JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

FOOD PACKAGING IN PERSPECTIVE OF MICROBIAL ACTIVITY: A REVIEW

Syeda Anum Zahra, Yasha Nazir Butt, Sitara Nasar, Sadia Akram, Qindeel Fatima, Jannat Ikram*

Address(es): *Syeda Anum Zahra,*
Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, 54590, Pakistan.

*Corresponding author: anum.zahra103@gmail.com

doi: 10.15414/jmbfs.2016.6.2.752-757

ARTICLE INFO

Received 5. 3. 2016
Revised 16. 4. 2016
Accepted 21. 4. 2016
Published 3. 10. 2016

Review

OPEN ACCESS

ABSTRACT

A successful packaging technique demands certain barriers for microbes, achieved through controlled conditions to indicate microbial growth, levels of oxygen, harmful bacterial and fungal toxins, moisture levels, and the indicators for temperature and time. Active food packaging is greatly being applied these days for food safety against harmful microbes. Food is protected from biological, physical, and chemical damages caused by pathogenic microbes through different technologies of packaging including modified atmosphere packaging and controlled atmospheric packaging through antimicrobial films. Moreover, it is essential to use selective materials suitable for different food stuffs for the maintenance of nutritional value of foods. Use of various gas scavengers and bio-based package designing are also greatly helpful towards enhanced shelf life of food products.

Keywords: Active packaging, preservation, antimicrobial packaging, gas scavengers

INTRODUCTION

For many years, advances in food packaging system has revolutionized methods of preservation, trends in distribution, improvement of quality, and prolonged shelf life of food, for good health and safety of consumers. Today, Packaging department dwells next to the Food Industries as an important section with significant 2% Gross National Product (GNP) of developing nations **(Ozdemir and Floros, 2004)**. The downstream processing of foods is greatly dependent on protective antimicrobial processing by optimizing factors including oxygen, moisture and water activity, sunlight exposure, and microbial contaminants. These controlled factors may help us pursue active packaging system through oxygen scavenging, reduction and prevention of food spoiling microbes, moisture absorption, and the adequate generation of ethanol and carbon dioxide **(Suppakul, 2003)**. The packaging material may either be rigid or flexible, to pack different kinds of foods. **Mauriello *et al.* (2005)** interpreted that microbes like *Micrococcus luteus* can be inhibited by effective coatings such as nisin treated films. However, the packaging conditions vary for different types of microbes. New antimicrobials and polymer materials are being introduced to meet regulatory limitations quite viably. Edible films mark another technique to keep food safe for consumption. It makes use of organic acids, salts of organic acids, bacteriocins, fungicides, enzymes, and compounds like silver zeolites **(Quintavalla *et al.*, 2002)**.
The main objective of our study elucidates the best food packaging materials and techniques to prevent microbial food spoilage. In addition, this document gives an overview of better optimization of biological, physical, and chemical parameters towards reduced microbial activity.

TOPICS

Food Safety and Nutritional Quality

Foods are prone to deterioration due to many chemical, physical, biological, and microbiological reactions. Through food safety measures such as freezing, drying, chilling, vacuum packing, acidification, fermentation, preservative agents, modified atmosphere packages helps us prevent microorganisms which cause spoilage of food **(Davidson and Critzer, 2012)**. Spoilage varies from extremely hazardous involving toxicogenic microorganisms to the minor loss of texture or quality such as the loss of flavor or color. To keep food safety and quality up to mark, prevention of infectious pathogenic and toxicogenic bacteria is a must **(Da Cruz Cabral, 2013)**.
Food products subjected to lower temperatures of about 12°C have low microbial growth which is quite useful in chilling process; however, many microbes such as *Listeria monocytogenes* grow rapidly even below 1°C **(Cho and Irudayaraj, 2013)**. Water activity A_w control must be ensured for food safety by reducing it for increased shelf life and reduced microbial activity **(Lopez-Malo and Alzamora, 2015)**. To maintain conditions like water activity and temperature, special packaging is done to create a standardized internal environment for food devoid of any nutritional changes. Microbial activity can also be controlled by selective pH chosen against the optimum pH of microbes that effect specific kinds of foods. For many foods, pH of 4.5 is maintained to restrict multiplication of *Clostridium botulinum* **(Gould, 2000)**.

Major Food Borne Pathogenic Bacteria

Of many bacterial pathogens which cause damage to different foods and hence illnesses, the major ones include *Salmonella, Escherichia coli O157, Campylobacter, Listeria monocytogenes, Clostridium perfringens, Staphylococcus, Shigella,* and *Bacillus* **(Dhama *et al.*, 2013)**. The European Commission has set criteria with certain reference methods for permissible limits to govern the presence of microbes in foods and feedstuffs **(De Jong *et al.*, 2013)**.
The presence of microbes in foods is tested analytically by through different protocols accepted by the international bodies. The emergence of lab based technologies such as PCR (Polymerase Chain Reaction), HPLC (High performance Liquid Chromatography), ELISA (Enzyme Linked Immunosorbent Assay), flow cytometry, and biosensors have made it easier for us to identify and quantify the pathogens. Progress has also been made in determining the presence of toxins produced by various bacteria in foods. Bacterial microbes in a food sample are measured in CFU (Colony Forming Units)/ml or CFU/gm. The permissible limit of *E. coli* 0157 is 1000 CFU/g in the ready to eat foods such as vegetables and fruits **(McGrath *et al.*, 2012)**.

Active Packaging and Techniques

Active Packaging, a significant system of actively functioning packaging for foods, results in an extended shelf life for upkeep of the freshness of food, seek information regarding food quality, enhancement of food safety, and the convenience in shipping and transport of food. It is also linked to the smart packaging and intelligent packaging with dire importance for short lived and demanded refrigerated fresh foods **(Dainelli *et al.*, 2008)**. The technical approach is to consider a contact between package film and food along with specific internal atmospheric gases **(Kour *et al.*, 2013)**. This packaging system is gold standard and is being improved day by day due to advancements in packaging, material science, biotechnology, and new customer demands **(Kinsey, 2001)**.

In the U.S, actively packaged foods are termed as 'ESL' or extended shelf life refrigerated foods **(Holley and Patel, 2005)**. Actively packaged foods include conventional products such as luncheon meat and cured meats, partially processed refrigerated foods such as seafood, egg, meat, vegetable salads, fresh pasta and pasta sauces, high moisture fruits, and vegetables **(Vanderroost *et al.*, 2014)**. Active packaging is the sum of interacting factors occurring between foods products and intrinsic environment within the food packages, with the goal of increasing shelf life of food products **(Vercammen *et al.*, 2012)**. These factors exhibit great ability towards the removal of excess gases, absorption of excess moisture; introduction of antimicrobial substances directly into the matrix of the packaging material, highly monitored release of anti-oxidants minerals, and the control of vitamin activity since fresh foods respire and have microbial activity **(Garcia-Lomillo, 2014)**.

Some basic technologies used in active food packaging involves the control of temperature, irradiation, chemical treatment of food, modified atmosphere packaging (MAP), and the controlled atmosphere packaging (CAP) **(Caleb *et al.*, 2012)**. The MAP and CAP systems of packaging are accomplished either in completely taped up warehouses, shipping tanks, or even in an individual package. Different atmospheric factors are controlled and modified in a combination by vacuum puling linked to internal atmosphere. Certain conditions are met through regulation of the level of ethylene, increase in level of carbon dioxide from 0.03% up to 3-5%, and the lowering of oxygen level from normal 21% to 2-3%. However, the limitations associated with these technologies are risks of explosion and dehydration. In commercial processes, chilling treatment is given before filling the cans with food **(Rigaux *et al.*, 2014)**.

Later they are sealed and then gas generators are used to provide a controlled atmosphere. It is mostly done for fruits to extend shelf life. Some sellers have also manufactured portable units for shipping short shelf lived fruits. For example, polar-stream shipping system use liquid nitrogen (LN_2) to blush out and to keep transport vehicles cool for ensuring fresh production **(James *et al.*, 2015)**. Advancement in CAP storage is to use selectively permeable packaging films for maintaining specific inside atmosphere in required composition which is practiced at the harvest level **(Labuza and Breene, 1989).**

Smart Packaging

Intelligent packaging is a package function which switches on and off spontaneously with respect to the changes in environmental conditions and hence gives the idea about the status of the product to the customers or end users **(Butler, 2001)**. There are several chemical sensors and biosensors which have been used over several decades with their applications in various areas including food technology. Use of such sensors in the food packaging has resulted in a new type of technology called smart or intelligent packaging. This technology consists of multidisciplinary systems that require the expertise from different fields like chemistry, biochemistry, biotechnology, physics and food science and technology. Smart packaging monitors the food quality and safety till its consumption by utilizing various chemical or biosensor. These sensors can monitor food quality and safety, such as its freshness, microbial contamination, leakage, carbon dioxide level, oxygen level, pH, time or temperature. Thus, smart packaging can be considered as a system that helps in monitoring the conditions of packaged food during its storage, transport and distribution to provide the information about its quality **(Park *et al.*, 2015)**. In general, the term can be used for features concerning about product identity, its authenticity and traceability and theft protection as well as quality and safety related issues.

Smart packaging is different from active packaging in many ways as shown in the table 1. In case of active packaging the package functions get activated in response to some triggering event i.e. exposure to ultraviolet radiations, decrease in pressure etc., and the process continues unless the product is protected while in case of smart packaging intimate food quality is monitored by a variety of sensors. The main focus in case of active packaging is to prevent the product from deterioration or spoilage. While in case of smart packaging major goal is to inform the buyer or consumer about the product quality packaged inside.

Table 1 Model of the Packaging Functions, Source: Yam *et al.,* 2005

Active packaging	Smart packaging
Contain anti-microbial component	Have time and temperature indicators
Ethylene scavenging occurs	Contain microbial spoilage sensors/indicators
Automated heating or cooling processes	Physical shock indicators are present
Moisture absorbing	Have allergen sensor
Odor and flavor absorbing mechanisms	Leakage indicator
Oxygen scavenging occurs	Microbial growth sensors
Spoilage retarders are present	Pathogens and contaminants sensors/indicators

There are varieties of sensors and indicators which are used to monitor the food quality like TTIs (Time Temperature Indicators), ripeness indicators, chemical sensors, biosensors and RFID (Radio Frequency Identification Tags). Some of them are not commercialized yet but the most common among them are TTIs and RFID **(Heising *et al.*, 2014)**. TTIs play a critical role in measuring the safety and quality of a food product. They monitor the food quality and communicate the consumer whether the food product is safe to intake or not. This becomes extremely important when food is stored in conditions other than the recommended conditions for that particular food item.

For a food item that is recommended to be frozen TTI indicates if the food had been improperly placed under high temperature along with the duration of exposure and vice versa **(Pavelková, 2013)**. RFID on the other hand assist in the wireless monitoring of the packaged food items through various tags, readers, and by using computer systems. It is widely used on the industrial scale due to its numerous and wide range applications. It provides the facility to trace the packaged food items as well as it helps in improving the productivity of supply chains. Further improvements in RFID and its integration with food science are still require in order to develop smart food packaging for food safety **(Potyrailo *et al.*, 2012)**.

Antimicrobial Packaging and Efficacy

Active packaging, comprising of antimicrobial packaging, interrelates well with the product and space among food and packaging **(Zhou *et al.*, 2014)**. Antimicrobial packaging through antimicrobial agents have several types including the addition of pouches or pads, covering and adsorbing antimicrobials on polymer planes, holding antimicrobials onto polymer surfaces by ion or covalent bonds, and the utilization of polymers with the naturally occurring antimicrobial agents **(Rhim *et al.*, 2013)**.

Comprehending the technique of adding pouches or pads first, they are present at the bottom, either tightly attached or loosely bound at any interior of the package **(Wani *et al.*, 2015)**. They have addition of volatile antimicrobial chemicals into packages; or the addition of non-volatile antimicrobial chemicals directly into the polymers. Used in mainly three forms, usually oxygen absorber, moisture absorber, and vapor generators, they have several other characteristics. Oxygen absorber reduces the oxidation and growth of aerobic microbes; whereas, the moisture absorber decreases water content lower than that required by the microbes specially molds **(Erkmen, 2012)**. The vapor generators can either be of ethanol or other organic acids. These vapors accumulate to all free space and also inhibit microbial growth **(Rooney, 1995)**.

Secondly, most of the food spoilage occurs due to surface contamination, which gets inhibited by the addition of antimicrobial compounds like lactoperoxidases, lactoferrins, cecropins, hydroquinones, and metals such as copper which causes disruption. A well-off example also includes the synthesis of microbial enzyme which inhibits their growth **(Pereira de Abreu *et al.*, 2012)**.

Thirdly, there may be adsorption or coverings of antimicrobials in the polymer packages to prevent microbes. When polymers are subjected to very high temperatures, there are antimicrobial agents which cannot withstand high temperature during the formation. So, they are adsorbed onto the polymers after the heating process. Agents like these involve nisin methylcellulose coverings for polyethylene films or the nisin zein coats **(Appendini and Hotchkiss, 2002)**. Pretreatment of polymer structures before coating or adsorption increases the adsorption power. Moreover, NaOH treated films not only increases its adsorption capacity but they also have top inhibitory result against molds **(Weng *et al.*, 1999)**.

Fourthly, there may be antimicrobials bound with polymers through ionic and covalent bonds. There is an interaction between polymers and the antimicrobials which requires functional groups on antimicrobial agent and the polymers. These functional groups make a unique boding pattern with each other whereby which the antimicrobial agent becomes stuck to polymer surfaces. Antimicrobials having functional groups could be peptides, enzymes, polyamines, and organic acids **(Basterrachea *et al.*, 2015)**. Polymers having functional groups could be acetyl butyl or propyl. Binding may require a spacer molecule which links the polymer's exterior to the antimicrobials. These spacers help sufficient liberty of motion so that active part of agent can interact with microorganisms in the food. Spacers could be dextrans, ethylenediamine, or polyethylene Glycol (PEG). Reduction in antimicrobial activity could be possible due to charged protein or peptide configuration or denaturalization due to other components **(Lopez-Rubio *et al.*, 2004)**. Protection of active sites and introduction of dendrites to increase surface area of package are some good remedies for higher microbial control. Some examples include covalently immobilized chitinase, Lysozyme, or both, used against Gram positive bacteria **(Appendini and Hotchkiss, 1997).**

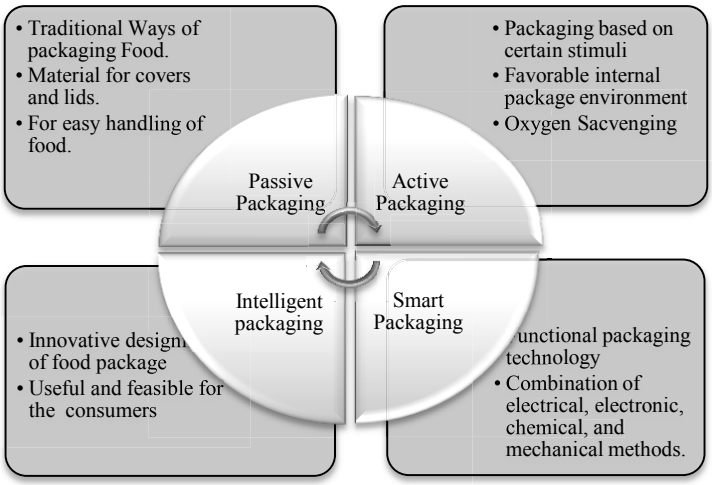

Figure 1 Distinctive types of Food Packaging Source

Significant food packaging parameters

There are many biological, physical, and chemical factors which directly affect the food properties. Chemical treatment is usually carried out for the shelf life extension of fruits and vegetables which involves spraying plant surface with hormones like gibberellins, auxins, and cytokines before harvesting to regulate ripening and other effects **(Vermeiren, 2003)**. However, chemicals used must get approval under Food and Drug Administration (FDA) regulations. Antimicrobial sprays, washes, fumigants like sulfur dioxide, and wraps that have been impregnated with antimicrobials to control rotting are also practiced. The use of carbon dioxide and chlorine dioxide gases for some foods is also mentioned **(Marsh, 2007)**.

Irradiation is another important parameter in which low dosage (<0.1 M Rad=1KGy=100kRad) of radiations can be used for shelf life extension of fresh foods. Fresh foods may always not be unprocessed. They may include fruits and vegetables and mushrooms capable of growing and maturing further but can be irradiated to inhibit these processes **(Siegrist, 2007)**. FDA regulated that any irradiated food must be labeled "treated by gamma rays or electrons" and there should be an international symbol for food products that undergo radiation treatment **(Moura *et al.*, 2004)**. Ultra violet radiations can also be used but only in wavelength range of 2200-3000nm with no ozone production. Subsequently, high fat foods must be treated under vacuum or inert gas to limit surface microbial growth. Chemical system also involves factors like control of carbon dioxide, ethylene, and water activity along with chemical agents, antimicrobial preservatives, and temperature **(Han, 2003)**.

Fresh salads and prepared foods require shelf life extension of minimum a week which can be accomplished by controlling the temperature. The United States mostly uses temperature control techniques to extend shelf life of fresh salads and prepared foods **(Norton and Sun, 2008)**. The use of open refrigerated cabinets should be prevented because they can cause rapid decay and can allow pathogens to grow. So closed refrigerated cabinets coupled with time temperature integrators to monitor distribution are mandatory for usage **(LeBail *et al.*, 2002)**.

