirable taste in the product as its human perception by senses to observe the taste of product. But if temperature is low and cooked food has not desirable taste characteristics due to under cooking. This is because when temperature is raised in extrusion cooking it results in cooking of product and removing and disruption of certain components which affect the taste of product in negative way. In this way in present study application of 155 ^{0}C in T_4 showed the value of the most acceptable taste. In this way it is very obvious that by increasing temperature gradually taste value increased significantly.

Texture of texturized vegetable protein patties

It is evident from results that treatments (temperature) have significant affect on sensory evaluated texture of texturized vegetable protein. These values varied from 5.85 to 6.15. It is very clear from results that extrusion temperature significantly affects the texture of sensory evaluated texture of texturized vegetable protein. The highest value of sensory evaluated texture (6.15) was observed for highest temperature (155°C) while lowest extrusion temperature (140°C) gave lower value for texture of product (5.85). This showed that with gradual increase in temperature of barrel texture was positively affected to a certain limit of temperature beyond which texture has not same results. The results were in accordance to the findings of Lin *et al*, 2000. According to him by increasing the barrel temperature the maximum bond disruption occurs and results in formation of more homogeneous laminate along barrel which produce more compact mass and hence imparting better textural characteristics. The results matched with earlier findings of Little and Hills, 1978. They proposed that by increasing the barrel temperature during extrusion of chickpea there was gradual increase in sensory evaluated texture of the product in all ratios.

Overall acceptability of texturized vegetable protein patties

The overall acceptability of texturized vegetable protein based patties of different treatments varied from 5.95 to 6.35. It is evident from the results that variation in barrel temperature of extruder during extrusion significantly affected the overall acceptability of the final product. The highest value of overall acceptability (6.35) was observed for highest temperature (155°C) while lowest extrusion temperature (140°C) gave lower value for overall acceptability (5.95). It is also clear from results that overall acceptability increases with gradually increase in temperature to 155^{0}C. Guy (2001) besides elaborating effect of die also proposed that when temperature of barrel is gradually increased there is positive effect on the extruded material from overall acceptability point of view of the final product. This is because the overall acceptability of product is directly related with other sensory attributes. When other sensory characteristics show positive value with increase in temperature then affect of temperature is also significant on overall acceptability of the final product to be

Table 3 Mean values for sensory evaluation of texturized vegetable protein

Treatment	Color	Flavor	Taste	Texture	Overall acceptability
T_1 (140°C)	6.33d	5.86d	6.87c	5.85d	5.95d
T_2 (145°C)	6.37c	5.91c	6.90c	5.89c	6.09c
T_3 (150°C)	6.39b	5.95b	6.93b	6.06b	6.16b
T_4 (155°C)	6.41a	5.99a	6.97a	6.15a	6.35a

evaluated for overall acceptability. If temperature is low the material will not be in laminating appearance and hence causing a hindrance in flow along the barrel of extruder. The results were in accordance to the findings of the Riaz, 2000. He proposed that by increasing temperature of barrel during extrusion for formation of texturized vegetable protein the sensory parameters color, flavor, texture and taste showed a linear trend as depicted in statically analysis so as over all acceptability is directly related with sensory attributes hence it is also significant with rise of temperature.

Conclusion

The research work was carried out to prepare texturized vegetable protein using indigenous source of protein by process of extrusion cooking. Different ranges of temperature were used to ensure the formation of product with more acceptable attributes. This temperature range was 140, 145, 150 and 155 ^{0}C. Effect of temperature variation was evaluated in different physical, nutritional and sensory characteristics of the final product. As for as the effect of temperature on physical attributes is concerned it showed a linear relationship between temperature and expansion ratio with values ranging 1.85, 1.88, 1.89 and 1.92 for four treatments T_1, T_2, T_3 and T_4 respectively.T_4 was most suitable value of temperature for maximum expansion. But for bulk density results were declining with increase in temperature having values ranging 0.63g.L, 0.61g/L, 0.59g/L and 0.57g/L for four treatments T_1, T_2, T_3 and T_4 respectively. The results for fat content and ash content of texturized vegetable protein were non significant. Moisture content was decreased by increasing barrel temperature of extruder. The protein of final product showed results ranging 22.39, 22.360, 22.34 and 22.33 for four treatments respectively. This showed by increasing barrel temperature there is a minute decrease in protein content as certain enzymes and proteineous pigments having obnoxious flavor are disrupted during extrusion cooking. Then TVP based patties were made and product was evaluated for sensory properties which include color, flavor, taste, texture and overall acceptability. The color, flavor, taste, texture and overall acceptability showed increasing trend with rise in temperature. The results for color showed trend ranging 6.33, 6.37, 6.39 and 6.41 and flavor was in range of 5.86, 5.91, 5.95 and 5.99. At low temperature treatment of 140^{0}C value of taste was 6.87 and at high temperature of 155 ^{0}C the value was 6.97. This showed that by increasing temperature cooking properties of dough's improved with better color, flavor and taste. Similar results were observed for texture and overall acceptability. The other attributes of texturized vegetable protein were evaluated like water solubility index, water and oil absorption and hardness and breaking strength. The results for water solubility index were 2.02, 2.05, 2.07, and 2.10 for four treatments T_1, T_2, T_3 and T_4 respectively. The water absorption capacity ranged 1.38g/g, 1.41g/g, 1.42g/g and 1.45g/g for four different levels of temperature. This showed an increasing trend which means by increasing barrel temperature proteineous and water absorption capacity is increased. The results for oil absorption capacity were also in almost similar fashion. Hardness and breakability showed a linear declining trend with rise in temperature and mean values ranged from 35.6 N to 16.03 N. Due to all attributes close to texturized vegetable protein made of soybean, the product was approved by sensory panel for use as color, texture and taste fulfilled the desired characteristics.

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PAK. J. FOOD SCI., 21(1-4), 2011:45-51
ISSN: 2226-5899

Antioxidant Potential of Bell Pepper (Capsicum annum L.)-A Review

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Abstract

The interests in the consumption of pepper fruits (Capsicum annum L.) is, to a large extent due to its content of bioactive compounds and their importance as dietary antioxidants. Peppers are used as a colourant, flavourant, and/or as a source of pungency. Peppers can be used fresh, dried, fermented, or as an oleoresin extract. It has both nutritional and nutraceutical importance. It contains an anticoagulant that helps prevent the blood clots that can cause heart attacks. Bell Pepper is good source of vitamin C. The benefits resulting from the use of natural products rich in bioactive substances has promoted the growing interest of food industries. Among the antioxidant phytochemicals, polyphenols deserve a special mention due to their free radical scavenging properties. Antioxidant compounds and their antioxidant activity in 4 different colored (green, yellow, orange, and red) sweet bellpeppers (Capsicum annuum L.) were investigated.The free radical scavenging abilities of peppers determined by the 2, 2~-diphenyl-1-picrylhydrazyl (DPPH) method. Natural antioxidants are preferred because synthetic antioxidants are considered carcinogenic. Antioxidants present in the (Capsicum annuum L.), protect the food or body from oxidative damage induced by free radicals and reactive oxygen.

Key words: Bell Pepper, Capsicum annum, Natural Antioxidants, Health benefits, dietary antioxidants

Introduction

Capsicum has its beginning since the beginning of civilizations. It is a part of human diet since 7500 BC. It was the ancient ancestors of the native peoples who took the wild chili Piquin and selected for the many various types known today. Native Americans had grown chili plants between 5200 and 3400 BC. This places chilies among the oldest cultivated crops of the Americas (Bosland, 1996). The genus Capsicum is one of the first plants being cultivated in the New World with beans (Phaseolus spp.), maize (Zea mays L.), and cucurbits (Cucurbitaceae) (Heiser, 1973). In the sixteenth century, Capsicum annuum and Capsicum frutescens were widely distributed from the New World to other continents via Spanish and Portuguese traders while the other species are little distributed outside South America (Andrews, 1995). Capsicum annuum is mostly used commercially.

Genus Capsicum is a member of family Solanaceae and has five species that are commonly recognized as domesticated: Capsicum annuum, Capsicum baccatum, Capsicum chinense, Capsicum frutescens, and Capsicum pubescens. However there are approximately 20 wild species that have been documented (Heiser, 1973). The classification system for this genus is somewhat confusing in the literature. In Spain, the Castilian word 'pimiento' refers to any Capsicum species, but in the USA, 'pimiento' or 'pimento' refers only to thick-walled, heart-shaped, non-pungent fruits from the species Capsicum annuum. The Hungarians call all Capasicum annuum fruits 'paprika', but paprika is defined in the world market as a ground, red powder derived from dried fruits with the desirable colour and flavour qualities. The word 'chile' is the common name for any Capsicum species in Mexico, Central America and the Southwestern USA. In Asia, the spelling 'chilli' is more common and is always associated with highly pungent varieties of Capsicum annuum and Capsicum frutescens, while the non-pungent sweet bell peppers are referred to as 'Capsicums' and it is native to Maxico. In American English, it is commonly known as the Chili Pepper or Bell Pepper. In British English, they are all called Peppers, whereas in Australian and Indian English, there is no commonly used name encompassing all its forms, the name Capsicum being commonly used for bell peppers exclusively. In Pakistan, it is locally known as Shimla Mirch (Grubben and Denton, 2004). Pungent fruits of all cultivated Capsicum species as a collective class are called 'chillies' in the Food and Agriculture Organization (FAO) Yearbook (Anon, 1997). Bird's eye chillies are grown primarily in East Africa, but they are merely small-fruited, highly pungent forms of Capsicum annuum or Capsicum frutescens. Different varieties of the genus Capsicum are widely grown for their fruits, which may be eaten fresh, cooked, as a dried powder, in a sauce, or processed into

oleoresin (Poulos, 1993). Three major products traded on the world market for use in food processing are paprika, oleoresin, and dried chilli (both whole and in powdered form).

• Oleoresin: A viscous liquid derived by polar solvent extraction from ground powder of any Capsicum species; there are three types of oleoresin: paprika (used for colour), red pepper (used for colour and pungency), and Capsicum (used for pungency).
• Paprika: A ground, bright red, usually non-pungent powder used primarily for its colour and flavour in processed foods; all paprika varieties are C. annuum; paprika fruits are used to produce paprika oleoresin.

• Chilli: Any pungent variety of any Capsicum species, but primarily C. annuum; chilli varieties may be used to produce red pepper oleoresin or Capsicum oleoresin.
• Pepper(s): Generic term describing the fruits of any Capsicum species, both pungent and non-pungent.

Peppers are used as a colourant, flavourant, and/or as a source of pungency. Peppers can be used fresh, dried, fermented, or as an oleoresin extract. They can be used whole, chopped, coarsely ground, or finely ground, with or without seeds. Various types of processed products containing primarily peppers include pickled fruits, chilli sauce, chilli powder (also known as cayenne powder), crushed red pepper flakes, fermented mash, paprika, and three types of oleoresin. Other processed products that contain a significant proportion of peppers include fresh and processed salsas, curry powders, barbecue seasonings, chili powder (a mixture of chilli powder, oregano, cumin, and garlic powder), and many other foods (Govindarajan, 1986).

The main source of pungency in peppers is the chemical group of alkaloid compounds called capsaicinoids (CAPS), which are produced in the fruit. The atomic structure of CAPS is similar to piperine (the active component of white and black pepper, Piper nigrum) and zingerone (the active component of ginger, Zingiber officinale). Capsaicin (C18H27NO3), trans-8-methyl-N-vanillyl-6-nonenamide), is the most abundant CAPS, followed by dihydrocapsaicin, with minor amounts of nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, and others. Capsaicin is a white crystalline, fat-soluble compound formed from homovanillic acid that is insoluble in water, odourless, and tasteless (Andrews, 1995). Varieties of chilli differ widely in CAPS content. The amount of CAPS in a given variety can vary depending on the light intensity and temperature at which the plant is grown, the age of the fruit, and the position of the fruit on the plant. The first test developed to measure pungency was the Scoville test, first developed in 1912 by Wilbur Scoville. It measures 'heat' as Scoville heat units (SHU) in a given dry weight of fruit tissue. Sweet peppers have 0 SHU, chillies with a slight bite may have 100 to 500 SHU, and the blistering habaneros have between 200,000 and 300,000. The red colour of mature pepper fruits is due to several related carotenoid pigments, including capsanthin, shown in Figure, capsorubin, cryptoxanthin, and zeaxanthin, which are present as fatty acid esters. The most important pigments are capsanthin and its isomer capsorubin, which make up 30–60% and 6–18% respectively, of the total carotenoids in the fruit. The intensity of the red color is primarily a function of the amount of these two pigments; the Hungarian and Spanish varieties used for paprika have very high amounts of capsanthin and capsorubin compared to other varieties (Govindarajan, 1985).