Biological parameters includes enzyme systems such as glucose oxidase used to oxidize glucose, whereas, alcohol oxidase utilized to oxidize ethanol. These systems involve controlled oxidation of reduced iron, photo catalysis with a dye, or the catalytic conversion of oxygen to water vapors by platinum in presence of hydrogen gas **(Hodges and Forney, 2000)**. Physical parameters include the usage of appropriate packaging material for different food products as well as absorption and adsorption through scavenging **(Siracusa *et al.*, 2008)**.

Packaging of Meat, Fish, and Fruits

Meat is one of those food products which require special packaging since it has short shelf life and in turn requires great attention. In its packaging, either absorbing pads or films with adsorbed antimicrobial agents are used. Absorbing pads makes use of organic acids or surfactants incorporated to avert microbial growth **(Hansen *et al.*, 1989)**. In case of antimicrobial films, there's adsorption of antimicrobial agents onto those films. Most films are prepared by polysaccharides, lipids, and proteins due to their advantages like biodegradability, endurance of physical stress, biocompatibility, and barrier properties against oxygen, edibility or aesthetic appearances **(Vasconez *et al.*, 2008)**. Films for meat products are also prepared due to its usefulness in many aspects. They may help lessen the problem of water loss during storing of frozen meats, hold fluid of new meat cuts when packed in selling plastic dishes, and decreased lipid oxidation processes which leads to rancidity **(Cutter, 2006)**.

There may be reduction of tan coloration by myoglobin oxidation, decrease in load of decay and pathogen microbes on the exterior of covered meats, and it may also restrict volatile flavor loss and external odor pick-up. **Ming *et al.*, (1997)** highlighted complete protection against *Listeria monocytogenes* in ham, beef or turkey breast obtained using nisin or pediocin immobilized on a cellulose casing. The package is a polymer film having heat resistant Pediococcus-derived bacteriocin synergistic to chelating agent to kill *L. monocytogenes* in food**.** Besides, in fish packaging, the initial quality of fish and the conditions in which it is stored, determines its shelf life. Empirical shelf life models have been suggested for initial product quality **(Tittlemier *et al.*, 2007)**. These models are not based on information of spoilage actions but beneficial shelf life estimation can be gained from time temperature profiles or quick methods of early product condition **(Koutsoumanis *et al.*, 2000)**.

Role of ethylene, oxygen, and other scavengers

There are many ways to remove undesirable substances present at the head-space of packaged food products. The top priority includes scavenge of oxygen, ethylene, carbon dioxide, and undesirable odors. Ethylene is absorbed onto oxidizing agents or organometallic substrates for antimicrobial activity. Ethylene is a growth hormone released by climacteric fruits during metabolism. It stimulates ripening and senescence. This in turn leads to fruit spoilage. So there is a need to control ethylene pressure as well **(Lopez-de-Dicastillo *et al.*, 2010)**.

Oxygen scavenging from the package within inner atmosphere involves the lowering of metabolism rate with reduced oxygen pressure for extended shelf life of food with only an exception of growth of anaerobic bacteria. However, growth of aerobic bacteria and molds can be prevented just like meat pigments exhibit purple color under low oxygen pressure and red under high oxygen pressure **(Brewer, 2004)**. Modified atmospheric packaging through vacuum conditions is commonly implied. Oxygen scavengers prevent rancidity, discoloration, loss of flavor, and loss of nutritional value. These scavengers are selected on the basis of greater absorption, non-toxicity, low cost, and great rate or absorption. Since oxygen scavenging sachets can be dangerous to human health so nowadays these oxygen removing components are being introduced in the films, crown corks, labels, and liners of packaging materials **(Lanciotti, 2004)**.

In many foods carbon dioxide is produced as a result of respiration and deterioration reactions. Increased pressure of this gas can cause package to burst. Carbon dioxide scavengers are henceforth used to omit excess gas through the usage of sachets. Many unlikely odors get trapped inside food packages and get released when packages are opened by the consumers. Activated carbon and silica gels are used as a remedy against such odors **(Skandamis, 2002).**

Use of Bio-based composites in food Packaging

Bio-based materials, derived from renewable resources like starch and other polymeric structures, are categorized according to the method by which they are produced. It may include production of the polymers from natural resources like starch, cellulose and wheat gluten from plants. Chemical synthesis of renewable bio-derived sources includes polylactate, a biopolyester produced by the polymerization of lactic acid, whereas, lactic acid is itself produced by the fermentation process of carbohydrate feedstock **(Hunjanen *et al.*, 1996)**. Polymer synthesis of bio-based materials is carried out with the help of microorganisms or genetically modified organisms. The best known polymers synthesized by this method are polyhydroxyalkonates, specifically polyhydroxy-butyrates, hydroxyl-valerate and hydroxy butyrate **(Siracusa, 2008)**. Polymers are being used directly or indirectly for packaging purposes.

Presently, cellulose is one of the bio-based materials being used for the exterior packaging layer in the form of paper and cardboard. Again, paper has limited advantages due to its poor water resistivity and therefore, can only be used for the packing material of dry products. In future, bio-based materials will be used as packaging material, because of its several benefits on mineral oil derived polymers **(Farris *et al.*, 2009)**. The food packaging industries are trying to produce such bio-based materials for food packaging as are more durable and resistant to environmental conditions such as water, pH, and temperature with better shelf life of product **(Weber *et al.*, 2002)**.

Bio-based materials should be stable, without any changes in its physical, mechanical and barrier properties. These materials must have efficient biodegradability which is the degradation of packaging material with the help of microbes, done either aerobically or anaerobically, after its disposal. The natural polymeric materials vary in their process of degradation while some proteins are considered to be non-degradable according to some definitions **(Cooke, 1990)**. Parameters affecting the stability of biodegradable material include water activity, oxygen, nutrients, pH, temperature, and storage time. Dry products can efficiently be stored for longer period of time; whereas, moist products have limited storage time **(Miller and Krochta, 1997)**. Before using bio-based materials for food packaging, its effect on food quality as well as on food safety must be examined.

For an improved mechanical strength of bio-based packaging of food, natural polymeric material is mixed with synthetic or chemically modified polymers **(Guilbert and Gontard, 1997)**. Recently hybrid organic and inorganic materials are used especially those which have silicates layer dispersed in polymeric matrix at nano-metric level **(Giannelis, 1996)**. These nano-hybrid composites are responsible of improved mechanical and oxidation stability, decreased solvent

uptake, self-extinguishing behavior and biodegradability. In addition, inorganic particles can impart different properties like color and odors and also act as reservoirs. Researchers are working with the objective to improve delivery methods of medicines or micronutrients in daily foods by making tiny edible capsules, or nano-particles that release their contents on demand at infected spots in the body. Nano-composites offer extra benefits to the packaging like low density, transparency, good flow, better surface properties and recyclability **(Koo *et al.,* 2005)**.

Future concepts of food Packaging

Many novel techniques apart from sterilizing and pasteurizing include new methods which give protection more than that obtained by inactivation **(Barbosa-Canovas *et al.,* 2008)**. A few techniques from a whole big list of new methods comprise of electric discharges of high voltage, ionization radiation, high light intensity, high hydrostatic pressure, ultrasonication through high heat and pressure, and the addition of bacteriocins **(Urzica, 2004)**. Researchers are now focusing on those procedures of food delivery which are of higher quality, free from additives but have natural composites, and are nutritionally healthier **(Gould, 2000)**. Of many novel packaging materials, cellulose based filter paper, graft copolymerized, along with silver nanoparticles has been studied for better antimicrobial safety of food especially against *E.coli* bacteria. **(Tankhiwale *et al.,* 2009)**.

RESULTS AND DISCUSSION

Antimicrobial preservation of foods

The ever increasing population has raised the demand for food to a dangerously higher level. Better and carefully selected packaging material with the use of latest technologies can save tons of food which is otherwise wasted every year due to improper packaging. To overcome the issues, companies have emerged in a competition to provide best food products and have raised great concern for a biotechnologist towards quality control. These companies have made use of various techniques to upgrade and maintain the nutritional quality of food products, and have devised efficient food packaging methods. It has been observed that safe packaging requires various composites incorporated in the polymers to prevent microbes. The most efficient methods to reduce microbes involve the control of biological, chemical, and physical parameters through active packaging. According to the findings, one of the most efficient packaging systems is the usage of bio-composites for bio-based packaging leading towards reduced microbial activity.

Selection of efficient packaging material

For better control and reduction of microbes, several packaging materials are being made for a variety of food stuffs. Figure 2 shows the estimated production of packaging materials in tons through a specific period, from 1960 to 2005. Based on these statistics it was inferred that the most abundantly used materials are paper and paperboard materials and that there are more packaging materials designed for non-durable materials than durable ones. Rigid packaging materials are least produced whereby which it can be inferred that flexible materials including polymers with films are more preferably being manufactured than the rigid ones for the antimicrobial packaging.

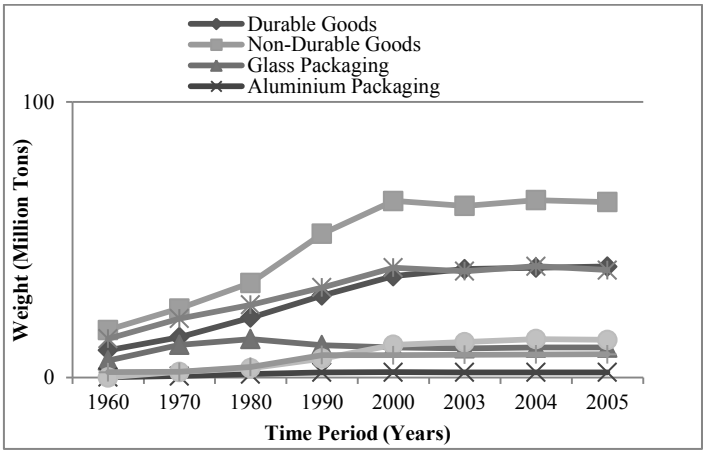

Figure 2 Weight of the Packaging Material Generated from 1960 to 2005. Source: (EPA 2006).

Intelligent Packaging Systems

Active and intelligent packaging systems require special treatments with specific substances. These treatments have great applications towards the preservation of a variety of foods as demonstrated in (Tab 2).

Table 2 Packaging systems and its applications

Microbial Species	Packaging Requirement & Treatment	Applications
Bacteria	BHT, BAH, and Tocopherol.	Dried and sacked food products are protected by special packaging conditions including flour and rice.
	Organic acids including sorbic acid and enzymes including lysozyme.	Meat and poultry products are well preserved.
Moulds	Ascorbic acid.	Maintain quality and freshness of vegetables and fruits.
	Sodium hydrogen carbonate.	Useful for fish, poultry, and meat department.
Yeast	Mixture of water and ethanol absorbed on SiO_2 powder	Preservation of bakery products.
	Ethanol vapors.	Preservation of dry fish.
Infectious Microbes	Preservative agents including bacteriocins, organic compounds, and inorganic compounds.	Reduced reductive and oxidative discoloration
	Control of humidity and water activity through adsorbents.	To maintain crispiness of food
Toxicogenic Microbes	Control of microstructure.	Reducing the movement of compounds with low MW.
	Vacuum modified atmospheric packaging.	To prevent oxidative rancidity
Other Growth Spoilage Microbes	Aluminum and stainless depositions on polyester films.	Foods subjected to refrigeration have good shelf life.
	Aluminum and stainless depositions on paperboard.	Ready to eat foods like popcorns and pizzas are preserved.

Legend: BHT – Butylated hydroxytoluene, BHA – Butylated hydroxyanisole, and MW – Molecular weight.
Source: (Tian *et al.,* 2013; Cooksey, 2005; Vermeiren *et al.,* 1999; and Kruijf *et al.,* 2002)

CONCLUSION

Microorganisms require specified parameters for their growth and development to cause the disease and effect the system and for those different requirements like carbon sources, moisture content, vitamins, and other important metabolites are to be controlled. By reducing one of these factors or optimum conditions we can reduce the microbial activity. Environment is the major factor for introducing the microbial activity in the container or the store of food. Through active packaging these microbes can be reduced or eliminated; however, it is important to choose best packaging material and antimicrobial control technique in

accordance to the type of food. Bio-based material packaging is now on the rise in food packaging industries due to its advantageousness.

Acknowledgment: We would like to thank Syeda Anum Zahra for assisting us with all our queries relevant to this review paper and for her valuable comments which significantly improved the manuscript. We would also pay thanks to the reviewers, for their great insight and time towards reviewing this manuscript.

REFERENCES

Appendini, P., & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. *Innovative Food Science & Emerging Technologies*, *3*(2), 113-126. http://dx.doi.org/10.1016/s1466-8564(02)00012-7

Appendini, P., & Hotchkiss, J. H. (1997). Immobilization of lysozyme on food contact polymers as potential antimicrobial films. *Packaging Technology and Science*, *10*(5), 271-279. http://dx.doi.org/10.1002/(sici)1099-1522(199709/10)10:5%3C271::aid-pts412%3E3.3.co;2-i

Barbosa-Cánovas, G. V., & Juliano, P. (2008). Food sterilization by combining high pressure and thermal energy. In *Food engineering: Integrated approaches* (pp. 9-46). Springer New York. http://dx.doi.org/10.1007/978-0-387-75430-7_2

Bastarrachea, L. J., Denis-Rohr, A., & Goddard, J. M. (2015). Antimicrobial food equipment coatings: Applications and challenges. *Annual review of food science and technology*, *6*, 97-118. http://dx.doi.org/10.1146/annurev-food-022814-015453

Brewer, S. (2004). Irradiation effects on meat color–a review. *Meat Science*,*68*(1), 1-17. http://dx.doi.org/10.1016/j.meatsci.2004.02.007

Butler, P. (2001). Smart packaging–intelligent packaging for food, beverages, pharmaceuticals and household products. *Mater World*, *9*(3), 11-13. http://dx.doi.org/10.1533/9780857098979.261

Caleb, O. J., Opara, U. L., & Witthuhn, C. R. (2012). Modified atmosphere packaging of pomegranate fruit and arils: a review. *Food and Bioprocess Technology*, *5*(1), 15-30. http://dx.doi.org/10.1007/s11947-011-0525-7

Cho, I. H., & Irudayaraj, J. (2013). Lateral-flow enzyme immunoconcentration for rapid detection of Listeria monocytogenes. *Analytical and bioanalytical chemistry*, *405*(10), 3313-3319. http://dx.doi.org/10.1007/s00216-013-6742-3

Cooke, T. F. (1990). Biodegradability of polymers and fibers-A review of the literature. *Journal of polymer engineering*, *9*(3), 171-212. http://dx.doi.org/10.1515/polyeng.1990.9.3.171

Cooksey, K. (2005). Effectiveness of antimicrobial food packaging materials.*Food additives and contaminants*, *22*(10), 980-987. http://dx.doi.org/10.1080/02652030500246164

Cutter, C. N. (2006). Opportunities for bio-based packaging technologies to improve the quality and safety of fresh and further processed muscle foods.*Meat science*, *74*(1), 131-142. http://dx.doi.org/10.1016/j.meatsci.2006.04.023

da Cruz Cabral, L., Pinto, V. F., & Patriarca, A. (2013). Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods. *International journal of food microbiology*, *166*(1), 1-14. doi:10.1016/j.ijfoodmicro.2013.05.026

Dainelli, D., Gontard, N., Spyropoulos, D., Zondervan-van den Beuken, E., & Tobback, P. (2008). Active and intelligent food packaging: legal aspects and safety concerns. *Trends in Food Science & Technology*, *19*, S103-S112. http://dx.doi.org/10.1016/j.tifs.2008.09.011

Davidson, P. M., & Critzer, F. M. (2012). Interventions to inhibit or inactivate bacterial pathogens in foods. In *Microbial Food Safety* (pp. 189-202). Springer New York. doi:10.1016/S0168-1605(03)00370-2

De Jong, A., Thomas, V., Klein, U., Marion, H., Moyaert, H., Simjee, S., & Vallé, M. (2013). Pan-European resistance monitoring programmes encompassing food-borne bacteria and target pathogens of food-producing and companion animals. *International journal of antimicrobial agents*, *41*(5), 403-409. http://dx.doi.org/10.1016/j.ijantimicag.2012.11.004

Dhama, K., Rajagunalan, S., Chakraborty, S., Verma, A. K., Kumar, A., Tiwari, R., & Kapoor, S. (2013). Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: A review.*Pakistan Journal of Biological Sciences*, *16*(20), 1076. http://dx.doi.org/10.3923/pjbs.2013.1076.1085

Marsh, K., & Bugusu, B. (2007). Food packaging—roles, materials, and environmental issues. *Journal of food science*, *72*(3), R39-R55. http://dx.doi.org/10.1002/0471743984.vse2979

Erkmen, O. (2012). 3 Modified-Atmosphere Storage of Foods. *Progress in food preservation*, 49. http://dx.doi.org/10.1002/9781119962045.ch3

Farris, S., Schaich, K. M., Liu, L., Piergiovanni, L., & Yam, K. L. (2009). Development of polyion-complex hydrogels as an alternative approach for the production of bio-based polymers for food packaging applications: a review. *Trends in food science & technology*, *20*(8), 316-332. http://dx.doi.org/10.1016/j.tifs.2009.04.003

García-Lomillo, J., González-SanJosé, M. L., Del Pino-García, R., Rivero-Pérez, M. D., & Muñiz-Rodríguez, P. (2014). Antioxidant and antimicrobial properties of wine byproducts and their potential uses in the food industry.*Journal of agricultural and food chemistry*, *62*(52), 12595-12602. http://dx.doi.org/10.1021/jf5042678

Gorrasi, G. (2007). Andrea Sorrentino*, Giuliana Gorrasi and Vittoria Vittoria.*Trends in Food Science & Technology*, *18*, 84e95. http://dx.doi.org/10.1002/adma.19960080104

Gould, G. W. (2000). Preservation: past, present and future. *British Medical Bulletin*, *56*(1), 84-96. http://dx.doi.org/10.1258/0007142001902996

Guilbert, S., Cuq, B., & Gontard, N. (1997). Recent innovations in edible and/or biodegradable packaging materials. *Food Additives & Contaminants*,*14*(6-7), 741-751. http://dx.doi.org/10.1080/02652039709374585

Han, J. H. (2003). Antimicrobial food packaging. *Novel food packaging techniques*, 50-70. http://dx.doi.org/10.1533/9781855737020.1.50

Hansen R., Rippl C., Midkiff D., & Neuwirth J. Ž1989. Antimicrobial absorbent food pad. US Patent 4 865 855.