CAPS in oleoresins are very stable compounds and generally do not break down, even during processing at high temperatures and during long storage periods. CAPS in dry products (fruits, powder, etc.) are not as stable as in oleoresins. The temperature at which the fruits are dried affects the CAPS content. For example, drying ripe fruits at 60ºC to a final moisture content of 8% decreases CAPS content approximately 10%. If the fruits are held for extended periods of time at 60ºC after reaching 8% moisture content as much as 50% of the CAPS may be lost. Once the fruits are dried, they typically lose 1–2% CAPS/month under cold (~16ºC) storage, and even more when stored under ambient conditions. Ground powder can lose as much as 5% CAPS/month depending on the fineness of the grind and the storage temperature (Bensinger, 2000). The red colour of paprika and chilli powder, on the other hand, is not as stable as oleoresin and CAPS, and much work has been done to optimize the processing and storage conditions for dried chillies and paprika to maximize the colour intensity for the longest period of time (Garcia-Mompean *et al*., 1999).

Peppers in food processing are used as food colorant, as source of pungency in food, as source of flavour, as source of pain relief for pharmaceutical use and as repellent. In many cases two or more of these properties are included in the same product; for example, paprika may be a source of color, pungency, and flavor.

People whose diets are largely colourless starches, such as rice or maize, use peppers to add color to their bland, achromatic diets. In various processed products paprika, paprika oleoresin, red pepper oleoresin, and dried chilli may all serve as a source of red color, but paprika and paprika oleoresins are the primary source of red color. Paprika is used in many products where no pungency is desired, but only the color, flavor, and texture of a finely ground powder is desired. These include processed lunchmeats, sausages, cheeses and other dairy products, soups, sauces, and snacks such as potato chips. Paprika oleoresin is used as a color and flavor additive in many products where the texture is important and small particles of paprika powder would be undesirable (Govindarajan, 1986).

Paprika is also important for its flavor in many products in addition to its color. Dried chilli is also valued for its contribution to flavor in chilli sauces and chilli powders. The flavoring principle is associated with volatile aromatic compounds and color. As a general rule, when the color of paprika or chilli powder fades, the flavor also disappears.

Peppers are well-known for their health benefits. Herbalists have long promoted peppers for their health-enhancing effects. These include clearing the lungs and sinuses, protecting the stomach by increasing the flow of digestive juices, triggering the brain to release endorphins (natural painkillers), making your mouth water, which helps to neutralize cavity-causing acids, and helping protect the body against cancer through antioxidant activity (Andrews, 1995).

CAPS stimulate sensory neurons in the skin and mouth cavity, creating a sensation of warmth that increases to

severe pain (type C nociceptive fibre pain) with higher doses. The neurons produce the neuropeptide Substance P (SP), which delivers the message of pain. Repeated exposure of a neuron to capsaicin depletes SP, reducing or eliminating the pain sensation in many people (Caterina *et al*., 1997). Thus the use of CAPS in pain relief has two modes of action: the sensation of heat, which may help sore muscles and arthritic joints feel better, and the depletion of SP, which reduces the pain sensation in the exposed area. Peppers have been reported to contain an anticoagulant that helps prevent the blood clots that can cause heart attacks (Andrews, 1995). Foods containing CAPS increase the thermic effects of food (TEF). The TEF is the slight increase in the body's metabolic rate after consumption of a meal. A meal containing foods with CAPS can increase the body's TEF up to 25% for three hours (Andrews, 1995). The role of CAPS in triggering the brain to release endorphins (natural painkillers) is well-known. As more CAPS are consumed, the body releases more endorphins, causing one to feel a mild euphoria – a natural high. Regular consumption has only a slight desensitizing effect. The Hungarian scientist Albert Szent-Gyorgyi won the 1937 Nobel Prize for isolating ascorbic acid, better known as vitamin C, from peppers. Peppers are also high in vitamin A, vitamin E, and potassium, and low in sodium. One hundred grams of fresh red chilli pepper has 240 mg of vitamin C (five times higher than an orange), 11,000 IU of vitamin A, and 0.7 mg of vitamin E. Vitamin C is sensitive to heat and drying but vitamin A is very stable, and paprika and dried chilli both contain relatively high amounts of this important nutrient (Govindarajan, 1986).

The chemical composition of foods is highly complex and comprises both volatile and non-volatile substances. Some of these substances contribute to the flavour of foods. Since the aroma component (volatile flavour) is usually responsible for the characteristic flavour of foods, the volatile compounds have received most attention (van Ruth *et al*., 2003). In the bell pepper 63 compounds were identified and included alcohols, aldehydes, ketones, acids, esters and sulphur- and nitrogen-containing compounds. The five most abundant compounds were 3-methylbutanal, 2-methylbutanal, 3-methylbutyric acid, acetone and hexanal (Chitwood *et al*., 1983).

Chemical constituents with antioxidant activity found in high concentration in plants (Velioglu *et al*., 1998) determine their considerable role in the prevention of various degenerative diseases (Diplock *et al*., 1998). Bell Pepper is good source of vitamin C and E, provitamin A, ascorbic acid and carotenoids (5.8 µgm/gm of fresh green wt.) (Materska and Perucka, 2005). Sweet peppers contain a very rich polyphenol pattern, which includes hydroxycianmates, flavonols and flavones (Marin *et al*., 2004). All these have great antioxidant activity. Natural antioxidants are preferred because synthetic antioxidants are considered carcinogenic (Branen, 1975).

Nutrition and health benefits

A wonderful combination of tangy taste and crunchy texture, bell peppers are the Christmas ornaments of the vegetable world with their beautifully shaped glossy exterior that comes in a wide array of vivid colors ranging from green, red, yellow, orange, purple, brown to black. Although peppers are available throughout the year, they are most abundant and tasty during the months of August and September (GMF, 2008).

Bell peppers offer a number of nutritional values. They are excellent sources of vitamin C and vitamin A. They also are a source of vitamin B6, folic acid, beta-carotene, and fiber. Red peppers also contain lycopene, believed important for reducing risk of certain cancers (GMF, 2008).

The proximate chemical composition of green bell pepper include dry mater (9.92%), total fat (0.33g), protein (0.99 g) , carbohydrate (10.63g), dietary fiber (2.73g) , vitamin C (133.00mg), calories (46.79cal), energy (195.58kj) (Durucasu and Tokusoglu, 2007).

Bell pepper have many health benefits like the protect us against free radicals, reduce risk of cardiovascular disease, promote optimal health, promote lung health, protect us against rheumatoid arthritis and seeing red may mean better eyesight (Ensminger and Esminger, 1986).

Essential oil functionality

The chemical composition of foods is highly complex and comprises both volatile and non-volatile substances. Some of these substances contribute to the flavor of foods. Since the aroma component (volatile flavor) is usually responsible for the characteristic flavor of foods, the volatile compounds have received most attention (Taylor *et al*., 2001). The fruits of Capsicum species have a relatively low volatile-oil content which has been reported to range from about 0.1 to 2.6% in paprika and similar large forms of C. annutn. The initial volatile-oil content of the freshly picked fruit is dependent largely upon the species and cultivar grown and the stage of maturity at harvest. The eventual volatile- oil content of the dried product, however, may be lower and is dependent upon the drying procedure, the duration and condition (whole or ground) of storage. Paprika powder, for example, usually contains less than 0.5% of volatile oil (van Ruth *et al*., 2003).

In the early stages of aroma research, most emphasis has been on development of methods to establish the chemical identity of the aroma constituents. The analytical task is rather complicated, as the fraction of aroma compounds of a simple food may be composed of 50–200 constituents, and these compounds are present in trace quantities. The large number of aroma chemicals complicates the task even further. Aroma science has benefited from the progress in the analysis techniques over the last decades, which led to long lists of volatiles (>6000) determined in foods (Maarse and Visscher, 1991).

Initially, the total volatile composition of a food was measured, for which extraction and distillation methods were employed, in combination with gas chromatography (GC). Later it appeared that the concentration of volatile compounds in a food does not necessarily reflect their concentration in air, as the concentration in air not only depends on the concentration in the food product but also on the interactions between the food matrix and the volatiles. The sensory perception of aroma is determined by the concentration of volatile compounds in the air phase. Therefore, headspace concentrations usually relate better to sensory properties than concentrations in the food product. Analysis methods, therefore, shifted from analysis of the compounds in the food to analysis of the volatile compounds in the air around the food (the headspace). Static and dynamic headspace measurements have become extensively used. This type of analysis developed further with the use of in-mouth analogues and in-mouth and in-nose measurements (van Ruth, 2001).

Indications that only a small fraction of the large number of volatiles occurring in food actually contributes to the aroma (Guth and Grosch, 1999) led to an interesting technique: gas chromatography–olfactometry (GC–O). The technique involves the sniffing of the gas chromatographic effluent by assessors in order to associate odour activity with eluting compounds, sometimes with a part of the effluent split to an instrumental detector. It is well known that many detectors are not as sensitive as the human nose for odour active compounds (Acree and Barnard, 1994)

The last few years have seen research groups developing methods to measure the change of the aroma profiles of foods during the time course of eating. Collection of air at the nostril(s) of subjects is the usual practice. Initially, these time–intensity measurements were conducted by trapping volatile compounds for short time intervals (e.g. 15 s). Absorbents and cryo-trapping have been used success-fully in combination with GC–mass spectrometry (GC–MS) (Taylorand and Linforth, 2000).

Color and pungency are the main quality parameters for assessing Capsicum varieties (Govindarajan *et al*., 1987). However, the majority of research has been focused on using aroma as an important parameter for assessing the quality of fresh fruits and vegetables (Guadayol *et al*., 1997). In the bell pepper 63 compounds were identified and included alcohols, aldehydes, ketones, acids, esters and sulphur- and nitrogen-containing compounds. The five most abundant compounds were 3-methylbutanal, 2-methylbutanal, 3-methylbutyric acid, acetone and hexanal (van Ruth *et al*., 2003). The volatile compound fractions of the pepper species have previously been isolated and more than 200 compounds were identified after hydro distillation and dynamic headspace sampling (purge and trap) procedures (Pino *et al*., 2006),

Later on characteristic volatile flavor compounds in healthy peppers (Capsicum annuum L.) were evaluated using a solvent-free solid injector coupled with a-gas chromatography-flame ionization detector (SFSI-GC-FID) and the results of evaluation were confirmed using GC–mass spectrometry (GC–MS). These compounds were compared with those obtained from peppers that were naturally infected or artificially inoculated with Colletotrichum spp. Parameters influencing the vaporization efficiency, including the injector temperature, pre-heating time and holding time, were optimized to improve the analytical efficiency. A total of 96 compounds (excluding eight capillary compounds), 17 of which were identified in healthy peppers, 49 of which were found in naturally infected peppers, and 61 of which were identified in artificially inoculated peppers, were separated and identified under the optimal conditions of an injector temperature of 250~C and 7-min preheating and holding times. Acetic acid and 2-furanmethanol were the major compounds detected in the volatiles of the healthy and diseased peppers. The major compound detected in both the healthy and naturally infected peppers was 3-hydroxypyridine, while hexadecanoic acid was the primary compound identified in the artificially inoculated peppers (In-Kyung Kim *et al*., 2007).

Antioxidants potential of Bell Pepper

Antioxidant means "against oxidation." Antioxidants work to protect lipids from peroxidation by radicals. They inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken (Dekkers *et al*., 1996). There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, where as natural antioxidants of plant region are classified as vitamins, phenolic compounds, or flavinoids (El-Ghorab *et al*., 2007).

Antioxidants protect the food or body from oxidative damage induced by free radicals and reactive oxygen species by (1) suppressing their formation; (2) acting as scavengers; and (3) acting as their substrate. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Ito *et al*., 1983).

There are two lines of antioxidant defense within the cell. The first line, found in the fat-soluble cellular membrane consists of vitamin E, beta-carotene, and coenzyme (Kaczmarski *et al*., 1999). Of these, vitamin E is considered the most potent chain breaking antioxidant within the membrane of the cell. Inside the cell water soluble antioxidant scavengers are present. These include

vitamin C, glutathione peroxidase, superoxide dismutase (SD), and catalase (Dekkers *et al*., 1996).

Natural antioxidants are extensively studied for their capacity to protect organism and cells from damage induced by oxidative stress (Dorman *et al*., 2008).

The supplementation of human diet with spices or herbs, containing especially high amounts of compounds capable of deactivating free radicals. The benefits resulting from the use of natural products rich in bioactive substances has promoted the growing interest of food industries (El-Ghorab *et al*., 2008).

Cultivars and growing conditions seem to play an important role in affecting the metabolism of antioxidant components and antioxidant capacity. Red sweet pepper (Capsicum annuum L.) is a vegetable known for its rich antioxidant content. Fresh sweet peppers have exceptionally high ascorbic acid, a 100 g serving supplying 100% of the current RDA of 60 mg/ day (Simmone *et al*., 1997).

Bell peppers, among vegetables, have become extremely popular for the abundance and the kind of antioxidants they contain. Among the antioxidant phytochemicals, polyphenols deserve a special mention due to their free radical scavenging properties. These compounds whose levels vary strongly during growth and maturation are also important because of their contribution to pungency, bitterness, colour and flavour of fruits (Estrada *et al*., 2000).

The attractive red color is due to the various carotenoid pigments, which include β-carotene with pro-vitamin A activity and oxygenated carotenoids such as capsanthin, capsorubin and cryptocapsin, which are exclusive to this genus and are shown to be effective free radical scavengers (Matsufuji *et al*., 1998). Red peppers also contain moderate to high levels of neutral phenolics or flavonoids, namely quercetin, luteolin and capsaicinoids (Hasler, 1998).

Ten cultivars of red sweet peppers grown over two consecutive years were compared with regard to ascorbic acid, total reducing content, β-carotene, total antioxidant activity and free radical scavenging activity. Cultivar Flamingo had the highest ascorbic acid content followed by cultivars Bomby and Parker. All cultivars fulfilled 100% RDA requirement for vitamin C. Torkel and Mazurka excelled in terms of β-carotene. Flamingo had the highest total reducing content and antioxidant activity. There was no effect of harvest year on antioxidant activity; however, ascorbic acid, total reducing content (mainly phenolics) and β-carotene differed significantly. A weak correlation was observed between total reducing content and antioxidant activity as measured by ferric reducing antioxidant power (FRAP) and free radical (1,1-diphenyl-2-picrylhydrazyl, or DPPH) scavenging assays (Deepaa *et al*., 2006).

Changes in total phenolics, antioxidant activity (AOX), carotenoids, capsaicin and ascorbic acid were monitored during three maturity stages in 10 genotypes of sweet pepper (green, intermediate and red/ yellow). All the antioxidant constituents (phenolics, ascorbic acid and carotenoids) and AOX, when expressed on fresh weight basis in general, showed an overall increasing trend during maturity in all the genotypes studied. On dry weight basis, phenolic content declined in majority of the genotypes during maturity to red stage. With maturation, most of the cultivars showed a declining trend with regard to capsaicin content while total carotenoids and β-carotene content increased significantly (Deepaa *et al*., 2007).

Antioxidant compounds and their antioxidant activity in 4 different colored (green, yellow, orange, and red) sweet bellpeppers (Capsicum annuum L.) were investigated. The total phenolics content of green, yellow, orange, and red peppers determined by the Folin-Ciocalteau method were 2.4, 3.3,3.4, and 4.2 μmol catechin equivalent/g fresh weight, respectively. The red pepper had significantly higher total phenolics content than the green pepper. Among the 4 different colored peppers, red pepper contained a higher level of β-carotene (5.4 μg/g), capsanthin (8.0 μg/g), quercetin (34.0 μg/g), and luteolin (11.0 μg/g). The yellow pepper had the lowest β-carotene content (0.2 μg/g), while the green one had undetectable capsanthin and the lowest content of luteolin (2.0 μg/g). The free radical scavenging abilities of peppers determined by the 2,2~-diphenyl-1-picrylhydrazyl (DPPH) method were lowest for the green pepper (2.1 μmol Trolox equivalent/g) but not significantly different from the other 3 peppers (Sun *et al*., 2007).

Conclusion

Nutritionally, sweet peppers are good source of mixture of antioxidants including ascorbic acid, carotenoids, flavonoids and polyphenols it is essential that compositional studies in plant food be carried out to take into account various factors such as cultivars, seasons and pre- and post-harvest conditions that may affect the chemical composition of plant foods.

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PAK. J. FOOD SCI., 21(1-4), 2011:52-55
ISSN: 2226-5899

Characterization of Different Groundnut Varieties Grown in Pakistan

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Abstract

Groundnut (Arachis hypogaea L.) is an annual legume which is also known as peanut, earthnut, monkeynut and ground bean. Among the major oil seed crops, groundnut has some specific advantages because of its use in many food products. The present study is planned to characterize different groundnut varieties, grown under similar conditions, for their chemical composition and nutritional assay. Fatty acids profile of groundnut will be determined through Gas Chromatography. The oleic acid (C18: 1) contents of groundnut varieties Bari-2000, P.W, 2KCG020, 2KCG017, 96CG005, Golden, No. 334, Chico, 01CG009 and 02CG005 were 43.6, 41.9, 36.4, 40.5, 38.0, 37.5, 35.8, 31.6, 30.4 and 32.9 g/100g respectively.

Keywords: Legumes, Groudnut, GC, Fatty acids

Introduction

Groundnut is a member of the genus Arachis in the subtribe Stylosanthinae of tribe Aeschynomeneae of the family Leguminosae and it is also known as peanut, earthnut, monkeynut, manilanut and ground bean. The only species in the genus of significant economic importance is A. hypogaea L., an annual herb that forms underground fruits. There are two subspecies of A. hypogaea, distinguished primarily on branching pattern and distribution of vegetative and reproductive axes. Subspecies hypogaea has two varieties (hypogaea and hirsuta), whereas Subspecies fastigiata has four (fastigiata, vulgaris, peruviana and aequatoriana). The botanical name of groundnut is derived from the Greek word arachis meaning 'legume' and hypogaea meaning 'below ground', referring to the formation of pods in the soil (Pattee and Stalker, 1995).

Groundnut consists mainly of protein 22–30% (Pancholy et al., 1978) and oils 44–56% providing high energy source 5.64 cal/g (Cobb and Johnson, 1973). Peanut oil mainly composed of unsaturated fatty acids and is consequently susceptible to lipid oxidation (Ahmed and Young, 1982). The ratio of polyunsaturated fatty acids to saturated fatty acids of peanuts has been reported as 1.8 compared to 2.9 for soybean oil, 4.3 for corn oil and 8.7 for safflower oil (Carpenter et al., 1974). Peanut oil principally contains less linoleic acid (a relatively unstable fatty acid) than other seed oils. Oleic, linoleic, and palmitic acids account for ca. 90% of the fatty acid profile of peanuts, although five other fatty acids are present in at least 1% proportions (Ahmed and Young, 1982).

Groundnut has traditionally been used as a source of oil; however, its worldwide annual protein harvest has reached nearly 4.5 million tons. In recent years, several cereals and legumes-based foods using peanuts as protein supplements have been developed to alleviate protein calories malnutrition problem. Peanut in the form of flour, protein isolates, and meal in a mixed product have been found to be very desirable from a sensory quality point of view. Peanut protein is deficient with respect to certain essential amino acids, but its true digestibility is comparable with that of animal protein (Singh, 1991). Groundnut can be used in many food forms. With simple roasting and grinding process, Groundnut can be converted into a variety of quality food products. Among the peanut eating people of the world, roasting and salting is the most preferred way of eating. Of the various ready-to-eat (RTE) foods of peanuts, roasted nuts are the most popular ones. Peanuts are sold fresh as a vegetable, canned frozen, roasted in the shell, toasted and salted, used in more than 50% confections as bakery products and are also ground into butter for use in hundreds of recipes (Woodroof, 1983). Peanuts were commonly used in the form of various food items such as roasted, ground (or paste), peanut oil, boiled or raw, the most commonly utilized form is the roasted peanut followed by peanut paste (Rubico et al., 1987). Extruded products of peanut meal and legume flour are also becoming popular as human foods in some African countries (Singh, 1985).

Material and Methods

Procurement of raw material

Samples of ten available groundnut varieties i.e. Golden, Bari-2000, Chico, P.W, No.334, 2KCG020, 2KCG017, 96CG005, 01CG005, 02CG005 were purchased from Barani Agricultural Research Institute (BARI), Chakwal.

Chemical Analysis

The flour samples will be analyzed for the moisture, ash, fat, protein and fiber contents according to the methods No. 44-15A, 08-01, 30-10, 46-10 and 32-10, respectively given in AOAC (2001).

Oil extraction:

Groundnut kernels will be ground and oil will be extracted for 8 hours with diethyl ether in a soxhlet apparatus. Then, the solvent will be completely removed under reduced pressure in a rotary evaporator. Oil percentage will be determined by weigh difference AOAC (2001).

Fatty Acids Profile

Preparation of Reagents:

40ml methanol was taken in 50ml conical flask and was placed it on ice and then 10ml sulphuric acid was added in it saved for further use.

Method of preparation of Methyl Esters:

Methylation of fatty acids in the oils under study carried out according to the procedure described by Slover and Lanzaa (1979) with some modifications. The procedure adopted was as under: 200mg (0.2ml) oil was taken in 50 ml screw capped pyrex glass tubes having length 50 cm and internal diameter 1 cm. Then 2 ml methanolic sulphuric acid was added in each tube and glass vials were put in oven pre-heated at 80 oC for 1 hour and shaked after every 15 min. The glass vials were taken out, cooled and 2ml dist. water was added in each tube to stop the reaction. Then esterified fatty acids were extracted with 1ml petroleum ether thrice. After that the ether content was evaporated and remaining oily surface was injected into gas chromatography for fatty acid profile.

Gas Chromatography:

Gas chromatography is a separation technique, available for the separating of organic compounds. The separation of a mixture containing many volatile compounds may be achieved by introducing it as a single plug into a continuously moving gas flow, which passes through the column of material whose properties may be chosen to bring about this separation. In the time the individual components were separated and emerge from the column for evaluation. For the purpose of analysis the separated components are detected and electronically displayed on to a recorder in the form of peaks. The time of emergence of each component, referred to as its elution or retention time, is characteristic of that component and area under the peak is proportional to its quantity.
Quantitative analysis of the fatty acids methyl esters prepared from each oil sample was carried out on DB-Wax (30m x 0.25mm id x .25µm film) glass packed column using nitrogen as carrier gas by 6890 agilent technologies, USA gas chromatograph equipped with flame ionization detector (FID). The peaks and area was acquired with Chromatopac C-R4A, Shimazu, Japan.the gas chromatographic operating conditions were as under:

Gas Chromatographic conditions:

Hydrogen (H) was used as carrier gas at a constant flow rate of 30 mL min−1. The injector and detector (FID) temperatures were maintained at 150 and 250 °C, respectively. The column temperature was maintained at 190oC and air is flow at a rate of 300mL/min.The samples were injector one micro litre syringe (SGE, Australia).

Standard methyl esters of fatty acids
Fatty acid methyle esters (F. A. M. E. Mix GLC-1891 and 1893) Kit of Supelco Corporation 595 North Harrison Road, Bellefonte, USA. was taken as standards. From this Kit methyle esters of 8:0, 10:0, 12:0, 14:0 16:0, 18:0, 18:1, 18:2, and 18:3 fatty acids were analyzed by GC individually to determine their retention time. Then this mixture was analyzed and peak of each fatty acid was marked.

Statistical Analysis

The data obtained were analyzed statistically by using analysis of variance techniques as described by Steel *et al.,* (1997).

Result and Discussion

The moisture content of the raw peanuts (Table 1) showed small but significant (P<O.05) differences. Cultivar Golden contained the maximum (30.59) and cultivar Chico the minimum protein content (23.99). The fat content was higher (51.82) in cultivars Bari-2000 than the other cultivars which differ significantly among themselves. The fiber content of the cultivar No.334 was higher (4.79) than that of other cultivars. The ash content of all the cultivars was differed significantly among different varieties. These results are within the range for peanuts and agree with those of Khalil and Chughtai (1983) and Grosso et al., (2000).