Heising, J. K., Dekker, M., Bartels, P. V., & Van Boekel, M. A. J. S. (2014). Monitoring the quality of perishable foods: opportunities for intelligent packaging. *Critical reviews in food science and nutrition*, *54*(5), 645-654. http://dx.doi.org/10.1080/10408398.2011.600477

Hodges, D. M., & Forney, C. F. (2000). The effects of ethylene, depressed oxygen and elevated carbon dioxide on antioxidant profiles of senescing spinach leaves. *Journal of Experimental Botany*, *51*(344), 645-655.http://dx.doi.org/10.1093/jexbot/51.344.645

Holley, R. A., & Patel, D. (2005). Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiology*, *22*(4), 273-292. http://dx.doi.org/10.1016/j.fm.2004.08.006

Hujanen, M., & Linko, Y. Y. (1996). Effect of temperature and various nitrogen sources on L (+)-lactic acid production by Lactobacillus casei.*Applied microbiology and biotechnology*, *45*(3), 307-313. http://dx.doi.org/10.1007/s002530050688

James, C., Purnell, G., & James, S. J. (2015). A Review of Novel and Innovative Food Freezing Technologies. *Food and Bioprocess Technology*,*8*(8), 1616-1634. http://dx.doi.org/10.1007/s11947-015-1542-8

Kinsey, J. D. (2001). The new food economy: consumers, farms, pharms, and science. *American Journal of Agricultural Economics*, *83*(5), 1113-1130. http://dx.doi.org/10.1111/0002-9092.00259

Koo, O. M., Rubinstein, I., & Onyuksel, H. (2005). Role of nanotechnology in targeted drug delivery and imaging: a concise review. *Nanomedicine: Nanotechnology, Biology and Medicine*, *1*(3), 193-212. http://dx.doi.org/10.1016/j.nano.2005.06.004

Kour, H., Wani, N. A. T., Malik, A., Kaul, R., Chauhan, H., Gupta, P., ... & Singh, J. (2013). Advances in food packaging–a review. *Stewart Postharvest Review*, *9*(4), 1-7. http://dx.doi.org/10.2212/spr.2013.4.7

Koutsoumanis, K. P., Taoukis, P. S., Drosinos, E. H., & Nychas, G. J. E. (2000). Applicability of an Arrhenius model for the combined effect of temperature and CO2 packaging on the spoilage microflora of fish. *Applied and environmental microbiology*, *66*(8), 3528-3534. http://dx.doi.org/10.1128/aem.66.8.3528-3534.2000

Kruijf, N. D., Beest, M. V., Rijk, R., Sipiläinen-Malm, T., Losada, P. P., & Meulenaer, B. D. (2002). Active and intelligent packaging: applications and regulatory aspects. *Food Additives & Contaminants*, *19*(S1), 144-162. http://dx.doi.org/10.1080/02652030110072722

Labuza, T. P., & Breene, W. M. (1989). APPLICATIONS OF "ACTIVE PACKAGING" FOR IMPROVEMENT OF SHELF-LIFE AND NUTRITIONAL QUALITY OF FRESH AND EXTENDED SHELF-LIFE FOODS 1. *Journal of Food Processing and Preservation*, *13*(1), 1-69. http://dx.doi.org/10.1111/j.1745-4549.1989.tb00090.x

Lanciotti, R., Gianotti, A., Patrignani, F., Belletti, N., Guerzoni, M. E., & Gardini, F. (2004). Use of natural aroma compounds to improve shelf-life and safety of minimally processed fruits. *Trends in food science & technology*,*15*(3), 201-208. http://dx.doi.org/10.1016/j.tifs.2003.10.004

LeBail, A., Chevalier, D., Mussa, D. M., & Ghoul, M. (2002). High pressure freezing and thawing of foods: a review. *International Journal of Refrigeration*, *25*(5), 504-513. http://dx.doi.org/10.1016/s0140-7007(01)00030-5

López-de-Dicastillo, C., Gallur, M., Catalá, R., Gavara, R., & Hernandez-Muñoz, P. (2010). Immobilization of β-cyclodextrin in ethylene-vinyl alcohol copolymer for active food packaging applications. *Journal of Membrane Science*, *353*(1), 184-191. http://dx.doi.org/10.1016/j.memsci.2010.02.049

López-Malo, A., & Alzamora, S. M. (2015). Water Activity and Microorganism Control: Past and Future. In *Water Stress in Biological, Chemical, Pharmaceutical and Food Systems* (pp. 245-262). Springer New York. http://dx.doi.org/10.1007/978-1-4939-2578-0_18

Lopez-Rubio, A., Almenar, E., Hernandez-Muñoz, P., Lagarón, J. M., Catalá, R., & Gavara, R. (2004). Overview of active polymer-based packaging technologies for food applications. *Food Reviews International*, *20*(4), 357-387. http://dx.doi.org/10.1081/fri-200033462

Marsh, K., & Bugusu, B. (2007). Food packaging—roles, materials, and environmental issues. *Journal of food science*, *72*(3), R39-R55. http://dx.doi.org/10.1111/j.1750-3841.2007.00301.x

McGrath, T. F., Elliott, C. T., & Fodey, T. L. (2012). Biosensors for the analysis of microbiological and chemical contaminants in food. *Analytical and bioanalytical chemistry*, *403*(1), 75-92. http://dx.doi.org/10.1007/s00216-011-5685-9

Mauriello, G., De Luca, E., La Storia, A., Villani, F., & Ercolini, D. (2005). Antimicrobial activity of a nisin-activated plastic film for food packaging.*Letters in applied microbiology*, *41*(6), 464-469. http://dx.doi.org/10.1111/j.1472-765x.2005.01796.x

Miller, K. S., & Krochta, J. M. (1997). Oxygen and aroma barrier properties of edible films: A review. *Trends in Food Science & Technology*, *8*(7), 228-237. http://dx.doi.org/10.1006/fstl.1998.051944(97)01051-0

Ming, X., Weber, G. H., Ayres, J. W., & Sandine, W. E. (1997). Bacteriocins applied to food packaging materials to inhibit Listeria monocytogenes on meats. Journal of Food Science, 62(2), 413–415. http://dx.doi.org/10.1111/j.1365-2621.1997.tb04015.x

Moura, E. A. B., Ortiz, A. V., Wiebeck, H., Paula, A. B. A., Silva, A. L. A., & Silva, L. G. A. (2004). Effects of gamma radiation on commercial food packaging films—study of changes in UV/VIS spectra. *Radiation Physics and Chemistry*, *71*(1), 201-204. http://dx.doi.org/10.1016/j.radphyschem.2004.05.034

Norton, T., & Sun, D. W. (2008). Recent advances in the use of high pressure as an effective processing technique in the food industry. *Food and Bioprocess Technology*, *1*(1), 2-34. http://dx.doi.org/10.1533/9781855737020.1.50

Ozdemir, M., & Floros, J. D. (2004). Active food packaging technologies.*Critical Reviews in Food Science and Nutrition*, *44*(3), 185-193. http://dx.doi.org/10.1080/10408690490441578

Park, Y. W., Kim, S. M., Lee, J. Y., & Jang, W. (2015). Application of biosensors in smart packaging. *Molecular & Cellular Toxicology*, *11*(3), 277-285. http://dx.doi.org/10.1007/s13273-015-0027-1

Pavelková, A. (2013). Time temperature indicators as devices intelligent packaging. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *61*(1), 245-251. http://dx.doi.org/10.11118/actaun201361010245

Pereira de Abreu, D. A., Cruz, J. M., & Paseiro Losada, P. (2012). Active and intelligent packaging for the food industry. *Food Reviews International*,*28*(2), 146-187. http://dx.doi.org/10.1080/87559129.2011.595022

Potyrailo, R. A., Nagraj, N., Tang, Z., Mondello, F. J., Surman, C., & Morris, W. (2012). Battery-free radio frequency identification (RFID) sensors for food quality and safety. *Journal of agricultural and food chemistry*, *60*(35), 8535-8543. http://dx.doi.org/10.1021/jf302416y

Quintavalla, S., & Vicini, L. (2002). Antimicrobial food packaging in meat industry. *Meat science*, *62*(3), 373-380. http://dx.doi.org/10.1016/s0309-1740(02)00121-3

Rhim, J. W., Park, H. M., & Ha, C. S. (2013). Bio-nanocomposites for food packaging applications. *Progress in Polymer Science*, *38*(10), 1629-1652. http://dx.doi.org/10.1016/j.progpolymsci.2013.05.008

Rigaux, C., André, S., Albert, I., & Carlin, F. (2014). Quantitative assessment of the risk of microbial spoilage in foods. Prediction of non-stability at 55° C caused by Geobacillus stearothermophilus in canned green beans. *International journal of food microbiology*, *171*, 119-128. http://dx.doi.org/10.1016/j.ijfoodmicro.2013.11.014

Rooney, M. L. (1995). Overview of active food packaging. In *Active food packaging* (pp. 1-37). Springer US. http://dx.doi.org/10.1007/978-1-4615-2175-4_1

Siegrist, M., Cousin, M. E., Kastenholz, H., & Wiek, A. (2007). Public acceptance of nanotechnology foods and food packaging: The influence of affect and trust. *Appetite*, *49*(2), 459-466. http://dx.doi.org/10.1016/j.appet.2007.03.002

Siracusa, V., Rocculi, P., Romani, S., & Dalla Rosa, M. (2008). Biodegradable polymers for food packaging: a review. *Trends in Food Science & Technology*, *19*(12), 634-643. http://dx.doi.org/10.1016/j.tifs.2008.07.003

Skandamis, P. N., & Nychas, G. J. E. (2002). Preservation of fresh meat with active and modified atmosphere packaging conditions. *International journal of food microbiology*, *79*(1), 35-45. http://dx.doi.org/10.1016/s0168-1605(02)00177-0

Suppakul, P., Miltz, J., Sonneveld, K., & Bigger, S. W. (2003). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of food science*, *68*(2), 408-420. http://dx.doi.org/10.1111/j.1365-2621.2003.tb05687.x

Tankhiwale, R., & Bajpai, S. K. (2009). Graft copolymerization onto cellulose-based filter paper and its further development as silver nanoparticles loaded antibacterial food-packaging material. *Colloids and Surfaces B: Biointerfaces*, *69*(2), 164-168. http://dx.doi.org/10.1016/j.colsurfb.2008.11.004

Tian, F., Decker, E. A., & Goddard, J. M. (2013). Controlling lipid oxidation of food by active packaging technologies. *Food & function*, *4*(5), 669-680. http://dx.doi.org/10.1039/c3fo30360h

Tittlemier, S. A., Pepper, K., Seymour, C., Moisey, J., Bronson, R., Cao, X. L., & Dabeka, R. W. (2007). Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging. *Journal of agricultural and food chemistry*, *55*(8), 3203-3210. http://dx.doi.org/10.1021/jf0634045

Urzica, A. C. (2004). High hydrostatic pressure inactivation of Bacillus subtilis var. niger spores: the influence of the pressure build-up rate on the inactivation. http://dx.doi.org/10.1007/978-3-662-05613-4_47

Vanderroost, M., Ragaert, P., Devlieghere, F., & De Meulenaer, B. (2014). Intelligent food packaging: The next generation. *Trends in Food Science & Technology*, *39*(1), 47-62. http://dx.doi.org/10.1016/j.tifs.2014.06.009

Vasconez, M. B., Flores, S. K., Campos, C. A., Alvarado, J., & Gerschenson, L. N. (2009). Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings. *Food Research International*, *42*(7), 762-769. http://dx.doi.org/10.1016/j.foodres.2009.02.026

Vercammen, A., Vanoirbeek, K. G., Lemmens, L., Lurquin, I., Hendrickx, M. E., & Michiels, C. W. (2012). High pressure pasteurization of apple pieces in syrup: Microbiological shelf-life and quality evolution during refrigerated storage. *Innovative Food Science & Emerging Technologies*, *16*, 259-266. http://dx.doi.org/10.1016/j.ifset.2012.06.009

Vermeiren, L., Devlieghere, F., Van Beest, M., De Kruijf, N., & Debevere, J. (1999). Developments in the active packaging of foods. *Trends in food science & technology*, *10*(3), 77-86. http://dx.doi.org/10.1016/s0924-2244(99)00032-1

Wani, A. A., Singh, P., Pant, A., & Langowski, H. C. (2015). Packaging Methods for Minimally Processed Foods. In *Minimally Processed Foods* (pp. 35-55). Springer International Publishing. http://dx.doi.org/10.1007/978-3-319-10677-9_3

Weber, C. J., Haugaard, V., Festersen, R., & Bertelsen, G. (2002). Production and applications of biobased packaging materials for the food industry. *Food Additives & Contaminants*, *19*(S1), 172-177. http://dx.doi.org/10.1080/02652030110087483

Weng, Y. M., Chen, M. J., & Chen, W. (1999). Antimicrobial food packaging materials from poly (ethylene-co-methacrylic acid). *LWT-Food Science and Technology*, *32*(4), 191-195. http://dx.doi.org/10.1006/fstl.1998.0519

Yam, K. L., Takhistov, P. T., & Miltz, J. (2005). Intelligent packaging: concepts and applications. *Journal of food science*, *70*(1), R1-R10. http://dx.doi.org/10.1111/j.1365-2621.2005.tb09052.x

Zhou, F. F., Liao, J. J., & Teng, J. (2014, November). Green Ecological Design Research of Tourism Product Packaging. In *Applied Mechanics and Materials* (Vol. 670, pp. 960-963). http://dx.doi.org/10.4028/www.scientific.net/amm.670-671.960

JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

METHOD FOR DETERMINING FRUIT HARVESTING MATURITY

*Marcin Bajcar, Bogdan Saletnik, Miłosz Zardzewiały, Barbara Drygaś, Maria Czernicka, Czesław Puchalski, Grzegorz Zaguła**

Address(es):
University of Rzeszow, Faculty of Biology and Agriculture, Department of Bioenergy Technologies, 4 Zelwerowicza St, 35-601 Rzeszow, Poland, (17) 785 50 46.

*Corresponding author: g_zagula@univ.rzeszow.pl

doi: 10.15414/jmbfs.2016.6.2.773-776

ARTICLE INFO

Received 5. 10. 2015
Revised 17. 2. 2016
Accepted 9. 5. 2016
Published 3. 10. 2016

Regular article

OPEN ACCESS

ABSTRACT

Accurate identification of fruit harvest timing is quite a complicated issue. We know several methods of determining harvest maturity in climacteric fruits; these include measurement of: firmness, total solids contents, respiration rate, ethylene emission measurement as well starch tests. On the other hand non-climacteric fruits (no collective maturity phase) are identified as suitable for harvesting by evaluating their colour. The first three aforementioned basic methods can be combined into a single tool, the so-called Streiff index. All the characteristics identified herein, i.e.: starch assay, total solids (including sugars, tannins, vitamins, organic acids, etc.), as well as firmness, corresponding to protopectin-pectin conversion, produce caloric response. Hence, the correlation between fruit maturity and the caloric response of the material provides the basis for an innovative test enabling assessment of harvest maturity in fruit. Caloric response is largely impacted by the changing structure of fruit dry mass. The calorific content of fruit during maturation process decreases due to the conversion of the water-insoluble starch, with a calorific value of 4200 cal/g, into soluble monosaccharides 3750 cal / g. The study presents a novel calorimetric method of assessing harvest maturity in fruit and the presentation is based on fruit of selected varieties of apples, tomatoes and strawberries. The calorimetric maturity thresholds have been determined for these fruits at the following levels: apples – 3930 cal/g s.m., strawberries – 3880 cal/g s.m. and tomatoes - 3910 cal/g.