Fatty Acids Composition

The results of fatty acids analysis by gas chromatography showed that five fatty acids viz. palmitic, caprilic, myristic, oleic, linoleic, linolenic acids were detected by comparing their retention times with the satadasrds. The values of fatty acids in different peanut varieties were presented in table 2. The oleic acid (C18: 1) contents of groundnut varieties Bari-2000, P.W, 2KCG020, 2KCG017, 96CG005, Golden, No. 334, Chico, 01CG009 and 02CG005 were 43.6, 41.9, 36.4, 40.5, 38.0, 37.5, 35.8, 31.6, 30.4 and 32.9 g/100g respectively. The maximum oleic acid (C18:1) content was found in groundnut variety Bari-2000 (43.6 g/100g) while lowest in groundnut variety 01CG009 (30.4 g/100g). The results for oleic acid (C18:1) content obtained in this study were comparable to the findings of Grosso et al., (2000) who

Table 1. Proximate composition of Different Pakistani Peanut Cultivars

VARIETIES	MOISTURE	PROTEIN	FAT	FIBER	ASH
02CG005	5.06 abc	24.1	49.9 abc	4.19 b	2.6
96CG005	4.86 abcd	28.4	42.65 cd	4.8 a	2.7
P.W	4.9 abcd	26.19	44 bcd	4.4 ab	3.00
2KCG017	5.26 a	29.50	46.65 abcd	4.1 b	2.8
Chico	4.73 bcd	23.99	41.38 d	4.50 ab	2.59
01CG009	5.2 ab	31.69	45.31 abcd	4.6 b	3.00
2KCG020	5.13 ab	27.3	45.35 abcd	4.29 b	2.6
No.334	5 abc	28.39	50.39 ab	4.79 a	2.3
BARI-2000	4.6 cd	26.2	51.82 a	4.19 b	2.60
Golden	4.46 d	30.59	43.3 bcd	4.5 ab	3.00

Table 2. Fatty acid composition (g/100 g of total fatty acids) of Different Pakistani Peanut Cultivars

Varieties	C 8:0	C 10:0	C12:0	C14:0	C 16:0	C18:0	C18:1	C18:2	C18:3
02CG005	ND	ND	ND	ND	9.6	1.5	32.9	45.3	0.46
96CG005	ND	ND	ND	0.02	8.5	2.66	38.0	44.6	0.56
P.W	ND	ND	ND	0.06	12.1	2.6	41.9	46.6	0.36
2KCG017	ND	ND	ND	0.08	10.5	1.6	40.5	48.8	0.45
Chico	ND	ND	ND	0.06	9.9	1.8	31.6	47.8	0.30
01CG009	0.03	ND	ND	ND	10.7	2.2	30.4	44.6	0.25
2KCG020	0.05	ND	ND	ND	11.7	1.9	36.4	42.6	0.39
No.334	ND	ND	ND	0.05	10.2	1.3	35.8	43.4	0.34
BARI-2000	ND	ND	ND	0.03	9.8	2.33	43.6	48.4	0.58
Golden	ND	ND	ND	ND	5.63	1.6	37.5	40.2	0.58

found the oleic acid (C18:1) content ranged from 29.3 to 46.1 g/100g in wild peanut species grown in Argentina. The linoleic acid (C18:2) contents of groundnut varieties Bari-2000, P.W, 2KCG020, 2KCG017, 96CG005, Golden, No. 334, Chico, 01CG009 and 02CG005 were 48.4, 46.6, 42.6, 48.8, 44.6, 40.2, 43.4, 47.8, 44.6 and 45.6 g/100g respectively. The maximum linoleic acid (C18:2) was found in groundnut variety 2KCG017 (48.8 g/100g) while lowest in groundnut variety Golden (40.2 g/100g). The results are comparable to the findings reported by Grosso et al., (1999) who reported the linoleic acid content ranged between 40.9 to 46.8 g/100g in Arachis hypogeae species originating from Uraguay. The linolenic acid (C18:3) contents of groundnut varieties Bari-2000, P.W, 2KCG020, 2KCG017, 96CG005, Golden, No. 334, Chico, 01CG009 and 02CG005 were 0.58, 0.36, 0.39, 0.45, 0.56, 0.58, 0.34, 0.30, 0.25 and 0.46 respectively. The maximum linolenic acid (C18:3) was found in groundnut variety Golden and Bari-2000 (0.58 g/100g) while lowest in groundnut variety 01CG009 (0.25 g/100g). Palmitic acid (C16:0) contents of groundnut varieties Bari-2000, P.W, 2KCG020, 2KCG017, 96CG005, Golden, No. 334, Chico, 01CG009 and 02CG005 were 9.8, 12.1, 11.7, 10.5, 8.5, 5.63, 10.2, 9.9, 10.7 and 9.6 g/100g respectively. The groundnut variety P.W showed maximum palmitic acid (C16:0) content (12.1 g/100g) while lowest palmitic acid content was found in groundnut variety Golden as 5.63g/100g. Capric acid (C8:0) was found in 01CG009 and 2KCG020 in concentration of 0.03 and 0.05 g/100g. Stearic acid (C18:0) content of groundnut varieties Bari-2000, P.W, 2KCG020, 2KCG017, 96CG005, Golden, No. 334,

Chico, 01CG009 and 02CG005 were 2.33, 2.6, 1.9, 1.6, 2.6, 1.6, 1.3, 1.8, 2.2 and 1.5 g/100g respectively.

Conclusion

In different peanut varieties grown in Pakistan, major fatty acids were detected while minor fatty acid such as Caprilic acid C 8:0, Capric acid C 10:0, Lauric acid C12:0 were not detected due to derivertization problems and some other problems.

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PAK. J. FOOD SCI., 21(1-4), 2011:56-66
ISSN: 2226-5899

Processing doughs for bread with improved nutritional properties through incorporation of dietary fibres

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Abstract

A well-balanced diet with emphasis on increased consumption of complex carbohydrates with low glycemic index is a major nutritional goal for public health. Bread can significantly contribute to this balance, all the more if its content in dietary fibres (DF) is increased. The main purpose of this work was to prepare breads with improved nutritional properties through incorporating fibers, without affecting overall acceptability. The glycemic index (IG) of breads was determined *in vitro* by α-amylolysis. The modifications of cellular structure during fermentation were observed by digital camera and analyzed in terms of porosity and stabilization. A decrease of dough stability due to DF addition was observed by the follow-up of bread dough proofing by image analysis. The increase of viscosity due to DF incorporation was balanced by an addition of water in order to reduce power consumption during dough mixing. The bread texture was assessed by multi-indentation and image analysis, for observing the effects of DF incorporation. These results suggest that DF mainly affect GI through the modification of texture rather than by a direct impact of their presence. However, it still remains unclear whether this is due to change of starch modification or an increase of crumb mechanical properties.

Key-words: Dietary fibers, Bread, Glycemic index, Texture, Multi-indentation, Image analysis

Introduction

Bread is an important source of nutrition for human diet contributing starch, vegetable proteins and fibres. The nutritional properties of bread can be improved by the addition of dietary fibres (DF) in order to decrease the glycemic index. The glycemic index or GI is a measure of the effects of carbohydrates on blood sugar levels. For most people, foods with a low GI have significant health benefits. Dietary fibres are the remnants of the edible part of plants, which are not digested and absorbed in the upper human intestine. They are mainly composed of plant cell walls, for instance in the different tissues of the wheat grain, and their variable structural features affect their properties and technological role (Saulnier *et al.* 2007). Because of this variety and in spite of the large amount of literature describing the effect of fibres in breadmaking, there is no evidence of its effect on IG, whereas the influence of the process might be significant (Rizkalla *et al.* 2007).

Wheat flour dough is a viscoelastic phase which plays an important role in the development of the alveolar structure of different cereal products. It also results in the modification of texture and nutritional properties of final products through incorporating dietary fibres (DF) into its constituents during dough processing which is a dynamic process and is difficult to understand. Dough development results from hydration of flour components and structural changes induced due to mechanical mixing (Belton 2005). Mixing is key step in bread making because the success of later steps of fermentation and baking depend on homogenized distribution of flour constituents and also on the creation of intermolecular associations between gluten proteins. The stability of these gas cells, which is crucial for the volume of the bread during proofing, is influenced by the composition and mechanical properties of the layer at the air-viscoelastic matrix interface particularly during dough processing. The addition of insoluble fibres can increase the viscosity of the dough as observed by measuring shearing properties of dough at large deformations, whereas the soluble fibres can increase the viscosity of liquid fraction. This increase in viscosity shows an adverse effect on the stability of dough, as it is related to bubble growth and dough expansion. The fibre rich breads are prepared in order to reduce this loss of stability. The duration of proofing is also important because a reduction in fermentation time can result in breads with lower volume, thus increasing the density of the final product (Ducasse 2009). Because the fibre

addition can affect the bread volume and crumb texture, along with decreasing the glycemic index, their control is still a daily challenge for the baking industry.

From a sensorial point of view, the addition of fibres in current French bread results in a loss of crispiness of the crust, an increase of density, a granular mouth feeling and a dark crumb. All these drawbacks may limit consumer's acceptability (Martin *et al.* 2008). The objectives of this study are two folds, firstly, studying various processing steps like dough mixing, proofing, rheological properties, and secondly, observing the effects of dietary fibres incorporation on dough processing steps along with textural and nutritional properties. In this context, dough processing steps will be studied in order to correlate the fibre incorporation with nutritional and sensorial properties of bread.

Materials and Methods

Dough mixing

Flour (Protein 12%) Brasseuil T65 type was a gift from Moulins Giraudeau (44), it contained 12% water (total basis). It was mixed with 66% water (weight/weight flour) in mixer with oblique arm (Mahot) for 12 mn at 80rpm to obtain 3 kg dough. Necessary adjustments were made to yeast content in order to modify density. 7.5% wheat fibres (fine bran, namely "shorts") were incorporated in order to get a ash level equivalent to a T80 type flour. To obtain bread in the format "baguette", two processes were applied: current French bread (PCF) and traditional French bread (PTF), the latter mainly having longer proofing times. The target specifications and properties are recalled in table 1.

Rheological properties

The rheological tests were performed at small and large deformations by conducting dynamic and biaxial extension measurements. The dynamic experiments (small deformation) were performed in a controlled strain dynamic mechanical and thermal analyser (DMTA MK 3E) provided by Rheometric Scientific, USA. The DMTA was used in compression mode at a frequency of 1Hz with deformation amplitude of 0.1% (Rouillé *et al.* 2005). After a period of rest time for 30 min, a cylindrical dough sample (~0.9g) was placed between two plates (Ø17mm) and coated with silicon to avoid moisture loss. Temperature was increased from 22 to 120°C and data was collected by means of computer programme which calculates dynamic properties E', E'' and tan δ= E''/E', where E' is the storage or elastic modulus and E'' the dissipative or viscous modulus. The second measurement (t_1) was taken after 90 min dough rest time.

In biaxial extension tests (large deformation), the compression is applied in lubricated conditions « Lubricated squeezing flow, LSF ». The dough samples of 5g used to measures the rheological properties. The samples were placed in small cylinders of Teflon lubricated with paraffin oil and kept at room temperature for 1H (Launay and Michon 2008). The homogenous samples were then removed from the cylinders (h= 14 mm) and placed between two parallel plates of Teflon (Ø = 20 mm), lubricated with paraffin oil (110-230 mPa.s). The upper plate is attached with movable crosshead of a traction/compression machine (INSTRON 1122), equipped with force sensor (Instron Corp., District, MY, US). The cylindrical dough samples were compressed until a final height of 1 mm, at constant speed (V = 5, 10 and 100 mm/min). The measurements were repeated 4 times for verifying the results.

Dough proofing

During proofing, the section and shape of dough were continuously measured by camera video (every 5mn) and image analysis in order to determine the evolution of dough porosity, assuming cylindrical symmetry, and dough stability, defined by the ratio height/maximum width (H/L). Breads (baguettes) were baked in a Bongard traditional electric deck oven (V=200L, P=21kW) at 250°C for 26 mn.