Keywords: fruits, harvesting maturity, calorific value

INTRODUCTION

The timing of fruit picking (harvest maturity) significantly impacts the duration of storage and quality parameters at the time of commercial operations. To make sure fruit maintain good quality and stability in storage, they should be collected at the most accurately defined time **(Blaszczyk, 2006)**. This is also one of those factors which can be controlled with precision **(Tomala, 2002)**. In order to determine harvest maturity in apples it is necessary to use assessment methods which enable precise evaluation of their condition. Generally these methods are not readily available for fruit farmers since they involve use of costly equipment (e.g. the procedure of measuring ethylene concentration in apple core). Others require a few measurements to be performed simultaneously, e.g. concentrations of ethylene, total solids and fruit firmness **(Łysiak, 1998)**. In recent years there have been attempts to use ultrasound waves in evaluating fruit ripeness and in accessing colour changes during the process of ripening **(Mizrach *et al.* 2000)**. Yet, the methods are still not recognized as fully legitimate and reliable in this type of measurements.

Harvest maturity in fruit designated for long-term storage (6-8 months) can be most effectively determined by measuring concentration of ethylene in the fruit core **(Tomala, 1995)**. The procedure requires the use of gas chromatograph, available at specialist laboratories. Harvest time can also be predicted with the use of induced ethylene method or by determining Streif index. As reported by previous studies, at harvest Streif index should be in the range 0.9–1.1 and it should not be lower than 0.8. Many years of observations suggest that during a normal period of ripening the time needed for a change in the index from 0.9 to 0.8 is usually approx. 7 days. Even though they are accurate, these methods require costly measuring instruments **(Łysiak 1998)**.

The easily available, affordable and simple methods include the test for starch. It involves observation of starch decomposition which occurs during the maturation process in apple fruit. Research examining this phenomenon showed close relationship between proportional content of starch and the degree of fruit maturity. Special model charts, which have been developed, are by convention applied to determine the so-called starch index, a function of starch content in a fruit at the cross-section perpendicular to its centre line **(Tomala 1995)**. Dark blue complexes, which emerge as a result of reaction between iodine and starch, represent areas occupied by starch; these are subsequently compared with model photographs and rated on a scale from 1 to 10. It is difficult to explicitly identify the area occupied by starch (the value of starch index SI results from it) due to the varied starch patterns encountered in different varieties. Precision of the assay also depends on the quality of lighting applied to the examined area of the sample, as well as individual subjective perception of the person performing the test **(Peirs *et al.*, 2002)**. Application of this simplified assay procedure leads to numerous errors. Hence, it is necessary to improve accuracy and reliability of measurements by using enhanced methods of reading and calculating the value of starch index. Other well-known and commonly applied methods include examination of the fruit flesh with a manual firmness tester and determination of refractive index of the total solids **(Zagula *et al.*, 2013)**.

Less frequently applied methods include the procedure of examining the image during starch decomposition with the use of three computerized video devices **(Domagała *et al.*, 2007)**. Another method applies a combined system of colour video camera and spectrophotometer to forecast the level of sugar contents in fruits, which is a critical parameter for the level of fruit maturity. The authors suggest the two methods could be combined into one joint algorithm. The entire procedure is highly effective and time-efficient – one measurement takes only 3.5 seconds **(Steinmetz *et al.*, 1999).** Finally, the question of expanding the visual system to include bicolour fruit – measurement of colour progression in multi-colour fruit is very important, yet highly complicated given the typically available and insufficiently accurate measurement instruments such as calorimeters and chronometers. The proposed system enables observation of a wider spectrum of the colour progression in a multi-colour fruit during the entire maturation process, and consequently its classification in terms of ripeness level **(Kang *et al.*, 2008)**. There are also methods which correlate the degree of fruit maturity with their sliding force and friction force **(Puchalski, 2001).**

The aim of the study was to find the individual method to indicate the optimum date of harvesting time for selected groups of fruit on the basis of physicochemical and mechanical properties.

MATERIALS AND METHODS

The material selected for the study comprised three species of fruit, most popular in the Central European market, These were represented by ‘Jonagold’ apples, ‘Elkat’ strawberries and ‘Bonaparte’ tomatoes. Typical harvesting times, as well

as parametres for determining harvest maturity in these fruit varieties are shown in Table 1.

Table 1 Harvest maturity time and basic parameters identifying harvest maturity in fruit **(Pieniążek, 1995)**

Variety (species)	Harvest maturity timing	Total solids content, %	Firmness, N	Starch index	Fruit colour
Jonagold (apple)	20.09-10.10	11.5-12	72-78	5-7	-
Elkat (strawberry)	10-30.06	7.5-8	-	-	Entire fruit has reached the target colour
Bonaparte (tomato)	Cyclic cultivation indoors (greenhouse)	5.3-6	14-15	-	2/3 of the fruit has reached the target colour

Potential harvest maturity timing was taken into account in determining the initial stage of the observation and the timeframes of the assessment were defined for the specific fruits in the way shown in **Table 2.**

Table 2 Dates of fruit picking or assessment (in 16 repetitions).

Object of study	Date of assessment		
	1	2	3
Jonagold (apple)	20.07.2014	01.09.2014	10.10.2014
Elkat (strawberry)	20.05.2014	10.06.2014	30.06.2014
Bonaparte (tomato)	20.05.2014	10.06.2014	30.06.2014

In accordance with the design, samples were collected three times during the process of fruit maturation, starting at the early stage after the green fruit has been formed and ending at the time of the potential harvest maturity. Each time analytical tests were carried out in 16 fruits of each variety, in accordance with Student's t-test identifying the significant number of repetitions in one group of fruit:

$$r \geq 2 \cdot \left[\frac{t_\alpha \cdot V}{d}\right]^2,$$

where:
r – explored number of repetitions;
 – Student's t-value at the significance level p = 0.95, equals 2,030;
d [%] – assessment precision reflected by the standard deviation in measurement results acquired in a series of repetitions of the same material – equal 2%;
V [%] – value of variability index for 35 pieces of fruit in a test sample – equal 2.7%.
The analytical tests conducted at each stage were designed to identify the level of fruit maturity based the following factors:
- contents of water, ash and volatile substances measured with the use of TGA 701 thermogravimetric analyser LECO, in compliance with standards: (PN-90/A-75101:2003); (PN-EN 1135:2002) ; (PN-C-04708-3: 1997).
- calorific value of dry matter with the use of AC 500 bomb calorimeter LECO, according to standard: PN-EN 14918:2010(U).
- the value of total soluble solids, identified by means of ATAGO refractometer (PN-90/A-75101:2002)
To achieve this the homogenized raw material was subjected to drying in a laboratory dryer, at the temperature of 105 °C, and then refined in a ball grinder and pelleted by means of Laormann apparatus. Calorific value was determined by incinerating the sample in oxygen atmosphere, in bomb calorimeter placed in water.
Statistical analyses of the obtained results were conducted by means of Statgraphics 4.1 Plus. Mean values were compared using Student's t-test and Duncan's test at the level of significance p = 0.05 for n = 16.

RESULTS AND DISCUSSION

Correlation tables were generated for the consecutive samples, to present the assessed analytical parameters (Tables 3-5).

Table 3 Results of analytical tests, with standard deviations, for the consecutive assessments of Jonagold apple variety

Date of test	Total solids, %	Content of water, %	Content of ash, %	Content of volatile substances, %
20.07.2014	$8.57^{a} \pm 0.04$	$82.45^{a} \pm 0.19$	$0.53^{b} \pm 0.07$	$0.73^{a} \pm 0.15$
01.09.2014	$9.80^{b} \pm 0.13$	$82.71^{a} \pm 0.14$	$0.44^{b} \pm 0.05$	$1.23^{b} \pm 0.08$
10.10.2014	$11.50^{c} \pm 0.07$	$82.67^{a} \pm 0.10$	$0.26^{a} \pm 0.01$	$3.81^{c} \pm 0.38$

Legend: the same letters (for each parameter) show there are no statistically significant differences at $p = 0.05$

In course of fruit and vegetable ripening, the quantity of monosaccharides in cell sap increases; these dissolve in water contributing to increased total soluble solids **(Dominguez *et al.*, 2012).** Statistically significant changes were observed in the value of total solids in Jonagold apple variety. During the maturation process the value of this parameter increased by nearly 3%, which is linked with the formation of simple sugars such as fructose and glucose in the fruit. Similarly, the contents of volatile substances, fragrances and essential oils showed a tendency for growth during fruit maturation. A comparison of the initial and final stage of the observation showed an over 5-fold increase in these rates. An opposite tendency was observed in the case of changes in the contents of ash, resulting from the processes of natural mineralization. On the other hand no statistically significant differences were identified in water content in the fruit. Apples at the final stage of fruit set, and at harvest maturity contain similar amount of water, oscillating around 82%. Because cells strive to achieve balance between osmotic pressure and emerging structural stresses the content of water may slightly change in course of ripening **(Frankel *et al.*, 2012).**

Table 4 Results of analytical tests (mean ± standard deviations) for the consecutive assessments of Elkat strawberry variety

Date of test	Total solids, %	Content of water, %	Content of ash, %	Content of volatile substances, %
20.05.2014	$5.87^{a} \pm 0.2$	$92.71^{a} \pm 0.05$	$0.32^{a} \pm 0.01$	$1.36^{a} \pm 0.08$
10.06.2014	$6.60^{b} \pm 0.07$	$92.66^{a} \pm 0.02$	$0.28^{a} \pm 0.03$	$1.64^{b} \pm 0.03$
30.06.2014	$7.30^{c} \pm 0.04$	$92.31^{a} \pm 0.15$	$0.29^{a} \pm 0.01$	$1.95^{c} \pm 0.03$

Legend: the same letters (for each parameter) show there are no statistically significant differences at $p = 0.05$

Statistically significant changes were observed in the value of total solids in Elkat variety of strawberries. During the maturation process the value of this parameter increased nominally by approx. 1.5%. Pineli et al. **(2011)** demonstrated that the change of green into red colour during vegetation of strawberries was a result of 2% increase in soluble solids. Similarly, the contents of volatile substances, fragrances and essential oils showed a tendency for growth during fruit maturation and reached the nominal value of 0.5%. On the other hand no statistically significant differences were identified in the contents of water and ash in the fruit. These parameters amounted to approx. 92% and 0.3%, respectively. According to Frankel and Hartman **(2012)** contents of water in strawberry and tomato prior to their harvesting (green fruit) changed only by 0.02% by the time the fruit turned red.

Table 5 Results of analytical tests (mean ± standard deviations) for the consecutive assessments of Bonaparte tomato variety

Date of test	Total solids, %	Content of water, %	Content of ash, %	Content of volatile substances, %
20.05.2014	4.53 [a] ± 0.04	93.12 [a] ± 0.15	0.43 [a] ± 0.01	0.88 [a] ± 0.06
10.06.2014	4.83 [b] ± 0.04	94.27 [a] ± 0.05	0.40 [a] ± 0.01	0.92 [b] ± 0.07
30.06.2014	5.3 [c] ± 0.04	94.47 [a] ± 0.06	0.45 [a] ± 0.01	0.96 [c] ± 0.02

Legend: the same letters (for each parameter) show there are no statistically significant differences at $p = 0.05$

Statistically significant changes were observed in the value of total solids in Bonaparte variety of tomatoes. During the maturation process the value of this parameter nominally increased to over 1%. According to Toor et al. **(2006)**, the contents of total solids in tomatoes depended mainly on cultivars. During the ripening process the amount of soluble substances increases nearly two-fold. During growth of tomato fruit there is also an increased proportion of sugars **(Dominguez *et al* 2012)**. Similarly, the contents of volatile substances, fragrances and essential oils showed a tendency for growth during fruit maturation, to finally reach the threshold value of 1%. On the other hand no statistically significant differences were identified in the contents of water and ash in the fruit. During the final stage these parameters reached the values of 94.5% and 0.45% respectively. Musse *et al.* **(2008)** reported that in the case of tomatoes changes in water content during the process of ripening was not statistically significant. These authors noticed that during vegetation, after eighteen days, before harvest, the content of moisture was at the level of 95.6% and two days before potential harvest it decreased to 95.0%.

The following results (**Figure 1 and 2**) show the trend in the changes of the calorific value of dry matter, which mainly consists in simple sugars, and starch, pectins, organic acids and pigments, i.e. these ingredients which, after the total moisture content has been evaporated, enter the matrix and produce calorific response during the test.

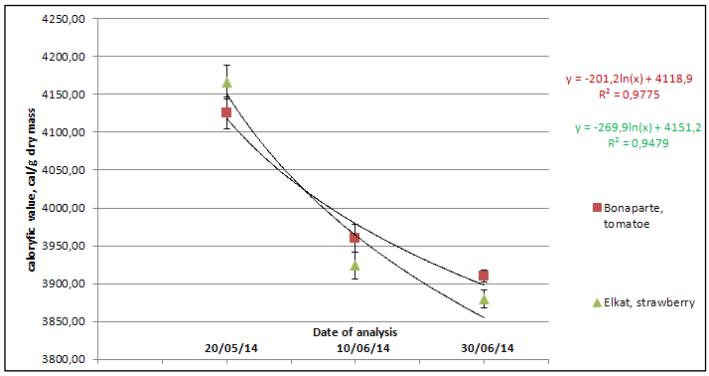

Figure 1 Changes in the calorific value of tomatoes and strawberries dry matter during consecutive analyses (for dates of analysis see Table 2).

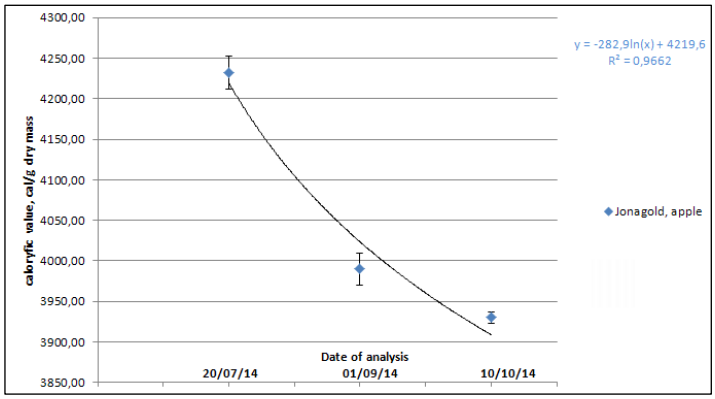

Figure 2 Changes in the calorific value of apples dry matter during consecutive analyses (for dates of analysis see Table 2).

It was observed during the consecutive analytic trials that the calorific value of the fruit dry matter tended to decrease from over 4100 cal/g for each fruit to the level of 3900 cal/g. Given the constant rates for moisture content in the specific fruits, shown in Tables 3-5, we can postulate there is a general tendency showing changes in the calorific value in fruit, calculated for the fresh matter. The calorific value decreased so visibly that it can be concluded the differences between the specific dates of assessment were statistically significant, at the significance level of 0.95. The flattening trend lines show that the decrease in the calorific value is gradually inhibited in the examined fruit; this might in fact indicate the optimum harvest maturity in these fruits.

As it was already mentioned, the changes in the fruit calorific value are mainly impacted by the caloric response of such components of the dry matter as simple sugars, and starch, fruit acids, pectins, and pigments. Each of these components contributes its unique energy value, which is characteristic for each group of components (**Tabel 6**). The highest calorific value is a characteristic of fats, found in fruits in trace amounts only; the prevailing components are starch (in immature fruits) with calorific value of approx. 4200 cal/g, and monosaccharides (at the later stage) with the calorific value of approx. 3950 cal/g.

Table 6 Calorific value of the main components of fruit dry matter **(Merrill *et al.*, 1973)**

Nutrient	Calorific value, cal/g
Starch	4200
Monosaccharides (fructose, glucose)	3950
Pectins	5890
Proteins	5000
Fats	9000
Organic acids	4200
Pigments	4200

Moreover, the dominant components of fruit dry matter include sugars (in the examined fruits sugars account for 92-98% of the dry matter), and at the initial stage, when the fruit is immature – starch, which later during the process of ripening is transformed into simple sugars. Calorific value of starch is higher than that of simple sugars (**Table 6**), therefore the present findings simply reflect the changes occurring in the fruits. More calorific starch is transformed into less calorific monosaccharides, and as a result the calorific value of the fruit decreases. Hence, it is possible to identify the moment when the calorific value of fruit dry matter is approx. 3900 cal/g, i.e. when starch has mostly broken down into glucose (possibly to be further transformed into fructose), which might be the way to determine the optimum timing for fruit harvest. In the case of apples the calorific value should be slightly higher than in tomatoes or strawberries, due to the fact that the latter are harvested at the time they are ready to for consumption. Unlike these, apples of Jonagold variety and other fruit of this type achieve ripeness for consumption during a prolonged process of storage.

Given the above, an application was filed to patent „The method of determining harvest maturity in fruit", which was approved by the Patent Office, and marked with symbol P.406446.

CONCLUSION

Authors created new method and new tool useful for determination of harvest date or maturity stage It seems important to create a table of calorific values for fruits in their harvest maturity time for each of their species and varieties

Acknowledgments: The study was carried out in the framework of the research project: „Innovation-oriented University of Rzeszów" No. 2/IUR/KI/2012 entitled: "Advanced calorimetric method of determining harvest maturity in fruits".