Structural and texture analysis

For α-amylolysis, bread pieces (≈0.25cm^3) are stirred within flaks containing 150mL water and .01µg amylase (ref 1.163.12, Merck) /mg starch in phosphate buffer to reach pH7, at 37°C. 2mL of suspension are removed every hour for the first seven hours and then at 24 and 28h; hydrolysis products (oses, oligosaccharides) are measured after centrifugation according to the procedure detailed by (Tollier and Robin 1979).

All measurements were triplicated, leading to a relative uncertainty <5%. Initial velocity and starch easily degradable fraction are determined after fitting amylolysis curve according to a pseudo 1st order kinetic model, in order to be compared to results from *in vivo* clinical assays (Rizkalla *et al.* 2007). Thermal and mechanical analyses of dough were performed by DSC and DMA as described in detail by (Chevallier *et al.* 2000) in order to assess starch gelatinization and gluten aggregation under conditions as close as possible of baking process (heating rate ≈5°C/mn). The shear behaviour of unleavened doughs was studied by adapting creep-recovery tests on plane/plane shear rheometer according to (Rouille *et al.* 2005). The shear behaviour was also studied with the help of liquid squeezing flow (LSF). Crumb grain (texture) was assessed by scanning (800dpi) about 10 slices x 3 baguettes per sample; the images were analysed, using Aphelion and MatLab softwares, and, following canonical analysis, similarity maps were drawn to compare crumb grain, detailed procedures being adapted from (Lassoued *et al.* 2007). Overall mechanical properties of baguette pieces are determined by multi-indentation test early set-up to assess crust crispness and crumb firmness by applying a compression/relaxation test with 10 to 20 adjustable pins,

from force-displacement curve as described in details by (Chaunier *et al.* 2008).

Results and Discussion

Nutritional properties: glycemic indices and starch modifications

Like for heat moisture treated starchy products, the kinetics of amylolysis of breads were comprised between those of native starch (low accessibility of enzyme) and amorphous extruded starch (easily accessible) (Fig 1a). Although significantly different, their similar shape allowed them to be fitted by the equation:

$$(\%\ hydrolysis) = a\ .\ Log\ (1+ t/b) \quad (1)$$

t (h) being the time of hydrolysis, and the initial velocity $V0 = a/b$ can be used to compare with values of *in vivo* glycemic index (Fig 1b).

Comparison between kinetics shows that bread glycemic index does not depend first on the DF content but that is also largely influenced by the breadmaking process, since all traditionally processed breads have lower IG (<60%) and V_0 values ($<12h^{-1}$). Results from amylolysis, namely V_0, are just fairly correlated with *in vivo* IG results. This is not surprising, since human digestion involves more phenomena including mastication and gastric emptying that even more sophisticated *in vitro* methods do not address, but at least, amylolysis gives a first mean of screening and relating nutritional properties to starch structural modifications. Regarding these latter, DSC results showed that in the crumb, starch was completely gelatinized whereas in the crust, the presence of fibres might have reduced this modification. The first result may be explained because the curve of moisture/temperature within bread (in the crumb) during baking (Wagner et al., 2007) early crosses the melting curve of starch; conversely, the uncertainty on the variations of temperature and moisture at bread surface (crust) limits the interpretation of the role of fibres during baking.

Table 1: Target characteristics and properties of bread "baguettes" and symbols used in figures

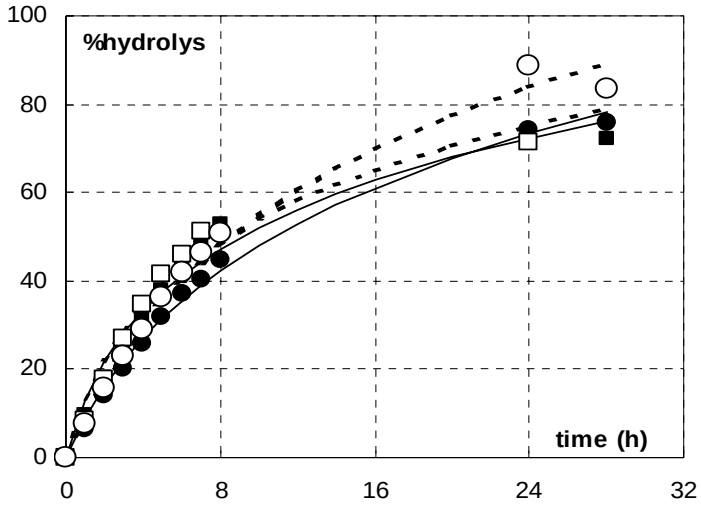
Sample #	Symbol	Type & composition	Density	Main Target Features
1	□	PCF	0.18	« Blank » high GI
2	■	PCF+fibres	0.20	Regular crumb grain T80
3	□	PCF « dense »	>0.3	High density, regular crumb grain
4	**O**	PTF	0.25	Irregular crumb grain
5	●	PTF+fibres	0.25	Irregular crumb grain T80
6	●	PTF+ modified fibres	0.25	Id.
7	●	PTF+fibres « dense »	0.35	High density, irregular crumb grain T80
8	**O**	Sourdough bread	0.35	High density, irregular crumb grain

Rheological properties

The results of power applied during mixing show that the doughs prepared through incorporation of fibers offer some resistance during to mixing (Fig 2). In order to verify these results, the experiments were performed to determine rheological behaviour of doughs. Typical DMTA result for flour-water dough modulus is presented as function of oven temperature (Fig 3). The dough transition occurs at 60°C, where a strong increase is clearly observed in the interval (75-90°C). The increase in elastic module is characteristic of gluten network and swelling of starch granules, as shown by the histograms. The elastic modulus (E') for fiber doughs is higher than normal doughs (Fig 3a), which can affect the final texture of bread. Furthermore, it increases with dough rest time both for fiber enriched and normal doughs.

The biaxial extension measurements, realized at different deformations $0.1< \varepsilon_b < 1.25$, verify the extensional behavior of doughs. The results of viscosity, presented as function of deformation rate (Fig 3b), show that the viscosity of fiber doughs is higher than normal doughs as they offer greater resistance to flow. The variations of viscosity are less influenced by the mixing conditions but the fiber addition can significantly affect the dough extensional behaviour. The values of n (flow index) and SHI (strain hardening index) are higher, being 0.33 and 1.72 (ε = 0.75), respectively, for normal doughs as compared to fibre enriched doughs (n= 0.27, SHI= 1.59).

(a)



(b)

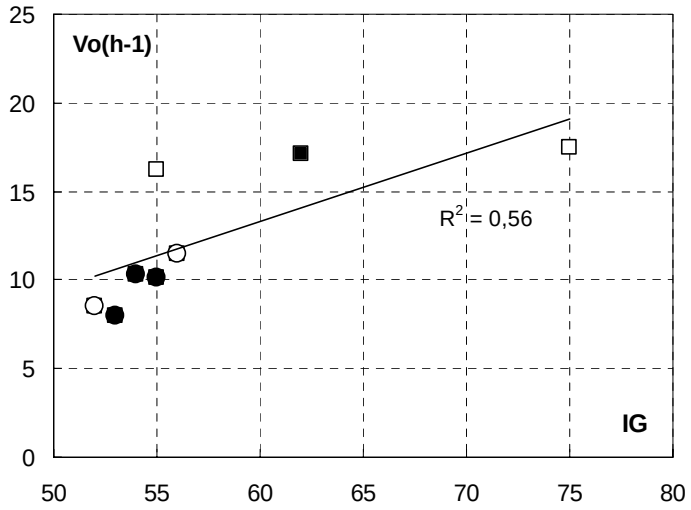


Figure 1: Results of kinetics of amylolysis for (a) breads without (1,4: □,O) and with fibres (2, 5 : ■,●) and their fit (dotted= without, full=with fibres) with eq.(1) and (b) comparison of initial hydrolysis velocity V_0 with in vivo IG (full legend in Table 1).

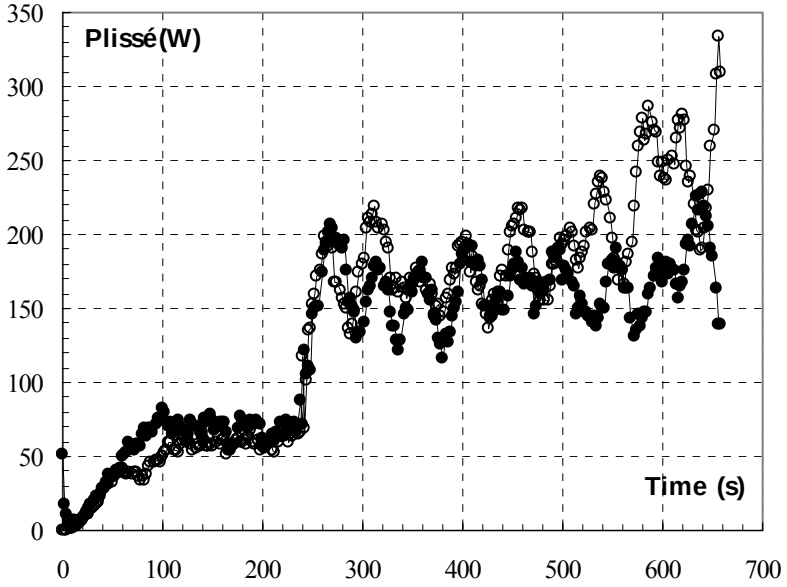
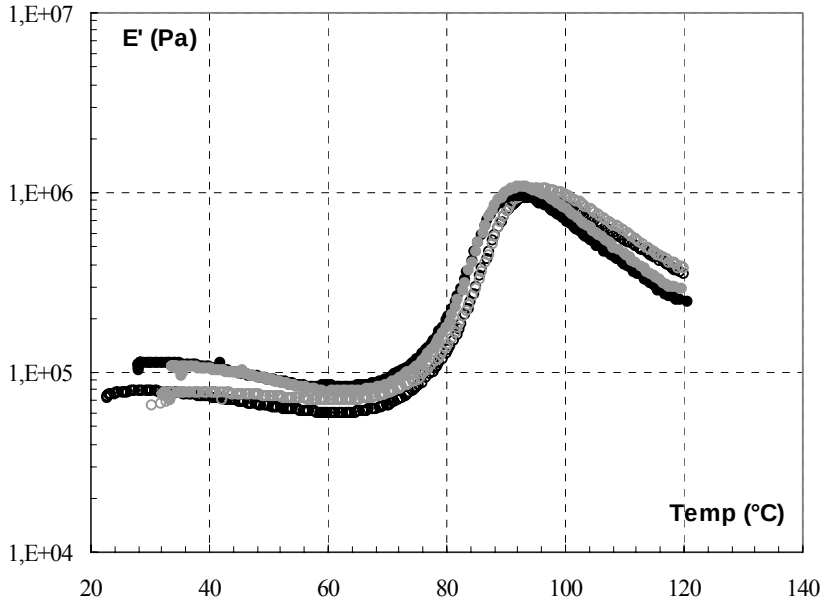


Figure 2: Representation of power curves during mixing. With (●) and without fibres (O)

(a)



(b)

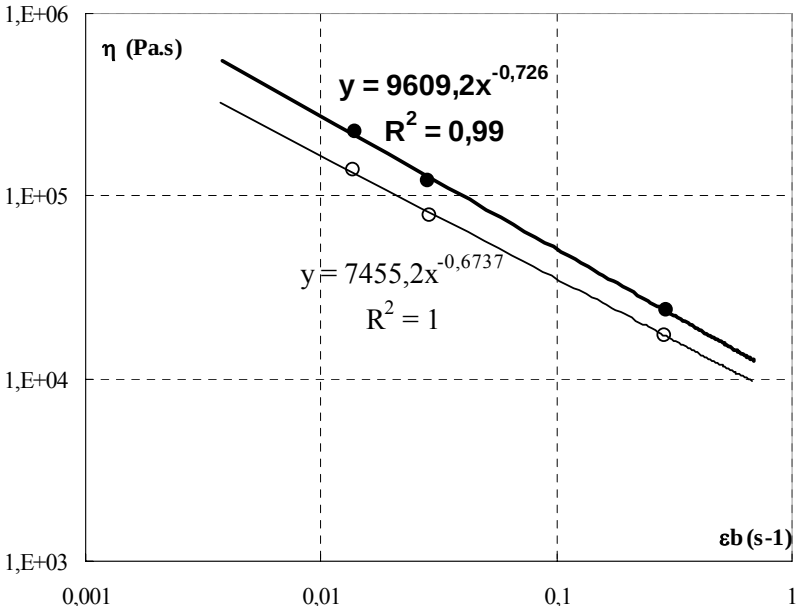
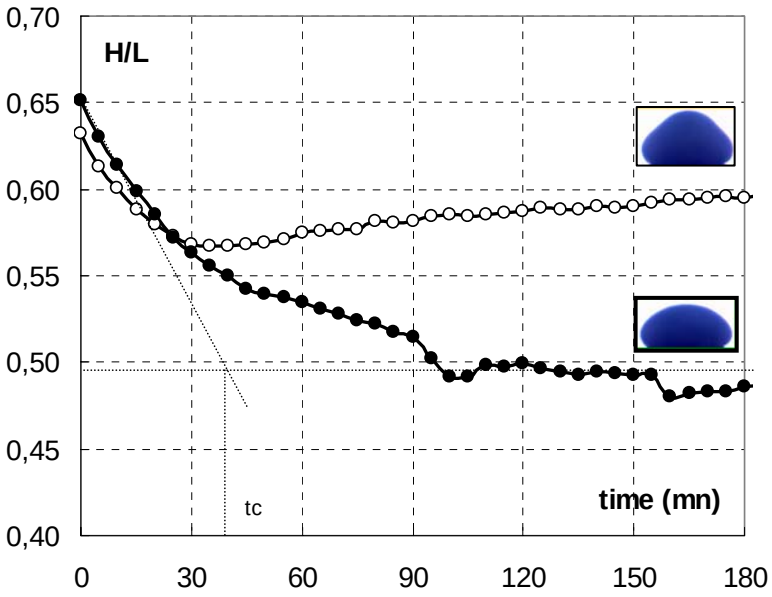


Figure 3: (a) Variation of elastic modulus E' with respect to temperature. (b) Viscosity vs deformation rate for doughs with (t_0 ●t_1 ●**)** and without fibres (t_0O t_1 O)

(a)



(b)

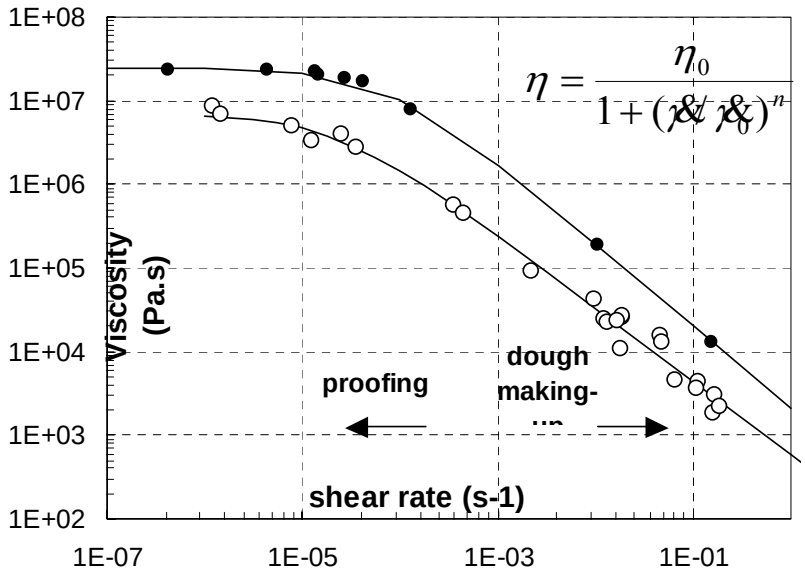


Figure 4: Curves of behavior (a) of stability and images during proofing and (b) of flow under shear (viscosity vs shear rate) for doughs with (●) and without (O) fibres

The fibre incorporation leads to an increase in the consistency index (K) from 7400 to 9600 Pa.s.

Dough behaviour

From the follow-up of dough development during fermentation, no significant influence of fibers addition was found for the evolution of porosity. The evolution of this property followed a classical sigmoid curve (Romano *et al.* 2007), with an inflection point close to (30mn, 40%) in both cases, with and without fibers. However, they did not display the same stability, as shown by the curves H/L (t) and the images of dough at the end of proofing (Fig 4a). In most cases, these curves can be well fitted (r^2>0.95) by an exponential decay, for time values ≤ 90 mn:

$$H/L(t) = [(H/L)_0 - (H/L)_\infty]. \exp(-t/t_c) + (H/L)_\infty \quad (2)$$

in which $(H/L)_0$ and $(H/L)_\infty$ feature the initial and long term values of stability, resp., and t_c the stability characteristic time.

The flow curves of the dough could be fitted by the Cross model (Fig 4b), inserted in the graph. The increase of viscosity due the addition of fibers, mainly at lower shear rate value ($\approx 10^{-4} s^{-1}$), may be attributed to a reinforcement of the dough due to the addition of solid particles or to the decrease of water in the viscoelastic gluten network (Rouillé et al., 2005). It may be the cause of the loss of stability of the fibers-enriched dough by precluding upwards bubbles migration or by destabilizing the interface between gas bubbles. These phenomena control the acquisition of cellular structure, and crumb texture. Finally, for higher shear rate values, the viscosity difference is less significant but suggests that higher mechanical power is required during mixing and making-up.

Mechanical properties and crumb appearance

The acquisition and analysis of images from bread cross sections allow to draw a similarity map of the crumb grain, or visual appearance, where the ellipses represent the variability along the baguettes (Fig 5a). When not overlapping, their location suggests that the main effect of process is that traditional bread has a coarser cellular structure with larger void cells than current one and that fibers addition increases crumb homogeneity and density. However, resulting from multi-indentation measurements, apparent modulus E (Pa) and residual stress σ_s (Pa), likely accounting for crust and crumb mechanical properties respectively, are well correlated (Fig 5b). This result suggests that neither the process nor the addition of fibers modify the texture contrast of "baguettes".

Conclusion: process recommendations and prospects

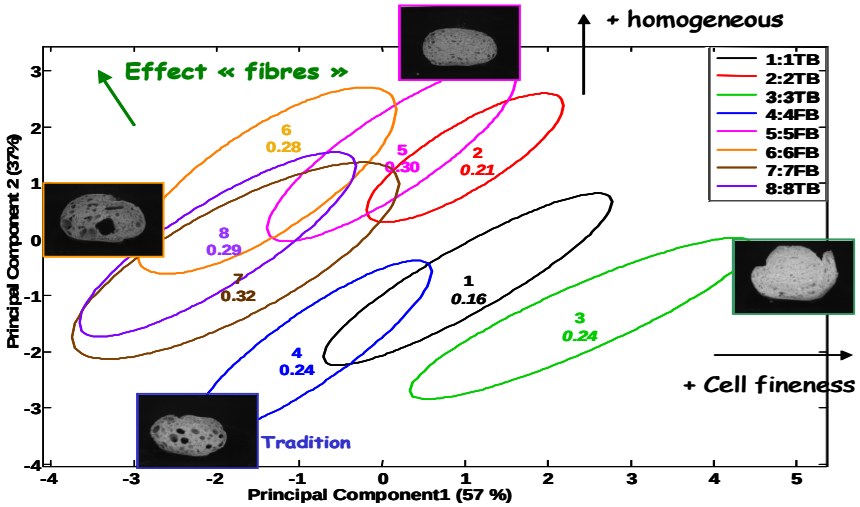
Taking into account these results as well as our expertise in breadmaking , it is suggested that when incorporating fibers, (1) the level of hydration of the dough is slightly increased (+3% total weight) in order to decrease dough viscosity, and (2) the total proofing time is decreased (-15mn) to reduce the loss of stability. As expected, the consequence is that the density of dough before baking, and hence, its final value, is increased. The resulting breads well illustrate this trend (Fig 6a), but the influence of breadmaking process is also significant.

This increase of density is particularly important as it seems to govern texture, by increasing modulus, in a similar way as Gibson-Ashby's model allows to predict the mechanical properties of cellular solids (Zghal and Scanlon 2001). The role of density is as much important as its increase leads to a decrease of *in vivo* glycemic index (Fig 6b). This increase may be explained either by a limited starch destructuration caused by a decrease of thermal flow within dough during baking, in agreement with (Wagner *et al.* 2007), or by a reduced bread fragmentation during chewing, which further hinders the accessibility to enzyme during digestion (Zghal and Scanlon 2001).

Acknowledgements

This work has been carried out in the frame of ANR-PNRA projects AQuaNuP (improving bread quality) and INCALIN (integrating knowledge in food industry), the participants of which are warmly acknowledged.

(a)



(b)

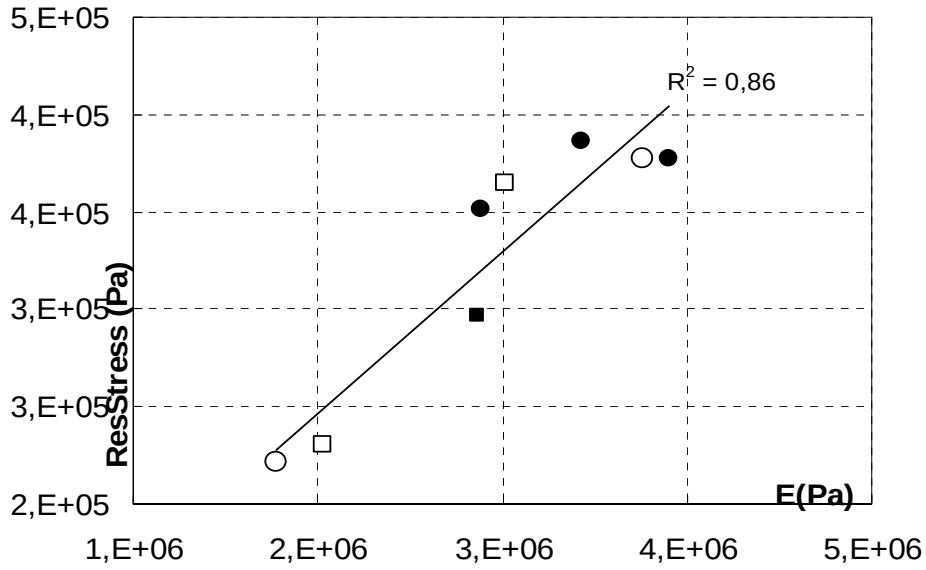
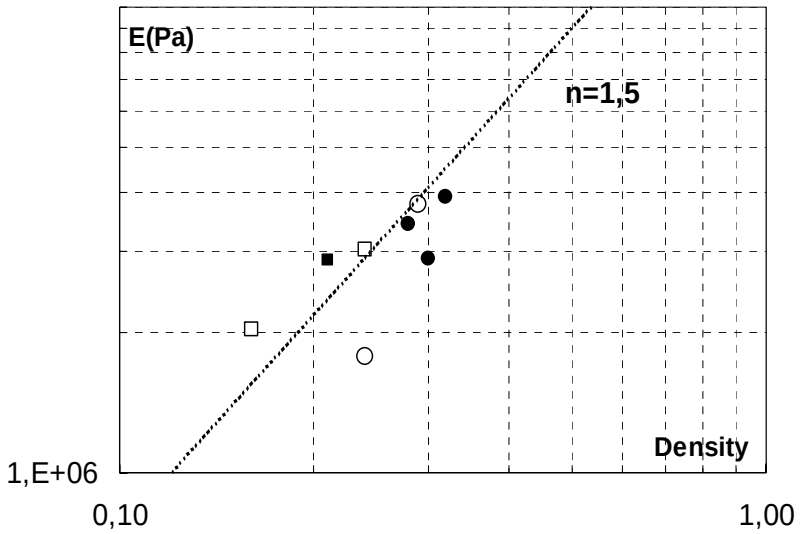


Figure 5: Texture of baguettes defined by (a) the similarity map of baguette crumbs and density and (b) the variations of their mechanical crust/crumb properties (same legend as Fig.1).