REFERENCES

Błaszczyk, J. (2006). Wpływ terminu zbioru na właściwości przechowalnicze jabłek odmiany 'Topaz'[Impact of harvest timing on storage properties of Topaz variety apples]. *Zeszyty naukowe instytutu sadownictwa i kwiaciarstwa.* 14, 87-93.

Domagała, D., Guz, T. (2007). Zastosowanie wybranych metod analizy obrazu podczas obserwacji rozpadu skrobi w jabłkach [Application of selected methods of image analysis during observation of starch decomposition in apples]. *Inżynieria Rolnicza,* 5 (93), 81-87.

Dominguez, E., Fernaindez, F., Carlos, J., Hernaindez, L., Perez, J. (2012). Tomato fruit continues growing while ripening, affecting cuticle properties and cracking. *Physol. Plant.,* 146, 473–486. http://dx.doi.org/10.1111/j.1399-3054.2012.01647.x

Frankel, CH., Hartman, T.G. (2012). Decrease in fruit moisture content heralds and might launch the onset of ripening processes. *Growing Systems. J Food Sci,.* 77(10), 365–376. http://dx.doi.org/10.1111/j.1750-3841.2012.02910.x

Kang, S.P., East, A.R., Trujillo F.J. (2008). Colour vision system evaluation of bicolour fruit. *Postharvest Biology and Technology*, 49. 152-155. http://dx.doi.org/10.1016/j.postharvbio.2007.12.011

Łysiak, G. (1998). Wstępna ocena pięciu metod określania terminu dojrzałości zbiorczej jabłek [Initial assessment of five methods of determining harvest maturity in apples]. XXXVII Polish Conferences, Skierniewice, 296-299

Merrill, A.L., Watt, B.K. (1973). Energy value of foods. *Agriculture Handbook,* 74, 3.

Mizrach,, Flitzanov, U., Akerman, M., Zauberman, G. (2000). Monitoring avocado softening in low temperature storage using ultrasonic measurements. *Computers and Electronics in Agriculture*, 26, 199-207. http://dx.doi.org/10.1016/s0168-1699(00)00072-7

Musse, M., Quellec, S., Lambert, M., Devaux, M., Lahaye, M., Mariette, F. (2008). Monitoring the postharvest ripening of tomato fruit using quantitative MRI and NMR relaxometry. *Post. Biol. Technol.,* 53, 22–35. http://dx.doi.org/10.1016/j.postharvbio.2009.02.004

P.406446. Zaguła, G., Puchalski, Cz., Czernicka, M. (2013). Sposób określania dojrzałości zbiorczej owoców [Method of determining harvest maturity in fruit]. *Biuletyn Urzędu patentowego RP*.

Peirs, A., Scheerlinck, N., Perez, A. B., Jancsók, P., Nicolai, B. M. (2002). Uncertainty analysis and modelling the starch index during apple fruit maturation. *Postharvest Biology and Technology*, 26, 199-207. http://dx.doi.org/10.1016/s0925-5214(02)00038-8

Pieniążek, S.A. (1995). Sadownictwo [Fruit farming]. Warszawa, ISBN 83-09-01622-0

Pineli, L.P., Moretti, C.L., Santos, M.S., Campos, A.B., Brasileiro, A.V., Cordova, A.C., Chiarello, M.D. (2011). Antioxidants and other chemical and physical characteristics of two strawberry cultivars at different ripeness stages. *J Food Comp Analysis,* 24, 11–16. http://dx.doi.org/10.1016/j.jfca.2010.05.004

PN-90/A-75101: (2003). Fruit and vegetable. Determination of dry weight by weight (Polish standard).

PN-C-04708-3 : (1997). Food colors. Determination of volatiles (Polish standard).

PN-EN 1135: (2002). Fruit and vegetable juices. Determination of ash content (Polish standard).

PN-EN 14918: (2010). Solid biofuels - Determination of calorific value (Polish standard).

Puchalski, C. (2001). Metodyczne aspekty badania tarcia i jędrności jabłek pod kątem ich jakości [Methodology aspects of assessing friction and firmness in apples from the point of view of their quality]. *Rozprawy Akademii Rolniczej im. H. Kołłątaja w Krakowie*, 275, 53-54.

Steinmetz, V., Roger, J.M., Molto, E., Blasco, J. (1999). On-line fusion of colour camera and spectrophotometer for sugar content prediction of apples. *J. Agric. Engng Res.,* 73, 142-155. http://dx.doi.org/10.1006/jaer.1999.0407

Tomala, K. (1995). Prognozowanie zdolności przechowalniczej i określenie terminu zbioru jabłek [Forecasting storage capacity and determining harvest maturity in apples].

Tomala, K. (2002). Dlaczego termin zbioru owoców jest taki ważny [Why is harvest timing so important] *Owoce, warzywa, kwiaty*. 16, 24-26.

Toor, R.K., Savage, G.P., Lister, P.D. (2006). Seasonal variations in the antioxidant composition of greenhouse grown tomatoes. *Food Comp. Anal*., 9, 1–10. *XXXVII Ogólnopolska Naukowa Konferencja Sadownicza [37th Polish Conference on Fruit Growing]* . Skierniewice, Wydawnictwo ISiK, 296-299. http://dx.doi.org/10.1016/j.jfca.2004.11.008

Zaguła, G., Puchalski, CZ. (2013). Zmiany glukozowo – fruktozowe w jabłkach poddanych oddziaływaniu stałych i wolnozmiennych pól magnetycznych [Changes in glucose and fructose contents of apples exposed to constant and slowly changing magnetic fields]. *Żywność. Nauka. Technologia. Jakość*, 2 (87), 162 – 172. http://dx.doi.org/10.15193/zntj/2013/87/162-172

JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

EFFECT OF GAC FRUIT POWDER ON QUALITY AND NITROSATION ACTIVITY OF MEAT PRODUCT

Pasakorn Uearreeloet[1], *Nattaya Konsue**[1]

Address(es): Nattaya Konsue,
[1]Program in Food Technology, School of Agro-Industry, Mae Fah Luang University, 57100, Chiang Rai, Thailand, +66-53916738.

*Corresponding author: nattaya.kon@mfu.ac.th

doi: 10.15414/jmbfs.2016.6.2.786-790

ARTICLE INFO

Received 30. 4. 2016
Revised 13. 5. 2016
Accepted 12. 7. 2016
Published 3. 10. 2016

Regular article

OPEN ACCESS

ABSTRACT

Nitrite is important for meat product quality especially for color stabilization. However, toxicity of nitrite according to nitrosation reaction raises interest of many researchers. To improve the quality and safety of meat product, nitrite was substituted with freeze dried gac fruit aril (GP). Frankfurters were produced with the combination of 50-150 mg/kg sodium nitrite and 0-4% GP and stored at 4˚C. Color, pH and lipid oxidation (thiobarbituric acid reactive substances, TBARS) were determined weekly for 4 weeks whereas sensorial qualities were evaluated after cold storage at 4˚C for one day. Aqueous determination of N-nitrosodimethylamine (NDMA) content was carried out by high-performance liquid chromatography (HPLC) at day zero before and after a two-step *in vitro* digestion. The results showed that replacement of nitrite with GP caused slightly increase in pH of frankfurter in a concentration dependent manner and falling of pH values were observed after 21 days storage. TBARS values also elevated but only when the highest level of GP (4%) was employed and storage time reached 28 days. Moreover, GP resulted in declining of L* (lightness) and greater of a* (redness) and b* (yellowness) values of frankfurter which these were paralleled with increasing of color acceptability score evaluated by panelists. These color values were unchanged throughout storage period at 4% GP content. Finally, NDMA only occurred after *in vitro* digestion and tended to increase in the presence of GP. It can be concluded that GP successfully improved color stability and acceptability of frankfurter. However, over dose of GP incorporation (4%) led to increasing of lipid oxidation and nitrosation reaction.

Keywords: Gac, *In vitro* digestion, Nitrosamine, Meat products, Lipid oxidation, TBARS

INTRODUCTION

Momordica Cochinchinensis Spreng is commonly known as gac. It is originated in Southeast Asia and most typical in Myanmar, Thailand, Laos, Bangladesh, Malaysia, Philippines, and Vietnam. All parts of the plant have attracted interest of researchers in recent years and are used for medicinal and nutritional purposes. The young fruit is rich in vitamin C, calcium, and fiber whereas gac aril of fully mature fruit contains superior amount of lycopene, flavonoid, and phenolic compounds and, therefore, high in antioxidant activities (**West and Poortvliet, 1993**). Being reported as a rich source of lycopene, compared to many fruits and vegetables especially tomato (**Kubola and Siriamornpun, 2011**), gac aril has intense red color which makes it a promising natural food colorant. Gac oil powder could be successfully incorporated into food products such as yogurt, pasteurized milk and cake to improve color retention, β-carotene and lycopene content (**Kha *et al.*, 2015**). Moreover, lycopene is a potent antioxidant and plays an important role in prevention of many diseases such as cancer, cardiovascular diseases as well as modulation of immune system (**Aust *et al.*, 2003**).
The reaction of nitrite with pigment in meat forms stable red color which is essential to consumer likeliness. The European Food Safety Authority (EFSA) stated that addition of nitrite is necessary to inhibit pathogenic microorganisms in particular *Clostridium botulinum* and the maximum level is 150 mg/kg (**FAO/WHO, 1996**). However, nitrite or nitrogen oxides can react with secondary amines in meat to form nitrosamine, the reaction of which is known as nitrosation. A multistep process of nitrosation includes nitrous acid (HNO_2) production by the addition of a hydrogen ion to the nitrite ion in acidic condition of gastric cavity following with water molecules removed to form an ultimate toxicant, nitrosonium ion ($N{\equiv}O^+$). Nitrosamine has shown toxicological significance in human by formation of DNA adducts and its carcinogenic effect has been well established. Ohshima and Bartsch (**Ohshima and Bartsch, 1999**) demonstrated that suitable condition of nitrosation rather involved with many factors including pH, amine substrate and nitrosating agent. Color is an important factor for the customer acceptance in meat-based food, therefore, many researchers have studied the stability of color of fresh and processed meat and optimized the use of nitrite in order to maintain good color and enhance product safety (**Feiner, 2009**). Inhibition of nitrosation has been reported; the addition of nitrosation inhibitor such as ascorbic acid, isothiocyanate and tocopherols to the curing mixture has resulted in significant reduction in the levels of nitrosamine (**Biaudet *et al.*, 1996**). Meat products contain high lipid content that makes it prone to lipid oxidation. Not only primary cause of flavour changes, lipid oxidation is considered as an important factor for color and nutritive value alteration in meat products as well as generation of toxic and carcinogenic compounds (**Jensen *et al.*, 1998**). Control of oxidation in meat product has become increasingly important to prevent quality deterioration especially during chilling storage of processed meat.
Therefore, this study was aimed to evaluate the possibility of gac aril powder application as a natural colorant in meat products and its effects on product quality. Moreover, degree of nitrite substitution on nitrosamine content in meat products before and after enzymatic *in vitro* digestion was also determined.

MATERIAL AND METHODS

Chemicals and reagents

Pancreatin, pepsin, N-nitrosodimethylamine, trichloroacetic acid (99%), 1,1,3,3 tetramethoxypropane (99%), 2-Thiobarbituric acid (98%), HPLC grade methanol and acetonitrile were obtained from Sigma-Aldrich (Singapore). HPLC grade water was acquired from Milli-Q water purification system (Millipore, Bedford, USA.).

Freeze dried gac fruit aril preparation

Fully mature gac fruits were purchased from local farm in Chiang Rai, Thailand. Gac arils were removed from the seeds and milled in a blender to obtain homogenous aril. The aril paste was freshly frozen at -40 ˚C. Then under freeze-drying system at 10^{-2} Pa, the frozen aril was dried overnight to obtain pure gac aril powder. The powder was blended and sieved to achieve homogenous fine powder. Gac fruit powder (GP) was stored in a cool dry place for further utilization.

Frankfurter preparation

Production of frankfurters was carried out according to Laemthong Food Products Co. Ltd. procedure. The frankfurters were divided into 9 treatments with different levels of nitrite (50, 100, and 150 mg/kg) and freeze dried gac fruit aril (0, 2, and 4 g/100 g). Briefly, lean pork (80 % w/w) and pork fat (20 % w/w) purchased from local market were chilled and finely chopped in a bowl chopper at the holding temperature below 5ºC prior to all ingredients were added including nitrite and GP. Liquid smoke at 75 ºC for 30 min was employed after casing in a 10 cm long with 3 cm diameter following with cooking in 100 ºC water bath until internal temperature reached 80 ºC. Cooked frankfurters were instantaneously cooled down in ice-cold water and vacuum packed. Samples were stored at 4 ˚C and taken at day 0, 7, 14, 21, and 28 for physicochemical analysis. Sensory evaluation was performed at one day after storage.

Color measurement

Determination of color was carried out as described previously (**Eyiler and Oztan, 2011**). Redness (a*), yellowness (b*), and lightness (L*) of inner cuts of samples were achieved by using Hunter Lab Color Quest XE (Hunter Associates Laboratory, Inc., USA.).

pH measurement

A 10-g of sample was homogenized at 5,000 rpm with 50 mL of distilled water. The homogenate was filtered through a filter paper and then pH of sample was assessed by using Digital pH-meter.

Determination of thiobarbituric acid reactive substances (TBARS)

TBARS assay was slightly modified as described previously (**Meineri *et al.*, 2013**). Samples (10 g) were homogenized with 10% trichloroacetic acid (20 mL). The homogenate was centrifuged at 600 rpm for 5 min at controlled temperature of 4 ºC. The supernatant was subsequently filtered through Whatman No.1 filter paper. To 1 mL of filtrate, 1 mL of a 0.02 M 2-thiobarbituric acid solution (TBA) was added prior to heating up in boiling water for 20 min. The pink color of sample was developed according to the reaction of TBA and oxidation products and was spectrophotometric measured at 538 nm. The content of malondialdehyde (MDA) was expressed as mg MDA/kg calculated using calibration curve of 1,1,3,3-tetramethoxypropane standard.

***In vitro* digestion**

The method for *in vitro* digestion was modified from **Boisen and Fernández (1997)**. The two steps digestion mimicking the protein digestion processes in the stomach and small intestine was carried out. Firstly, 0.1 M of pH 6.0 phosphate buffer was added into the flask containing ground sample (0.5 g). The mixture was continuously mixed using magnetic stirrer. A-10 mL HCl (0.2 M) was added prior to the pH adjusted to 2.0. The combination was digested for 2 h at 39 ºC with pepsin (25 mg/mL) in the presence of 0.5 mL of 0.5% chloramphenicol in ethanol. Subsequently, 10 mL of 0.2 M phosphate buffer (pH 6.8) and 5 mL of 0.6 M NaOH solution were incorporated and pH was adjusted to 6.8. Finally, pancreatin (100 mg/mL) was added and left for 4 h at the same temperature. The complete digestion samples were further subjected to N-nitrosodimethylamine analysis.

Determination of N-nitrosodimethylamine

N-nitrosodimethylamine (NDMA) in samples was analyzed by HPLC (HPLC Waters Alliance 2695 with PDA and ELSD) equipped with reverse phase C18 column (150 mm x 3.9 mm, 5 µm particle size) according to **Krauss and Hollender (2008)** with some changes. A gradient mobile phase was adopted where ratio of 0.1 mM ammonium acetate/methanol at 85/15 was increased to 0/100 in 18 min. Injection volume of sample was 20 µL and mobile phase flow rate was 100 µL/min. Standard curve was produced employing NDMA standard solutions at a concentration range of 0-10 mg NDMA/kg.

Sensory evaluation

Sensory evaluation was carried out on day 1 of refrigerated storage using the 9-point Hedonic Scale with 20 panelists. Frankfurters were heated by steaming and halved before serving with 3 digit random numbers. Four attributes i.e. color, texture, odor, and taste were evaluated in the numerological scale, 1 and 9 referred to dislike extremely and like extremely, respectively. Overall acceptability served as indication of preference by the panel, but it was not used to infer consumer acceptance.

Statistical analysis

Three replicates were used in the experiment. Factorial design was employed and analysis of various (ANOVA) of the obtaining data were analyzed where factors that significantly different between means were determined using Duncan's new multiple range test. Differences were considered significant at the $p<0.05$ level.

RESULTS AND DISCUSSION

Effects of nitrite and GP on pH and color

Table 1 shows the effect of nitrite and GP on pH of frankfurter during storage period (0, 7, 14, 21, and 28 days). The results illustrated that nitrite alone led to similar pH values of frankfurter at day 0 being 6.28, 6.09 and 6.23 at 50, 100 and 150 ppm of nitrite, respectively. These results are in agreement with previous report by Deda and colleagues (**Deda *et al.*, 2007**). However, considering increasing the level of GP, it can be seen that pH value increased in concentration dependent manner with increasing levels of GP, especially when 100 and 150 ppm nitrite were combined with GP. This is thought to be a result of high pH of gac aril powder. The pH values of samples were quite stable during day 0 to day 14 but markedly decreased after two weeks (Table 1). Chilling storage at 4 ºC is designed to maintain microbiological quality of processed meat where the shelf life of which is generally up to 14 days. Spoilage microorganisms, especially lactic acid producing bacteria, grow slowly and falling of pH observed after this period. Among different nitrite levels, 150 ppm maintained pH at higher level compared to 50 and 100 ppm after 21-day storage. Similarly, it was previously reported that sodium nitrite could inhibit growth of lactic acid bacteria isolated from vacuum-packed cooked ring sausages, the effect was very limited at 50 and 100 ppm but strong inhibition was observed at the dose as high as 400 ppm (**Korkeala *et al.*, 1992**).GP showed little effect on pH values. Inhibition of pH changes were obtained when nitrite was added at high level.