(a)



(b)

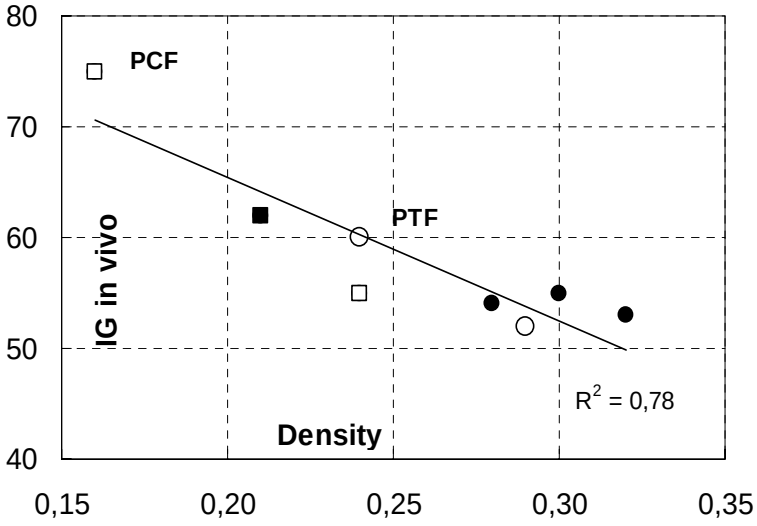


Figure 6: Variations with density of (a) crust/crumb apparent modulus (dotted line = Gibson-Ashby's power law) and (b) in vivo IG (same legend as Fig.1).

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PAK. J. FOOD SCI., 21(1-4), 2011:67-71
ISSN: 2226-5899

Evaluation of quality of mango *(Mangifera indica* L.*)* squashes available in Lahore market

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Abstract

This study was conducted to evaluate the quality of five different mango squashes available in Lahore market. Physio-chemical characteristics including moisture, ash, total carbohydrates, total solids, total soluble solids (TSS), pH, acidity, total sugars and ascorbic acid contents were evaluated. The results showed that there were significant differences among squashes of all brands for physio-chemical parameters. Sample (S3) showed the highest TSS, total sugar, total carbohydrate and total solids. The value of TSS of S4 and S5 were not according to Pakistan Standard Specification for fruit squashes. The squashes which possessed higher value of total sugar, total carbohydrates and total solids contained high pulp content and exhibited better quality. Sample (S2) exhibited the highest ascorbic acid contents. The organoleptic evaluation was done for color, taste and flavor. The results showed significant difference in color of different brands but taste and flavor of different brands exhibited non significant differences from each other. Two samples (S2 and S3) were better than other three but all the mango squashes were acceptable regarding organoleptic quality.

Key words: mango, squash, acidity, sugars and ascorbic acid.

Introduction

Mango (*Mangifera indica L.*) which belongs to the family *Anacadiacease*, is one of the most cultivated fruit in the world (Ashoush and Gadallah 2011). Pakistan's mango production in 2009-10 was 1847,000 tones (Anonymous, 2010). Mango has a prominent position in the fruit processing industry of Pakistan. The bulk of mango fruit is consumed as fresh fruit. It is perishable in nature and cannot be stored for long time. Therefore, it is also processed in many forms to make pulp, juices, chutneys, pickle, jams, nectars, squashes, mango toffees, canned mango slices and frozen mango slices etc. It is also used to prepare jellies, ice creams, milk shake, fruit cocktail and in topping products.

Mango is the most relished fruit. It is cherished not only due to its pleasant taste, aroma but also for its nutritional contribution to our diet. It serves as a good source of energy and provides vitamins A, C and minerals like iron and phosphorus (Malik, 1994).

The fruit flesh of a ripe mango contains about 15% sugar, up to 1% protein. Mango has antioxidant, anticancer and anti-cardiovascular abilities. Because of the high iron content they are suggested for treatment of anemia and are beneficial to women during pregnancy and menstruation. Mangoes contain an enzyme with stomach soothing properties similar to pepsin. This comforting enzyme helps in digestion.

Juicy verities of mango are preferred for making squash. Squashes are sweetened juices containing some pulp. The term "cordial" is often used interchangeably with squash. Fruit squashes contain a minimum of 25% by volume of fruit juice and are intended to be drunk after dilution. Although sulphur dioxide is the usual preservative for squashes, benzoic acid is often used. The acidities are usually in the range of 1.5 to 2.5 per w/v citric acid. The squash is filled into washed and sterilized bottles, leaving about 1 inch head space. The bottles are closed with crown. The squash keeps in well for 1 to 1.5 years without much change in color or taste (Gupta, 1993). The present study was conducted to evaluate the quality of Mango Squashes available in Lahore market. It was investigated by evaluating the physio-chemical parameters and sensory evaluation of squashes.

Materials and Methods

Locally manufactured Mango Squashes were purchased from local market. All these brands had common packing materials (Pet bottles). Mango Squashes selected for study were: Shezan mango squash, Mitchell's mango squash, PCSIR mango squash, Kinza mango squash and Tops mango squash.

Physio-chemical analysis

Moisture, ash contents, total solids, total soluble solids (TSS), pH, total titrable acidity, total carbohydrates, total sugars and ascorbic acid were determines as under:

Moisture: Moisture contents of samples were determined according to the method reported by AOAC (2005) by using oven drying method. Sample (5 g) was taken in a pre-weighed crucible and dried at 70˚C for 16 to 18 hours.

Ash content: Ash content of the sample was determined according to Kirk and Sawyer (1999). Sample (5 g) was

dried in an oven at 70˚C for 16 to 18 hours and placed in muffle furnace for 4 to 6 hours at 525˚C.

Total solid: Total solid content of the mango squashes were determined as described by the Kirk and Sawyer (1999). Sample (5 g) was dried at 70ºC under vacuum.

Total soluble solids: Total soluble solids (ºBrix) were estimated by using Refractometer (Reichert) as described by Kirk and Sawyer (1999).

Acidity and pH: Acidity of the mango squashes was determined as described by Kirk and Sawyer (1999). Sample (10 ml) was taken in 250 ml beaker and approximately 100 ml water was added. The solution was titrated with 0.1N NaOH to pH 8.2 by using pH meter (Inolab, WTO). The pH of all samples was also measured by using pH meter in accordance with Kirk and Sawyer (1999).

Total Carbohydrates Total carbohydrates were determined by titrimetric method as reported by Ranganna (1987). Sample (10 ml) was taken, 1 ml 6 N HCl was added and made up the volume 40–50 ml with distilled water. Boiled it (30 minutes) for inversion. Neutralized by adding 7 drops of 40% NaOH and made up volume 100 ml with distilled water.

Benedict's solution (5 ml) was taken in a flask, added 40–50 ml distilled water and boiled. Sample solution (volume used = V) was taken in burette and titrated with benedict's solution till color of benedict's solution changes to water and white color ppt are formed.

$$\text{Total carbohydrates} = \frac{12 \times 100 \times 100}{V \times 10 \times 1000}$$

Total Sugars: Total sugars were determined by titrimetric method as reported by Ranganna (1987). Same procedure was adopted as described for Total carbohydrate except boiling for 2-3 minutes for inversion.

Ascorbic Acid: Ascorbic acid was determined by titrimetric method as reported by Kirk and Sawyer (1999). Sample (5 ml) of mango squash (W) was taken in 100 ml volumetric flask. The volume was made up with 0.4% oxalic acid.

Standard ascorbic acid solution (1 ml) + 1.5 ml of 0.4% oxalic acid was titrated against freshly prepared dye (Indophenol dye 0.026% w/v, volume R), until brick red end point.

Sample solution (5 ml) was titrated against freshly prepared dye until brick red end point. The ascorbic acid contents were calculated by the following formula.

$$\text{mg/ 100 ml ascorbic acid} = \frac{R1 \times V \times 100}{R \times W \times V1}$$

Where: R1 = volume of dye used.

V1 = volume of aliquot taken for titration

V = volume of aliquot made by 0.4% oxalic acid.

Organoleptic evaluation

The Mango Squashes were evaluated organoleptically by a panel of four judges for color, taste and flavor as described by Larmond (1977).

Results and discussion

The study was conducted to evaluate the quality of Mango Squashes by studying their physio-chemical parameters and organoleptic characteristics.

Physio chemical characteri-stics

The percentage of moisture present in different squashes is given in Table 1. The highest value of moisture is 74.27% and lowest 46.26% for S5 and S3, respectively. Statistical data regarding moisture contents showed that the differences among different squashes were highly significant with respect to moisture contents.

The data on ash contents of locally manufactured mango squashes is given Table 1. It is evident from data that the lowest value of ash 0.124% and the highest 0.268% was exhibited by S1 and S3, respectively. The statistical analysis of the data indicated that the differences with respect to ash contents are highly significant for different brands of mango squashes.

The highest value of total solids is for S3 and lowest is for S5 which are 53.74% and 25.73%, respectively. Fig. 1 shows the differences in total solids among different samples.

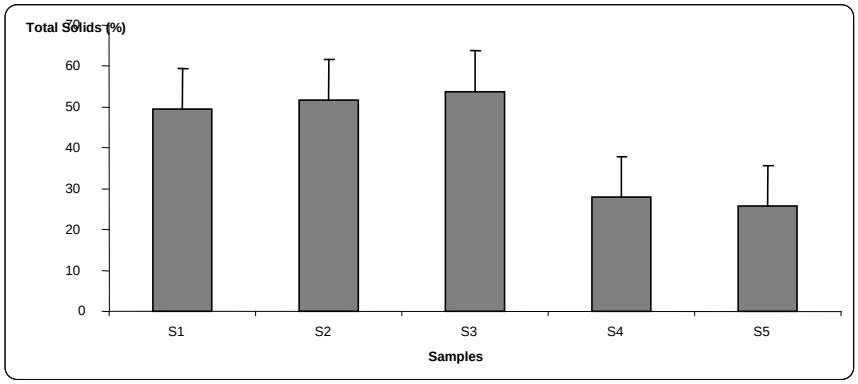

Fig 1. Total solid (%) of different samples of squashes.

It is evident from the statistical data regarding total solid contents that the differences among different squash varieties were highly significant with respect to total solids (Table 1).

Table 1. Physio-chemical characteristics of locally manufactured different brands of mango squashes.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	F value
Moisture (%)	50.50	48.30	46.26	72.07	74.27	11902.204**
Ash contents (%)	0.124	0.231	0.268	0.208	0.217	158.625**
Total solids	49.44	51.70	53.74	27.93	25.73	20582.744**
Total soluble solids (TSS)	49.9	51.0	52.5	29.0	27.0	36431.075**
pH	2.81	2.74	3.06	2.72	2.95	316.950**
Acidity (%)	1.988	1.799	1.694	1.811	1.785	855.583**
Total carbohydrates (%)	60.00	60.06	63.00	28.80	23.61	675.827**
Total sugars (%)	48.67	50.00	55.41	28.35	23.08	841.167**
Ascorbic acid (mg/ 100 ml)	23.11	26.31	23.11	23.29	24.00	5.341*

** = Highly significant, * = Significant

Total soluble solids (TSS) give information about the soluble sugar present in the squash. TSS values of different squashes are presented in Fig. 2.

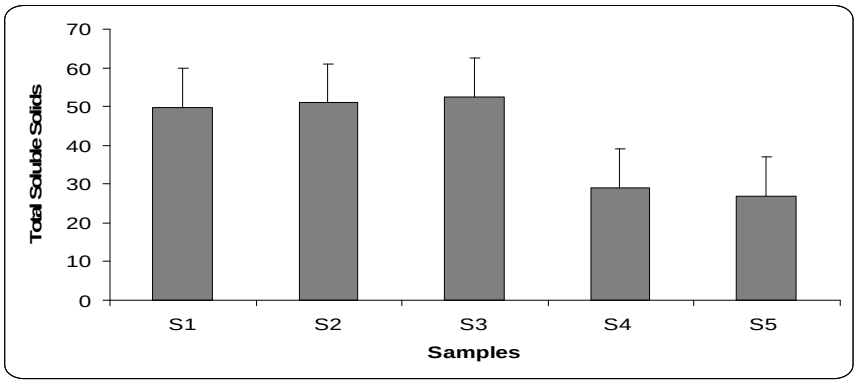

Fig. 2. Total soluble solids (TSS) in different squashes.

The squashes having high value of TSS indicated that they have more sugar contents than those having lower value. It is evident from the statistical data regarding TSS that the differences among different squash varieties were highly significant with respect to TSS (Table 1). The data showed that S3 Mango Squash has the highest value (52.5) and S5 Mango Squash has the lowest value (27.0). Hussain *et al*. (2005) observed that mango squash exhibited TSS values 44.66 to 53.74.

The values of pH of different brands of mango squashes are presented in Table 1. The pH value of sample S3 is highest (3.06) among other brands and S4 has the lowest value (2.72). Hussain *et al*. (2005) reported that mango squash prepared from different varieties of mangoes showed pH 2.15 to 2.56. High pH value may be due to low acidity. It is clear from the statistical data regarding pH that the differences among different squash varieties were highly significant with respect to pH.