Color L*, a*, and b* values of samples were affected by both nitrite and GP content (Table 2). At high concentration of nitrite, lightness (L*) increased whereas that was reduced in the presence of higher GP level. The a* (redness) and b* (yellowness) values, on the other hand, increased significantly with increasing GP content. These results indicate that the color of meat product is more red-yellow in the presence of GP. This was not surprising because GP is a rich source of lycopene compared to other natural sources, 70 times more than tomato (**Ishida *et al.*, 2003**). Tonucci and colleagues reported, in the tomato pulp powder, that lycopene is the predominant carotenoid responsible for the red color of the powder and its derived products (**Tonucci *et al.*, 1995**). However, the added GP increased b* value which inferred that the products was more likely in orange color. The orange color developed is possibly a result of reaction between fat and lycopene which may turn lycopene from a reddish color into yellow as also reported by **Hayes *et al.* (2013)**. Moreover, following 28-day storage there were obvious changes of lightness (L*), redness (a*) and yellowness (b*) of frankfurter in the samples containing low levels of GP (0 and 2%) whereas no color change was recorded in 4% GP incorporated samples. This infers that GP improved color stability of frankfurter throughout the shelf life of product. Though natural colorant is readily for degradation, lycopene is lipid soluble pigment that considerably highly stable. It is well established that the most important factors in food processing that cause degradation of lycopene are heat, light and oxygen (**Shi *et al.*, 1999**). During storage of frankfurter, destruction of lycopene was retarded because oxygen was excluded under vacuum packaging and temperature was well controlled at 4 ºC.

Effects of nitrite and GP on lipid oxidation

Addition of nitrite at low levels did not affect lipid oxidation of frankfurter as indicated by TBARS values ($p > 0.05$) (Table 3). Lin and colleagues (**Lin *et al.*, 2011**) reported, in different meat product, that nitrite led to a significant reduction of TBARS values. Study in Italian sausage, **de Oliveira *et al.* (2011)** found that without antioxidant incorporated, TBARS values of samples decreased when 100 mg/kg nitrite was added. However, at 200 mg/kg nitrite, adverse effect was noted especially when nitrite was combined with antioxidant. It was suggested that the antagonistic effect observed was due to an interaction between nitrite and chemical compounds present in natural antioxidant derived from plant extract. Similarly, addition of GP tended to increase the TBARS values at the highest level studied (Table 3). The negative effects of GP on lipid oxidation are also presumably attributed to high fatty acid content of gac aril in combination with polyunsaturated fatty acids in the muscle and tissues (**Buckley *et al.*, 1989**). Chemical composition of gac aril has been well established. The gac fruit aril contains 22% fatty acid by weight which composes of 32% oleic, 29% palmitic, and 28% linoleic acids (**Ishida *et al.*, 2003**). From the composition of gac aril, it can be said that high fatty acid may be one of the causes of oxidation. Moreover, significant amount of lycopene in gac fruit aril might also be the case since it has been reported that 20 µg/mL lycopene is a pro-oxidant of triglycerides (**Haila *et al.*, 1996**). However, all treatments had TBARS value less than 2 mg MDA/kg up to 28 days of storage. According to **Greene and Cumuze (1982)**, the minimum TBARS value which causes off-flavor in meat products is 2 mg MDA/kg.

Therefore, it is important to point out that GP, at the range of study, showed no major effect on lipid oxidation and as a result, no effect on sensory attribute was observed. Moreover, the TBARS values were only slightly changed until 21 days of storage and a significant increase was detected at 28-days ($p<0.05$).

Table 1 Effect of nitrite and GP on pH of frankfurter products

Factors		pH				
Nitrite (ppm)	**GP(g/100g)**	**0 d**	**7 d**	**14 d**	**21 d**	**28 d**
50	0	6.28 ± 0.13^{bcB}	6.47 ± 0.02^{bA}	6.70 ± 0.09^{aAB}	5.43 ± 0.14^{bcC}	4.83 ± 0.04^{bD}
	2	6.55 ± 0.07^{aA}	6.21 ± 0.01^{eB}	5.67 ± 0.06^{dC}	5.28 ± 0.09^{cD}	4.56 ± 0.04^{eE}
	4	6.28 ± 0.12^{bcA}	6.32 ± 0.03^{dA}	6.15 ± 0.04^{bB}	5.26 ± 0.06^{cC}	4.71 ± 0.02^{cdD}
100	0	6.09 ± 0.14^{dA}	5.89 ± 0.07^{fB}	5.48 ± 0.01^{eC}	4.97 ± 0.02^{dD}	4.23 ± 0.05^{gE}
	2	6.30 ± 0.02^{bcA}	6.35 ± 0.06^{cdA}	6.02 ± 0.01^{cB}	4.86 ± 0.64^{dC}	4.58 ± 0.05^{eD}
	4	6.41 ± 0.11^{abcB}	6.67 ± 0.04^{aA}	6.21 ± 0.04^{bB}	5.45 ± 0.23^{bcC}	4.93 ± 0.02^{aD}
150	0	6.23 ± 0.10^{dAB}	6.40 ± 0.01^{bcA}	6.14 ± 0.05^{bB}	5.74 ± 0.23^{aC}	4.77 ± 0.06^{bcD}
	2	6.30 ± 0.010^{bcB}	6.47 ± 0.04^{bA}	6.17 ± 0.03^{bC}	5.56 ± 0.09^{abD}	4.42 ± 0.06^{fE}
	4	6.43 ± 0.01^{abA}	6.43 ± 0.01^{bcA}	6.01 ± 0.09^{cB}	5.62 ± 0.25^{abC}	4.66 ± 0.03^{dD}

^{abc}Mean values in the same column bearing different superscripts are significantly different ($p<0.05$).
^{ABC} Mean values in the same row bearing different superscript are significantly different ($p<0.05$).

Effects of nitrite and GP on N-nitrosodimethylamine

Nitrite is converted to nitric oxide when antioxidant such as ascorbic or caffeic acid is incorporated hence formation of nitrosamine is limited. The findings in the current study demonstrated that NDMA was not detected before *in vitro* digestion application (Table 4) implying that formation of NDMA requires the acid condition of gastric as reported by Meineri and colleagues (**Meineri *et al.*, 2013**). However, prior to gastric digestion system, NDMA was generated. As expected, increasing level of nitrite led to elevation of NDMA content in samples ($p< 0.05$). Surprisingly, the addition of GP tended to increase NDMA value, however, without statistical significance ($p>0.05$). Similar results were reported by **Biaudet *et al.* (1996)**. It was suggested that nitrogen oxide, a product from reduction of nitrite, can further react with unsaturated lipids and results in transnitrosation of secondary amine. Therefore, in a corporation with high fatty acid content of GP, nitrite enhanced NDMA formation. In addition, **Combet *et al.* (2007)** also reported the results of *in vitro* assay that ascorbic acid inhibited NDMA formation by 5 folds in aqueous solution. However, addition of 10% lipid converted ascorbic acid from inhibiting to promoting nitrosation. This may be explained by nitric oxide, formed by ascorbic acid, can regenerate nitrosating species by reacting with oxygen in the lipid phase. Moreover, it can be seen that increasing of nitrosamine content in the presence of antioxidant was concentration dependent (Table 4). **Walter *et al.* (1976)** reported that N-nitrosopyrrolidine (NPYR) in fried bacon was suppressed when ascorbate was incorporated at a low concentration of 300 mg/kg while adverse effect was observed at the higher level. Obviously, high concentration of antioxidant leads to increasing production of nitric oxide and simultaneously promote the reaction with unsaturated lipids and as a result secondary amine reaction increased.

Sensory evaluations of frankfurter products

The results of sensory evaluation (Table 5) showed that nitrite and GP had a marked significant effect ($p<0.05$) on color of products whereas taste, texture and overall acceptance were slightly different and no effect on odor was observed. Addition of GP increased acceptability of color and texture but slightly lower the score of product taste ($p<0.05$). The results of greater color acceptability as affected by GP is inferring that the red-yellow color of frankfurters was preferable. However, the taste of the frankfurter, on the other hand, was lower when 4% GP was incorporated and the results indicated that taste of frankfurter affected overall acceptability the most, as can be seen from the highest and lowest overall acceptability were noted in the sample that had the highest and lowest taste score, respectively. The reason for lower taste score in the presence of GP was thought to be due to the unique flavor of gac aril which might interact with flavoring agents added into frankfurter according to standard recipe. However, this could be easily overcome by adjusting the content of herbs and spices.

Table 2 Effect of interaction between nitrite and GP on color parameters (L*, a* and b*) of frankfurter products

Factors		L*		a*		b*	
Nitrite (mg/kg)	**GP(g/100g)**	**0 d**	**28 d**	**0 d**	**28 d**	**0 d**	**28 d**
50	0	58.69 ± 1.31^{c}	58.72 ± 0.70^{ans}	6.53 ± 0.24^{e}	$4.87\pm0.88^{e*}$	10.74 ± 0.69^{e}	$12.03\pm0.41^{c*}$
	2	55.23 ± 0.48^{d}	55.24 ± 1.13^{bns}	19.58 ± 0.61^{b}	$17.59\pm0.47^{b*}$	23.74 ± 0.94^{b}	23.20 ± 0.22^{bns}
	4	53.12 ± 1.19^{e}	51.71 ± 1.20^{cns}	23.04 ± 0.75^{a}	22.87 ± 1.72^{ans}	24.92 ± 0.86^{b}	26.00 ± 1.39^{ans}
100	0	63.05 ± 1.05^{b}	$60.86\pm1.04^{a*}$	5.56 ± 0.13^{e}	$5.79\pm0.28^{e*}$	10.93 ± 0.46^{e}	11.91 ± 0.36^{cns}
	2	56.23 ± 0.73^{d}	55.20 ± 1.73^{bns}	18.26 ± 0.33^{c}	18.95 ± 0.50^{bns}	22.33 ± 1.17^{c}	23.40 ± 0.91^{bns}
	4	52.99 ± 0.70^{e}	53.78 ± 2.07^{bcns}	23.71 ± 0.83^{a}	22.03 ± 2.02^{ans}	26.46 ± 1.04^{a}	24.64 ± 1.08^{abns}
150	0	65.79 ± 1.03^{a}	$60.61\pm0.32^{a*}$	5.72 ± 0.19^{e}	5.87 ± 0.22^{cns}	8.25 ± 0.08^{f}	$10.68\pm0.41^{c*}$
	2	58.74 ± 1.19^{c}	$54.08\pm1.22^{bc*}$	12.99 ± 0.53^{d}	$17.19\pm1.54^{b*}$	17.63 ± 0.86^{d}	$24.39\pm1.58^{ab*}$
	4	54.94 ± 0.65^{d}	53.30 ± 2.43^{bcns}	22.88 ± 0.91^{a}	22.26 ± 3.08^{ans}	25.10 ± 1.33^{b}	26.17 ± 1.84^{ans}

^{abc} Mean values in the same column bearing different superscripts are significantly different ($p<0.05$).
*=Mean values are significant different between 0 and 28-day ($p<0.05$).
ns=non-significant difference between 0 and 28-day

Table 3 Effect of nitrite and GP on TBARS value (mg MDA/kg sample) in frankfurter products

Factors		TBARS (mg MDA/kg)				
Nitrite (mg/kg)	GP(g/100g)	0 d	7 d	14 d	21 d	28 d
50	0	0.99±0.11^{cB}	1.13±0.12^{cB}	1.02±0.02^{eB}	1.27±0.23^{dB}	2.23±0.04^{cdA}
	2	1.48±0.06^{bD}	1.77±0.02^{abC}	1.25±0.09^{cdeE}	1.98±0.15^{bB}	2.08±0.01^{dA}
	4	1.86±0.20^{aCD}	2.08±0.06^{aBC}	1.47±0.06^{bcD}	2.47±0.45^{aAB}	2.73±0.19^{aA}
100	0	1.10±0.01^{cC}	1.36±0.06^{bcB}	1.08±0.06^{eC}	1.35±0.02^{cdB}	2.26±0.20^{cdA}
	2	1.42±0.07^{bBC}	1.48±0.26^{bcB}	1.20±0.17^{cdeC}	1.47±0.05^{cdB}	2.34±0.05^{bcdA}
	4	1.71±0.23^{aB}	1.71±0.09^{abB}	1.70±0.16^{abB}	1.63±0.05^{bcB}	2.45±0.21^{abcA}
150	0	1.45±0.05^{bC}	1.53±0.10^{bcC}	1.13±0.16^{deD}	1.91±0.19^{bB}	2.57±0.18^{abA}
	2	1.86±0.12^{aAB}	1.82±0.72^{abB}	1.44±0.09^{bcdB}	1.94±0.05^{bAB}	2.48±0.17^{abcA}
	4	1.91±0.11^{aB}	1.86±0.34^{abB}	1.81±0.42^{aB}	2.57±0.09^{aA}	2.64±0.07^{aA}

^{abc}Mean values in the same column bearing different superscripts are significantly different ($p<0.05$).
^{ABC} Mean values in the same row bearing different superscript are significantly different ($p<0.05$).

Table 4 Effect of nitrite and GP on N-nitrosodimethylamine contents (mg NDMA/kg sample) in frankfurter products

Factors		NDMA (mg /kg)	
Nitrite (mg/kg)	GP (g/100g)	Before *in vitro* digestion	After *in vitro* digestion
50	0	ND	2.48±0.76^{ab}
	2	ND	3.21±0.94^{abc}
	4	ND	2.88±0.94^{abc}
100	0	ND	2.17±0.32^{c}
	2	ND	1.69±0.01^{c}
	4	ND	2.38±0.33^{c}
150	0	ND	3.30±0.10^{abc}
	2	ND	4.19±0.90^{ab}
	4	ND	4.26±1.13^{a}

^{abc}Mean values in the same column bearing different superscripts are significantly different ($p<0.05$).
ND = Not detectable

Table 5 Effect of nitrite and GP on sensory quality of frankfurters

Nitrite (mg/kg)	GP(g/100g)	Color	Odor	Taste	Texture	Overall acceptability
50	0	5.10±1.68^{bc}	5.30±1.75^{ns}	5.85±1.78^{a}	4.85±1.76^{ab}	5.85±1.39^{a}
50	2	6.50±1.47^{a}	5.50±1.64^{ns}	3.95±1.47^{bc}	4.15±1.70^{ab}	4.85±1.27^{ab}
50	4	5.95±1.85^{ab}	5.35±1.81^{ns}	5.00±1.72^{ab}	5.10±1.94^{a}	5.40±1.96^{ab}
100	0	4.65±1.18^{c}	5.35±1.78^{ns}	4.85±1.60^{abc}	4.70±2.05^{ab}	4.90±1.48^{ab}
100	2	6.00±1.95^{ab}	5.30±1.72^{ns}	4.65±1.42^{bc}	4.50±1.76^{ab}	5.15±1.56^{ab}
100	4	5.70±1.75^{abc}	5.25±1.37^{ns}	4.45±1.90^{bc}	4.75±2.07^{ab}	4.80±1.83^{ab}
150	0	4.70±1.62^{c}	5.45±1.76^{ns}	4.40±1.70^{bc}	3.75±1.48^{b}	4.75±1.48^{ab}
150	2	5.05±2.11^{bc}	4.65±1.78^{ns}	4.20±1.80^{bc}	4.05±2.00^{ab}	4.75±1.80^{ab}
150	4	5.80±1.85^{abc}	4.85±1.81^{ns}	3.70±1.87^{c}	3.90±1.62^{ab}	4.25±1.77^{b}

^{abc}Mean values in the same column bearing different superscripts are significantly different ($p < 0.05$).
NS = Non-significant

CONCLUSION

GP successfully improved the color of frankfurter as can be observed from the results of sensory evaluation and supported by increasing of a* and b* values. However, high level of GP had slight effect to increase TBARS value and N-nitrosodimethylamine content in the products. Finally, it can be concluded that GP is an alternative way to improve color of frankfurter products. Nonetheless, the addition of GP more than 2g/100g may increase lipid oxidation and N-nitrosodimethylamine formation.

Acknowledgments: The authors would like to thank Laemthong Food Products Co.Ltd. (Food Products Business) for financial support. Thanks also goes to Mae Fah Luang Unversity for providing technical support.

REFERENCES

Aust, O., Ale-Agha, N., Zhang, L., Wollersen, H., Sies, H. & Stahl, W. (2003). Lycopene oxidation product enhances gap junctional communication. *Food* Chemistry, 41, 1399-407.http://dx.doi.org/10.1016/s0278-6915(03)00148-0

Biaudet, H., Pignatelli , B. & Debry, G. (1996). N-nitroso compounds. Hand book of food analysis. New York:Marcel Dekker.

Boisen, S.& Fernandez, J. A. (1997). Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim. *Feed Sci. Technol, 68*, 277-286.http://dx.doi.org/10.1016/s0377-8401(97)00058-8

Buckley, D.J., Gtay, J.I., Asghar, A., Booren, A.M., Crackel, R.L., Price, J.F.& Miller, E. R. (1989). Effects of dietary antioxidants and oxidised oil on membranol lipid stability pork product quality. *Journal of Food* Science, 54, 1193–7.http://dx.doi.org/10.1111/j.1365-2621.1989.tb05952.x

Combet, E., Paterson, S., Iijima, K., Winter, J. Mullen, W,. Crozier, A., Preston, T.& McColl K.E.L. (2007). Fat transforms ascorbic acid from inhibiting to promoting acid-catalysed N-nitrosation. Gut, 56(12), 1678-1684. http://doi.org/10.1136/gut.2007.128587

Deda, M.S., Bloukas, J. G. & Fista, G.A. (2007). Effect of tomato paste and nitrite level on processing and quality characteristics of frankfurters. *Meat Science, 76*(3),501-508. http://dx.doi.org/10.1016/j.meatsci.2007.01.004

de Oliveiraa, T. L. C., de Carvalhob, S. M., de Araújo Soaresa, R., Andradeb, M. A., Cardosob, G., Ramosc, E. M. & Piccolia, R. H. Antioxidant effects of Satureja montana L. Essentail oil on TBARS and color of mortadella-type sausages formulated with different levels of sodium nitrite. *LWT - Food Science and Technology*, 45 (2), 204–212.http://dx.doi.org/10.1016/j.lwt.2011.09.006

Eyiler, E. & Oztan, A. (2011). Production of frankfurters with tomato powder as a natural additive. LWT - *Food Science and Technology*, *44*(1), 307-311.http://dx.doi.org/10.1016/j.lwt.2010.07.004

Feiner, G. (2006). Meat products handbook: practical science and technology.England:Woodhead Publishing Ltd.http://dx.doi.org/10.1201/9781439824245

FAO/WHO. (1996).Toxicological evaluation of certain food additives and contaminants. Geneva. World Health Organization, Joint FAO/WHO Expert

Committee on Food Additives, WHO Food Additives Series No. 35, Geneva, Switzerland.
Nollet, L.M.L.& Toldra, F. (2009). Handbook of Processed Meats and Poultry Analysis. New York: CRC press.http://dx.doi.org/10.1201/9781420045338
Greene, B. E. & Cumuze, T.H. (1982). Relationship between TBA numbers and inexperienced panelists' assessments of oxidized flavor in cooked beef. *Journal of Food Science*, *47*(1), 52-4.http://dx.doi.org/10.1111/j.1365-2621.1982.tb11025.x
Haila, K.M., Lievonen, S. M. & Heinonen, M.I. (1996). Effects of lutein, lycopene, annatto, and γ-tocopherol on autoxidation of triglycerides. *Journal of Agricultural and Food Chemistry,* *44*(8),2096-1000.http://dx.doi.org/10.1021/jf9504935
Hayes, J.E., Canonico, I. & Allen, P. (2013). Effects of organic tomato pulp powder and nitrite level on the physicochemical, textural and sensory properties of pork luncheon roll. *Meat Science*, *95*(3), 755-762.http://dx.doi.org/10.1016/j.meatsci.2013.04.049
Ishida, B.K., Turner, C., Chapman, M.H.& McKeon, T.A. (2003). Fatty acid and carotenoid composition of gac (*Momordica cochinchinensis* Spreng) fruit. *Journal of Agricultural and Food Chemistry,***52**(2), 274-279.http://dx.doi.org/10.1021/jf030616i
Jensen, C., Lauridsen, C. & Bertelsen, G. (1998). Dietary vitamin E: Quality and storage stability of pork and poultry. *Trends in Food Science &Technology, 9*(2),62-72.http://dx.doi.org/10.1016/S0924-2244(98)00004-1
Kha, T.C., Nguyen, M.H., Roach, P. D. & Stathopoulos, C.E. (2014). Microencapsulation of gac oil: optimisation of spray drying conditions using response surface methodology. *Powder Technology, 264*,298-309.http://dx.doi.org/10.1016/j.powtec.2014.05.053
Kha,T.C., Nguyen, M.H., Roach, P. D. & Stathopoulos, C.E.(2015). A storage study of encapsulated gac (*Momordicacochinchinensis*) oil powder and its fortification into foods.*Food and Bioproducts Processing, 96*:113–125.http://dx.doi.org/10.1016/j.fbp.2015.07.009
Korkeala, H., Alanko, T. &Tiusanen T. (1992). Effect of sodium nitrite and sodium chloride on growth of lactic acid bacteria.*Acta Vet Scand. 33*(1), 27-32.
Krauss, M.& Hollender, J. (2008). Analysis of nitrosamines in wastewater: exploring the trace level quantification capabilities of a hybrid linear ion trap/orbitrap mass spectrometer. *Analytical Chemistry, 80*(3), 834-842.http://dx.doi.org/10.1021/ac701804y
Kubola, J. & Siriamornpun, S. (2011). Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (*Momordica cochinchinensis* Spreng). *Food Chem, 27*(3),1138-45. http://dx.doi.org/10.1016/j.foodchem.2011.01.115
Lin, Y., Huang, M., Zhou, G., Zou, Y.& Xu, X. (2011). Prooxidant effects of the combination of green tea extract and sodium nitrite for accelerating lipolysis and lipid oxidation in pepperoni during storage. *Journal of Food Science, 76*(5):C694-C700.http://dx.doi.org/10.1111/j.1750-3841.2011.02187.x
Meineri, G., Medana, C., Giancotti, V., Visentin, S. & Peiretti, P.G. (2013). Effect of dietary supplementation of vitamin E in pigs to prevent the formation of carcinogenic substances in meat products. *Journal of Food Composition and Analysis*, *30*(2), 67-72.http://dx.doi.org/10.1016/j.jfca.2013.02.002
Ohshima, H. ,& Bartsch, H. (1999). Quantitative estimation of endogenous nitrosation in humans by monitoring N-nitrosoproline excreted in the urine. *Nitric Oxide Part C: Biological and Antioxidant Activities, 40-49.http://dx.doi.org/10.1016/s0076-6879(99)01067-8*
Shi, J., Maguer, M. L., Kakuda, Y., Liptay, A., & Niekamp, F. (1999). Lycopene degradation and isomerization in tomato dehydration.
Food Research International, *32*, 15-21.http://dx.doi.org/10.1016/S0963-9969(99)00059-9
Tonucci, L.H., Holden, J.M., Beecher, G.R., Khachik, F., Davis, C. S. & Mulokozi, G. (1995). Carotenoid content of thermally processed tomato-based food products. *Journal of Agricultural and Food Chemistry, 43*(3), 579-586.http://dx.doi.org/10.1021/jf00051a005
West, C.E.& Poortvliet, E.J. (1993). The carotenoid content of foods with special reference to developing countries. Institute IsaT: Virginia.
Walters, C. L., Edwards, M. W., Elsey, T. S. & Martin, M. (1976).The effect of antioxidants on the production of volatile nitrosamines during the frying of bacon. *Z LebensmUntersForsch. 162*(4), 377-385.http://dx.doi.org/10.1007/bf01122791

JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

THERMOANALYTICAL STUDY OF ACECLOFENAC FORMULATIONS WITH REGULAR MAIZE STARCH AND WAXY MAIZE STARCH

Maristela Denck Colman[1], Elaine da Silva Ramos[2], Cristina Soltovski de Oliveira[1], Tiago Andre Denck Colman[3], Egon Schnitzler[1]*

Address(es):
[1]State University of Ponta Grossa, Carlos Cavalcanti 4748, ZIP 84030-900, Ponta Grossa, Paraná, .Brazil,
[2]Federal Institute of Education, Science and Technology of Paraná - Campus Telêmaco Borba, Rod. PR 160, km 19.5, Telêmaco Borba, Paraná, Brazil
[3]Faculty of Science and Technology, Federal University of Grande Dourados, Rodovia Dourados-Itahum, Km 12, ZIP 79804-970, Caixa Postal 364, Dourados, MS, Brazil

*Corresponding author: tiagocolman@ufgd.edu.br

doi: 10.15414/jmbfs.2016.6.2.828-831

ARTICLE INFO

Received 3. 11. 2015
Revised 10. 12. 2015
Accepted 12. 7. 2016
Published 3. 10. 2016

Regular article

OPEN ACCESS

ABSTRACT

Production of tablets in pharmaceutical industry often is required to mixture the active ingredient (drug) with an inert material. Starch is shown as a good alternative material to be used as excipient. Thus, in this investigation the techniques: thermogravimetry and derivative thermogravimetry (TG/DTG), differential scanning calorimetry (DSC as well as thermomicroscopy were performed with aim to verify existence of interactions between aceclofenac (2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxalacetic) with regular maize starch and waxy maize starch. Binary and physical mixtures of aceclofenac and starch in ratios 2:1, 1:1 and 1:2 (drug:starch) studied by the instrumental techniques showed that no interactions occurs between the drug and starches in any ratio, being maintained the properties of aceclofenac.

Keywords: Thermal analysis, aceclofenac, excipient, regular maize starch, waxy maize starch; interaction drug:excipient

INTRODUCTION

The excipients are inactive ingredients, these are substances they have no therapeutic activity and be used for bring stability in physicochemical properties as well as organoleptic characteristics of pharmaceutical products (Oliveira & Storpirtis, 1999). Excipients are so important that Robertson (1999) conducted a study which found 3816 of these substances in a sample of 12132 drugs.
Starch is a widely used and studied excipients, because in most cases the starch does not interact with active principle formulation (**Souza & Ferrão, 2006; Parisotto *et al.*, 2005**). However each botanical source of starch can give different characteristics as example higher amylose or amylopectin levels depending on the case (**Liu, Ramsden & Corke, 1997**).
The 2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxalacetic, is a white crystalline solid, insoluble in water, commonly known as aceclofenac. It belongs to the class of nonsteroidal anti-inflammatory drugs (NSAIDs); chemically being a derivative of aryl acetic acid. This is a non-selective cyclooxygenase (COX) reducing metabolites and providing anti-inflammatory, analgesic and antipyretic effects. It has been prescribed mainly in diseases such as rheumatoid arthritis, ankylosing spondylitis and osteoarthritis (**Yamazaki *et al.*, 1997; Shakeel *et al.*, 2007; Colman *et al.*, 2015**).
The objective of this study was to investigate the possible interactions occurring between aceclofenac drug and regular maize starch and waxy maize starch. The used techniques were: thermogravimetry/derivative thermogravimetry (TG/DTG), differential scanning calorimetry (DSC) and thermomicroscopy.

MATERIAL AND METHODS

Materials and methods

The sample used in this study was aceclofenac pharmaceutical grade (b), lot: 11020326A acquired in the city of Ponta Grossa –Paraná, Brazil. The regular maize starch (a) and waxy maize starch (f) were extracted using the methodology described in the literature (**Whistler, 1964; Colman *et al.*, 2012**).

Mixtures Aceclofenac : starch

The mixtures were made by dispersing the proportions of drug:starch in water to give a mixture with final mass of 1 g, the following proportions were used in ratios drug:starch (2:1 (c), 1:1 (d) and 1:2 (e)) for regular maize starch and (2:1 (g), 1:1 (h) and 1:2 (i) for waxy maize starch. After agitation and homogenization of the dispersion was performed for 10 min, followed by filtration and evaporation of water in oven with air circulation at 45 ° C for 24 h (**Colman *et al.*, 2015**).

Instrumental analysis

Thermogravimetry and derivative thermogravimetry (TG/DTG)

The thermogravimetric curves (TG) were obtained using the thermal analysis system TGA-50 (Shimadzu, Japan), where the samples were heated from 35 ºC to 650 ºC using open alumina crucibles with approximately 5.0 mg of the sample under a synthetic air flow of 150 mL min^{-1} at a heating rate of 10 ºC min^{-1}. The instrument was preliminarily calibrated according to the recommendations of the manufacturer. All mass loss percentages were determined using TA-60WS data analysis software, as well as first derivative (DTG) was calculated. This mathematic resource is useful in determining the main steps and temperatures of mass loss (**Cordoba *et al.*, 2013; Alberton *et al.*, 2014; Leone *et al.*, 2014; Ribeiro *et al.*, 2014; Colman *et al.*, 2015**).

Differential Scanning Calorimetry (DSC)

The DSC curves were obtained using the thermal analysis system model DSC-Q200 (TA-Instruments, USA). The curves were recorded under an N_2 flow of 50 mL min^{-1}, heating rate of 10 ºC min^{-1} and samples weighing about 5 mg in aluminium crucibles with perforated lid. The instrument was previously calibrated according to the recommendations of the manufacturer (standard Indium 99.99% purity, *m.p.* = 156.6ºC, ΔH = 28.56 J g^{-1}). The results were calculated using Universal Analysis 2000 software (**Cordoba *et al.*, 2013; Colman *et al.*, 2015; Malucelli *et al.*, 2015**).

Thermomicroscopy

The images were obtained by coupling a digital microscope upon DSC cell. The microscope is equipped with color CMOS sensor and lens glass 2M pixel resolution with magniffication 800x. The software AMCAP V9.016 was used for capturing images.

RESULTS AND DISCUSSION

Mixtures Aceclofenac : regular maize starch

The TG/DTG curves are show in Fig. 1 and results for regular maize starch (a), aceclofenac (b), mixtures (c-e) are show in Table 1. The thermal decomposition of regular maize starch (a) occurs in three stages of mass loss. The first step according to the literature (**Liu *et al.*, 2009**), is associated with dehydration, followed by stability. Once anhydrous the sample is stable up to 223 °C, after this temperature the decomposition and oxidation of organic material takes place in two consecutive stages of mass loss, with final residue corresponding to 0.18% of initial mass.

The aceclofenac (b) is anhydrous and thermally stable up to 161 °C, after that temperature its thermal decomposition occurs in two consecutive stages of mass loss, with final residue corresponding to 0.74% of its initial mass.

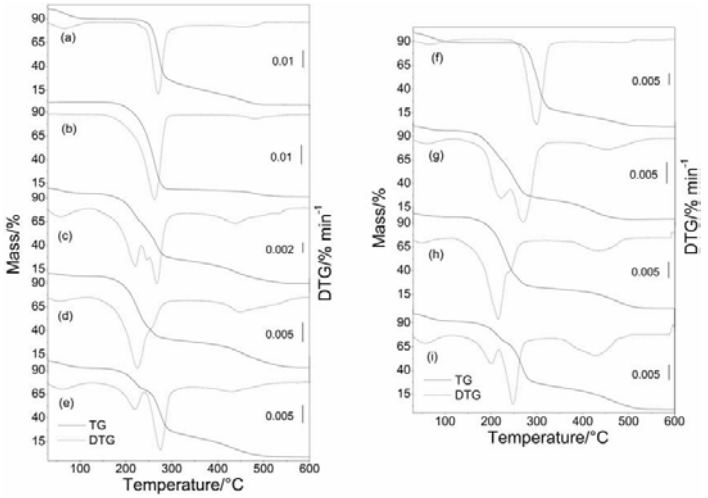

Figure 1 TG/DTG curves of: regular maize starch (a), aceclofenac (b), (c) mixture aceclofenac:regular maize starch (2:1), (d) mixture aceclofenac:regular maize starch (1:1) and (e) mixture aceclofenac:regular maize starch (1:2), waxy maize starch (f), aceclofenac (b), (g) mixture aceclofenac:waxy maize starch (2:1), (h) mixture aceclofenac:waxy maize starch (1:1) and (i) mixture aceclofenac:waxy maize starch (1:2).

The TG/DTG curves of mixtures aceclofenac: regular maize starch show thermal decomposition in three (d) and four (c, e) steps. Due to the sum of steps of thermal decomposition of the starch and aceclofenac, the first step is attributed to dehydration of the starch present in the sample portion. The final residue corresponds to 0.56% (c) 0.95% (d) and 0.58% (e) of the initial mass sample, respectively.

Table 1 Results TG and DTG of: regular maize starch (a), aceclofenac (b), (c) mixture aceclofenac:regular maize starch (2:1), (d) mixture aceclofenac:regular maize starch (1:1) and (e) mixture aceclofenac:regular maize starch (1:2)

Sample		TG and DTG Steps				
		1st	Stability	2nd	3rd	4th
(a)	ΔT/°C	30-107	107-223	223-366	366-523	-
	Tp/°C	66.72	-	269.83	442.43	-
	Δm/%	10.01	-	73.98	15.83	-
(b)	ΔT/°C	161-371	-	371-545	-	-
	Tp/°C	262.49	-	480.2	-	-
	Δm/%	92.38	-	6.88	-	-
(c)	ΔT/°C	30-100	100-134	134-234	234-335	335-561
	Tp/°C	57.64	-	220.35	246.54 (shoulder) 267.80	437.91 536.21
	Δm/%	5.36	-	31.07	39.48	23.53
(d)	ΔT/°C	30-99	99-142	142-350	350-570	-
	Tp/°C	96.90	-	225.11 255.58 (shoulder)	449.38	-
	Δm/%	2.73	-	70.06	26.26	-
(e)	ΔT/°C	30-110	110-154	154-241	241-348	348-539
	Tp/°C	61.55	-	219.40	274.58	431.20
	Δm/%	7.37	-	22.21	4+8.85	20.99

(*) Δm mass loss/%, ΔT temperature range, Tp peak temperature

DSC curves are shown in Fig. 2 and the obtained results in Table 2. The sample (a) does not present any thermal event at the temperature range. The profile of DSC curve show endothermic peak due to melting of aceclofenac (b), for mixtures there were no major changes in temperature related to the portion of aceclofenac melting. The values of melting enthalpy decreased proportionally with the increase in regular maize starch samples.