The data about acidity of locally manufactured mango squashes is given in Table 1. The values of total acidity of all mango squashes are above one percent which is according to Pakistan Standard Specification for fruit squashes. The highest acidity was 1.988% and the lowest 1.694% in sample S1 and S3, respectively. The statistical analysis of data showed that the differences among different brands of mango squashes were highly significant with respect to total acidity. These results are in agreement with Hussain *et al*. (2005). They observed that mango squash showed acidity 0.933 to 1.149%.

The values of total carbohydrates were 60.00, 60.06, 63.00, 28.80, and 23.61% for S1, S2, S3, S4 and S5, respectively (Table 1). The highest value was 63.00% for S3 and lowest value of total carbohydrates was 23.61% for S5. The statistical analysis of the data showed that the differences among different brands of squashes were highly significant with respect to total carbohydrates. The differences in total carbohydrates are also evident from Fig. 3.

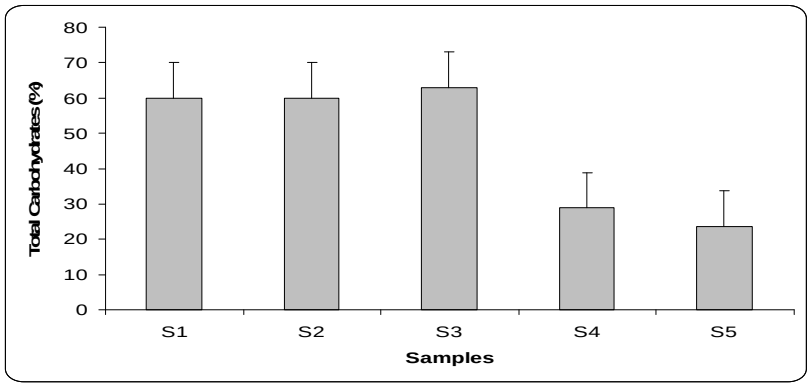

Fig3. Total carbohydrate (%) in different squashes

Organoleptic evaluation

The locally manufactured mango squashes of different brands were organoleptically evaluated for color, flavor and taste by a panel of four judges. The data about organoleptic evaluation of locally manufactured mango squashes is given in Table 2.

Table 2. Organoleptic evaluation of mango squashes.

Sample	Color	Flavor	Taste
Sample 1	8.12	6.50	6.75
Sample 2	7.67	6.25	6.50
Sample 3	7.75	7.12	7.62
Sample 4	7.00	7.25	7.00
Sample 5	6.87	6.25	6.00
F value	6.516**	$1.170^{N.S}$	$0.286^{N.S}$

Maximum mean score for S1 was 8.12 and minimum mean score for S5 was 6.87 (Table 2). Statistical analysis of data on color of squashes indicated highly significant differences among different varieties of mango squashes.

Flavor is the blend of taste and smell perceptions noted when food is in the mouth. The overall flavor impression is the result of the tastes perceived by the taste buds in the mouth and the aromatic compounds detected in the nose (Rathore *et al*. 2007). The data on flavor of different mango squashes is given in Table 2. The highest value is 7.25 for S4 and lowest value is 6.25 for both S2 and S5. Statistical data regarding flavor indicated that the differences among different squash varieties were non significant with respect to flavor.

Maximum scores were awarded to S3 for taste followed by S4, S1, S2 and S5. The maximum score for taste was 7.62 (S3) and minimum score was 6.00 (S5). It is evident from the statistical data that the differences in taste of different squashes were non significant. Two samples (S2 and S3) were better than other three but all the mango squashes were acceptable regarding organoleptic quality. Ahmed *et al.* (1993) while preparing the squashes from pure and mixed fruit juices found that pure squashes were preferred over mixed fruit squashes. But all squashes were acceptable after 3-4 months of storage at 22-36 °C. Babar (1999) observed that taste and flavor were not affected in mango drink after a storage period of 45 days.

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PAK. J. FOOD SCI., 21(1-4), 2011: 72-75
ISSN: 2226-5899

Nutritional facts and reducing power activity of Bitter Gourd (*Momordica charantia L*) tea

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Abstract

Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids, DNA and can initiate degenerative disease. Antioxidants in vegetables, fruits and tea are the core factors for the observed efficacy of these foods in dipping the incidence of chronic diseases including heart disease and some cancers. Bitter gourd or Karela (*Momordica charantia*) belongs to the family *cucurbitaceae*. It is high in vitamins and an excellent source of iron and calcium. Fruits, young shoot and flowers are used as vegetables. It is also known to cure diabetes, arthritis, rheumatism, asthma, warts, abscesses and ulcers. In the present study bitter gourd tea was developed and its nutritional facts and antioxidant potentials were assessed through reducing power assay (RPA). From this study it was found that Bitter gourd tea had low fat (0.23 ± 0.02 g/100g) but high fiber (14.87± 1.65 g/100g) and protein (21.83± 1.94 g/100g), A concentration dependent reducing potential were exhibited by the bitter gourd tea and these results indicated that it may used as a potential source of natural antioxidant.

Key words: Nutritional facts, antioxidant potential, RPA, Bitter gourd tea, BHA

Introduction

Herbal and natural products have been used for centuries in every culture all around the world. The search for natural antioxidants, especially of plant origin, had increased greatly in recent years. Plants have almost ability to synthesize aromatic substances, most of in aerobic cells and reflect the interaction between which are phenols or their oxygen substituted derivatives. In many cases, these substances serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive, protect molecular damage and herbivores (Shyamala and Vasantha, 2010; Vaya *et al.* 1997).

Reactive oxygen species (ROS) capable of damaging DNA, proteins, carbohydrates and lipids are generated in aerobic organisms. These ROS include superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), and single molecular oxygen. The deleterious reactions triggered by these ROS are controlled by a system of enzymic and non-enzymic antioxidants which eliminate pro-oxidants and scavenge free radicals (Ogunlana *et al.* 2008).There are some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated fruiting is almost through out the year chiefly during hydroxyanisole (BHA), commonly used in foods have some side effects (Branien, 1975). In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human disease (Evans *et al.* 1997).

Bitter gourd, also known as balsam pear or Karela, (*Momordica charantia*; *Cucurbitaceae* family) is a vegetable indigenous to subtropical and tropical regions of South America and Asia. *Momordica* means, "to bite" referring to the jagged edges of the leaf, which appear as if bitten. All parts of the plant, including the fruit, taste bitter. The fruit is oblong and resembles a small cucumber, young fruit is emerald green that turns to orange-yellow when ripe (Saeed *et al.* 2010; Vijayalakshmi *et al.* 2009; Grove and Yadav, 2004). The fruit is considered as tonic, stomachic, stimulant, emetic, antibilous, laxative and alterative. The fruit is useful in gout, rheumatism and subacute cases of the spleen and liver diseases. It is supposed to purify blood and dissipate melancholia and gross humours. It has also been shown to have hypoglycemic properties (anti-diabetic) in animal (Shih *et al.* 2009; Lans and Brown, 1998). This investigation was carried out with an objective to study the nutrient analyses, organoleptic evaluation of the developed bitter gourd tea and to investigate its antioxidant property by reducing power assay.

Materials and Methods

Reagents: In this study, the authors used commercial distilled water (DW) unit (Favorit- W4L, Nottingham, UK) and UV-1700 Pharmaspec, spectrophotometer, Shimadzu, Japan. Po tassium ferricyanide [$K_3Fe(CN)_6$, NaH_2PO_4, $NaHPO_4$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA) while trichloroacetic acid (TCA), BHA, sodium carbonate (Na_2CO_3) and ferric chloride $FeCl_3$were purchased from Across Chemical Co. All remaining chemicals were of analytical grade and were used as received.

Plant material: The bitter gourd (*Momordica charantia*) and other raw materials used in this study was purchased from local market.

Preparation of green tea extract: Bitter gourd tea (1g) was extracted for 15 min with 100 ml of distilled water (DW) at 80°C. Then, the extracts (bitter gourd tea) were filtered using a filter paper and stored at +4°C until they are used.

Nutritional Facts: Moisture, ash, fiber, protein and lipid contents in bitter gourd were determined according to AOAC methods (AOAC, 2005).

Reducing power: The reducing power of bitter gourd tea was determined according to the method previously described (Oyaizu, 1986). Different concentrations of bitter gourd tea extract (0.1– 0.5 mg) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml. 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical analysis: Data analyses were processed using Microcal Origin 6.0 software (Microcal Software, Inc., Northampton, MA, USA). All the experiments were performed three times and the values were represented as mean ± SD.

Results and Discussion

Nutrient Analysis

Knowledge of the physico-chemical properties of food is fundamental in analyzing the characteristics of food during the processing. The study of these food properties and their responses to process conditions are necessary because they influence the treatment received during the processing and also because they are good indicator of other properties and qualities of food (Rao and Das, 2003). In the present investigation certain physico-chemical properties of developed bitter gourd tea were analyzed to ensure the quality of products. The moisture content bitter gourd tea was 5.45%, while the content of total ash was 4.04% (Table 1). Percentage of crude protein and fiber in bitter gourd tea were 21.83 & 14.87 respectively while the content of fat 0.23% was found in bitter gourd tea.

Table: 1. Nutritional Facts of Bitter gourd tea

Sr. No.	Parameters	Values (%)
1	Moisture	5.45± 1.32
2	Ash	4.04± 1.03
3	Fat	0.23± 0.02
4	Fiber	14.87± 1.65
5	Protein	21.83± 1.94

Data are represented ± SD (on dry basis)

Reducing Power Assay

ROS including superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH) are generated during normal aerobic metabolism and can be increased under the oxidative stress. ROS are considered to cause oxidative damage to DNA, lipids, proteins and other bio-logical macromolecules, which can lead to cancer and other diseases (Floyd *et al.* 1989; Pansarasa *et al.* 1999). The reducing power of the bitter gourd tea were range from 0.721±0.05-1.722±0.18 (absorbance value) at concentration 0.1-0.5 mg/ml (Fig. 1), which was higher than that of BHA, that were range from 0.263±0.01 1.171±0.12 absorbance valu-e at same concentration (Fig. 2).

The power of certain antioxidants is associated with their reducing power (Jayaprakasha et al 2001). Duh (1998) reported that the reducing properties of antioxidants are generally associated with the presence of reductants.

Conclusions

Findings of this study revealed that bitter gourd tea had promising sources of protein, fiber and natural antioxidants.

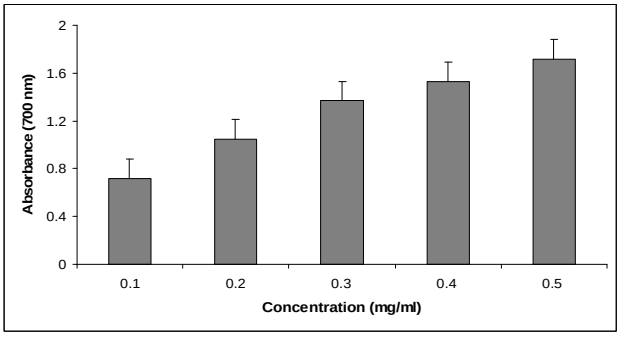

Fig.1. Reducing Power Activity of Bitter gourd tea

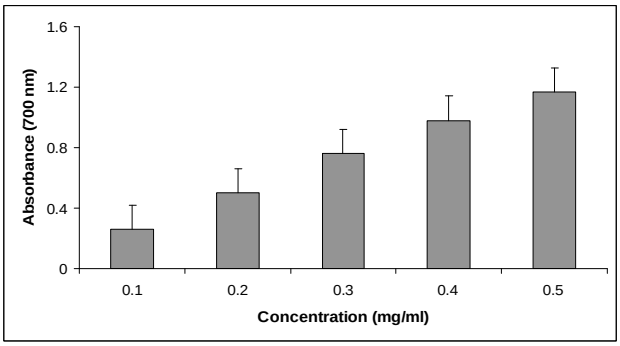

Fig.2. Reducing Power Activity of Standard Antioxidant BHA

Acknowledgements

The authors thank to International Foundation for Science. This study was supported by a research grant (# E/4631-1) from International Foundation for Science (IFS), Sweden.

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