Table 2 DSC results of: regular maize starch (a), aceclofenac (b), (c) mixture aceclofenac:regular maize starch (2:1), (d) mixture aceclofenac:regular maize starch (1:1) and (e) mixture aceclofenac:regular maize starch (1:2), waxy maize starch (f), aceclofenac (b), (g) mixture aceclofenac:waxy maize starch (2:1), (h) mixture aceclofenac:waxy maize starch (1:1) and (i) mixture aceclofenac:waxy maize starch (1:2)

DSC regular maize starch					DSC waxy maize starch				
Samples	T_{on}/°C	T_p/°C	T_{end}/°C	ΔH/J g^{-1}	Samples	T_{on}/°C	T_p/°C	T_{end}/°C	ΔH/J g^{-1}
(a)	-	-	-	-	(f)	-	-	-	-
(b)	152.68	156.12	159.99	158.6	(b)	152.68	156.12	159.99	158.6
(c)	153.28	154.79	157.31	79.04	(g)	150.36	155.88	161.45	78.85
(d)	151.62	154.46	158.12	49.40	(h)	151.35	154.50	158.65	53.59
(e)	150.94	153.49	156.17	24.55	(i)	150.79	153.24	156.42	28.71

(*) "T_{on}" Onset temperature, "T_p" Peak temperature, "T_{end}" Endset temperature, "ΔH" Melting enthalpy

The thermomicroscopy show in Fig. 2 that the melting occurs in aceclofenac fraction of the mixtures. The greater amount of aceclofenac and the greater reflection of light at temperatures above 165 ° C were observed. This occurs because the fused fraction of aceclofenac sample (b) has a greater capacity to reflect light from the microscope. It can be observed that the fraction of the sample corresponding to the starch which no visible change in the temperature range studied, suggesting the formation of a physical mixture, without chemical interaction between the regular maize starch and aceclofenac.

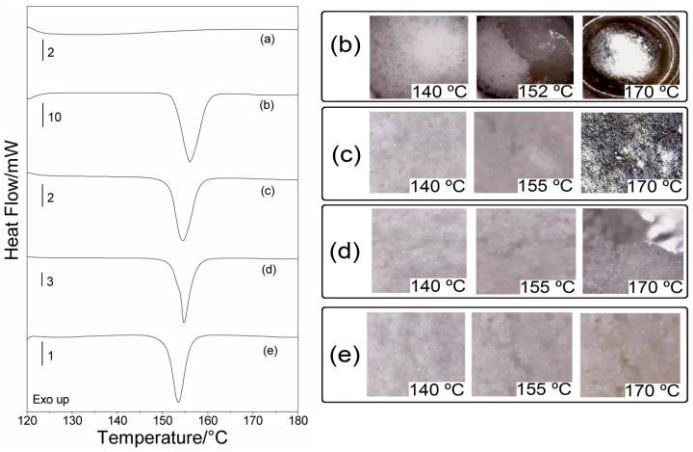

Figure 2 DSC curves (left) and Thermomicroscopy (right): regular maize starch (a), aceclofenac (b), (c) mixture aceclofenac:regular maize starch (2:1), (d) mixture aceclofenac:regular maize starch (1:1) and (e) mixture aceclofenac:regular maize starch (1:2).

Mixtures Aceclofenac:waxy maize starch

The TG/DTG curves are shown in Fig. 1 and TG/DTG results for waxy maize starch (f), aceclofenac (b), mixtures (g-i) are show in Table 3. The thermal decomposition of waxy maize starch (a) occurs in three stages of mass loss. The first step according to the literature (**Malucelli *et al., 2015;* Oliveira *et al., 2013;* Liu *et al., 2009***), is associated with dehydration, the anhydrous sample is stable up to 232 °C, after this temperature the decomposition and oxidation of organic material takes place in two consecutive stages of mass loss, with final residue corresponding to 0.62% of the initial mass.

Table 3 Results TG and DTG of: waxy maize starch (f), aceclofenac (b), (g) mixture aceclofenac:waxy maize starch (2:1), (h) mixture aceclofenac:waxy maize starch (1:1) and (i) mixture aceclofenac:waxy maize starch (1:2)

Sample		TG and DTG Steps				
		1st	Stability	2nd	3rd	4th
(f)	ΔT/°C	30-122	122-232	232-351	351-521	-
	Tp/°C	68.18	-	298.3	481.11	-
	Δm/%	11.31	-	72.83	15.24	-
(b)	ΔT/°C	161-371	-	371-545	-	-
	Tp/°C	262.49	-	480.2	-	-
	Δm/%	92.38	-	6.88	-	-
(g)	ΔT/°C	30-95	95-142	142-356	356-543	-
	Tp/°C	59	-	219.61 268.30	451.05	-
	Δm/%	4.41	-	74.21	20.54	-
(h)	ΔT/°C	30-86	86-141	141-351	351-536	-
	Tp/°C	50.12	-	231.07 259.1 (shoulder)	468.51	-
	Δm/%	5.28	-	73.95	19.98	-
(i)	ΔT/°C	30-101	101-146	146-233	233-353	353-534
	Tp/°C	58.32	-	215.28	266.36	400.86
	Δm/%	7.73	-	22.61	47.08	22.17

(*) Δm mass loss/%, ΔT temperature range, Tp peak temperature

The TG/DTG curves of mixtures aceclofenac: waxy maize starch (g-i) profile show thermal decomposition that occurs in three (g-h) and four (i) steps. The DTG curves shows the existence of two consecutive and overlapping steps, associated with the second mass loss observed in the TG curve, this is not observed for waxy maize starch (f), so the thermal decomposition mixtures (g-i) presents a step more than the starch (f).

The first step is attributed to dehydration of the starch present in the sample portion. The anhydrous samples show some behaviour in thermal stability. The decomposition and oxidation of organic matter takes place in three consecutive steps of mass loss, with formation of final residue corresponds to 0.84% (g) 0.79% (h) and 0.41% (i), respectively, of the initial mass sample.

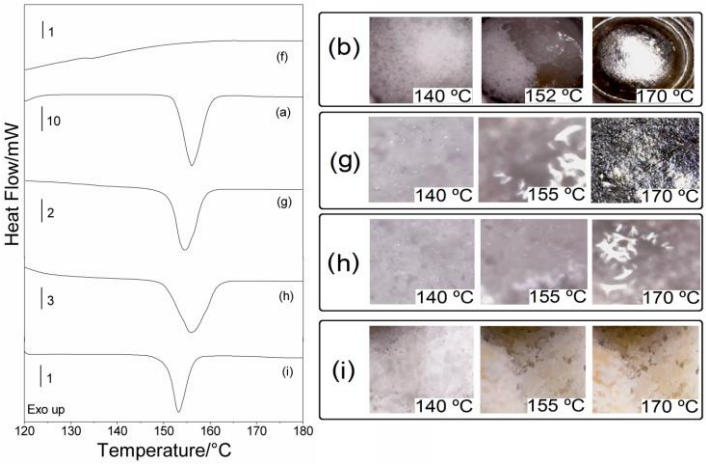

Figure 3 DSC curves (left) and Thermomicroscopy (right): waxy maize starch (f), aceclofenac (b), (g) mixture aceclofenac:waxy maize starch (2:1), (h) mixture aceclofenac:waxy maize starch (1:1) and (i) mixture aceclofenac:waxy maize starch (1:2)

DSC curves show in Fig. 3 and results of DSC are show in Table 2. The waxy maize starch (f) does not present any thermal event at the temperature range of studied, the profile of curves show melting pick of aceclofenac (b), as well as, for mixtures aceclofenac:regular maize starch, for the DSC study of mixtures aceclofenac:waxy maize starch (g-i) there were no major changes in temperature related to the portion of the aceclofenac melting, the melting enthalpy decreased proportionally the increase in regular waxy starch samples. The thermomicroscopy shows Fig. 3 that the melting occurs in aceclofenac fraction of the mixtures, suggesting the formation of a physical mixture, without chemical interaction between the waxy maize starch and the aceclofenac.

CONCLUSION

Thermoanalytical techniques were important analytical tools for evaluating possible interactions between drugs and excipients. The profile of the TG and DTG curves suggests that the decomposition occurs as physical mixtures of two substances. The found results of DSC and thermomicroscopy suggesting the formation of a physical mixture, without chemical interaction between the regular and waxy maize starch and aceclofenac. This fact confirm that these two starchs can be used as excipient in formulations containing aceclofenac.

Acknowledgments: The authors acknowledge the CNPq and CAPES, Brazil, for research funding.

REFERENCES

Alberton, C., Colman, T. A. D., Souza, J. A., Oliveira, C. S., Andrade, M. M. P., Schnitzler, E. (2014). Thermal analysis, rheology, x-ray diffractometry and atomic force microscopy in the evaluation of binary mixtures of" starch-hydrocolloids". *Journal of Microbiology, Biotechnology and Food Sciences*, *3*(4), 305-309.

Colman, M. D., Oliveira, C. S., Colman, T. A. D., & Schnitzler, E. (2015). Avaliação termoanalítica e estrutural de misturas binarias aceclofenaco:amido de pinhão. *Brazilian Journal of Thermal Analysis, 4*(1-2),44 – 47. http://dx.doi.org/10.1016/10.18362/bjta.v4.i1-2.82

Colman, T. A. D., Bicudo, S. C. W., Lacerda, L. G., Carvalho-Filho, M. A. S., Demiate, I. M., Bannach, G., Schnitzler, E. (2012). Characterization of wheat starch by thermoanalytical, rheological and atomic force microscopy techniques, de pinhão. *Brazilian Journal of Thermal Analysis*, 1, 62-65.

Cordoba, L., Ribeiro, L., Colman, T., Oliveira, C., Andrade, M., Fernanda Janaína Oliveira Gomes Da Costa, & Schnitzler, E. (2013). Effect of hydrochloric acid in different concentrations and temperatures up to some properties of organic cassava starch. *Brazilian Journal of Thermal Analysis*, *2*(1), 6-11. http://dx.doi.org/10.18362/bjta.v2i1.8

Leone, R. S., Colman, T. A. D., Schnitzler, E., Ellendersen, L. N., Masson, M. L. (2014). Evolved gas analysis (coupled TG–DSC–FTIR) applied to the thermal behaviour of inulin. *Journal of Analytical and Applied Pyrolysis*, 108, 323-326. http://dx.doi.org/10.1016/j.jaap.2014.04.009

Liu, H., Ramsden, L., & Corke, H. (1997). Physical properties and enzymatic digestibility of acetylated ae, wx, and normal maize starch. *Carbohydrate Polymers,* 34(4), 283-289. http://dx.doi.org/10.1016/S0144-8617(97)00130-6

Liu, X., Yu, L., Liu, H., Chen, L., & Li, L. (2009). Thermal Decomposition of Corn Starch with Different Amylose/Amylopectin Ratios in Open and Sealed Systems. *Cereal Chemistry, 86*(4), 383-385. http://dx.doi.org/10.1094/CCHEM-86-4-0383

Malucelli, L., Lacerda, L. G., Filho, M. A. C. S. , Fernández, D., Demiate, I. M., Oliveira, C. S., & Schnitzler, E. (2015). Porous waxy maize starch. *Journal of Thermal Analysis and Calorimetry*, 525-532. http://dx.doi.org/10.1007/s10973-015-4483-6

Oliveira, C. S. , Andrade, M. M., Colman, T. A. D., Costa, F. J. G., & Schnitzler, E. (2013). Thermal, structural and rheological behaviour of native and modified waxy corn starch with hydrochloric acid at different temperatures. *Journal of Thermal Analysis and Calorimetry, 115*(1), 13-18. http://dx.doi.org/10.1007/s10973-013-3307-9

Oliveira, G. & Storpirtis, S. (1999). Toxicidade de excipientes: carência de informação nas bulas de medicamentos disponíveis no mercado brasileiro. *Revista Brasileira de Ciências Farmacêuticas*, *35*(supl. 1), 71.

Parisotto, G., Souza, J., Ferrão, M., Furtado, J., & Molz, R. (2005). Análise exploratória aplicada no estudo de medicamentos contendo piroxicam. *Revista Brasileira de Ciências Farmacêuticas, 41*(4), 499-505.

Ribeiro, L., Cordoba, L., Colman, T., Oliveira, C., Andrade, M., & Schnitzler, E. (2014). Influence of some sugars on the thermal, rheological and morphological properties of "pinhão" starch. Journal of Thermal Analysis and Calorimetry, 117(2), 935-942. http://dx.doi.org/10.1007/s10973-014-3778-3

Shakeel, F., Baboota, S., Ahuja, A., Ali, J., Aqil, M., & Shafiq, S. (2007). Nanoemulsions as vehicles for transdermal delivery of aceclofenac. *AAPS PharmSciTech, 8*(4), 191-199. http://dx.doi.org/10.1208/pt0804104

Souza, J., & Ferrão, M. (2006). Aplicações da espectroscopia no infravermelho no controle de qualidade de medicamentos contendo diclofenaco de potássio. Parte I: Dosagem por regressão multivariada. *Revista Brasileira de Ciências Farmacêuticas, 42*(3), 437-445.

Whistler, R. (1964). Preparation of starch and starch fractions - wholestarch corn starch isolation., In: *Methods in carbohydrate chemistry* (pp. 3-5). London: Academic Press.

Yamazaki, R., Kawai, S., Matsuzaki, T., Kaneda, N., Hashimoto, S., Yokokura, T., Okamoto, R,. Koshino, T. & Mizushima Y. (1997). Aceclofenac blocks prostaglandin E2 production following its intracellular conversion into cyclooxygenase inhibitors. *European Journal of Pharmacology, 329*(2-3), 181-187. http://dx.doi.org/10.1016/S0014-2999(97)89179-0

JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

CHEMICAL AND NUTRITIONAL PROPERTIES OF SOME COMMERCIAL AVAILABLE CORN AND WHEAT PRODUCTS

Muhammad Usman Khan[1] *, Majid Majeed*[2] *, Muhammad Tayyab*[3]*, Mohammad Ali Shariati*[4]*, Shilan Rashidzadeh*[5]

Address(es):
[1]Department of Energy Systems Engineering, University of Agriculture Faisalabad,38000,Faisalabd,Pakistan,+92312667362.
[2]National institute of Food Science and Technology, University of Agriculture, Faisalabad,38000,Faisalabd,Pakistan,+92312667362.
[3]Punjab Bio Energy Institute,University of Agriculture Faisalabad, Faisalabad,38000,Faisalabd,Pakistan,+92312667362.
[4]Research Department, LLC «Science & Education», Russia and Researcher All Russian Research Institute of Phytopathology, Moscow Region, Russia.
[5]Department of Food Science and Technology, Gorgan Payamenoor Univeristy, Gorgan, Iran.

*Corresponding author: engineer_usman_Khan@yahoo.com

doi: 10.15414/jmbfs.2016.6.2.863-866

ARTICLE INFO

Received 23. 6. 2016
Revised 17. 8. 2016
Accepted 15. 9. 2016
Published 3. 10. 2016

Regular article

OPEN ACCESS

ABSTRACT

Commercial corn and wheat products were obtained and investigated for, proximate composition, mineral composition and tocopherol content. Proximate composition shows moisture content in the range of 28.75-2.75%, ash (2.70-0.32%), fats (21.52 -0.75%), protein (11.54-0.940%), crude fiber (4.06-0.250%) and carbohydrates (85.80-55.96%). The data indicate that Commercial corn and wheat products vary greatly in term of protein, fats and crude fiber. White flour and whole wheat flour were determined to contain high protein content (>10% protein) while oil popped popcorn and biscuit contain high fats content of >20%. In minerals the level of iron is 9.56-51.21mg/kg, Ca (50-560 mg/kg), Zn (1.90-32.40 mg/kg), K (820-2946) and Mg (310-1512 mg/kg). Tocopherol analysis was also done by the HPLC and found that Alpha tocopherol showed higher concentration than gamma and sigma tocopherol. In tocopherols level of alpha tocopherol is 0.3385-7.135 mg/100g, Gamma tocopherol (0.000212-9.665 mg/100g)Sigmmatocopherol (0.00212-1.685 mg/100g).

Keywords: Proximate composition, minerals, tocopherol, maize, wheat

INTRODUCTION

Cereals are mostly grasses cultivated for their edible grains. These are grown at larger areas in Pakistan and cereal give more energy than any other type of crop worldwide. They are also a rich source of carbohydrate, protein, vitamins, fiber and minerals. In some developing countries, wheat or corn constitutes entire diet of poor people. In developed countries, cereal consumption is both more moderate but consumption is still substantial. In Pakistan wheat, maize and rice are grown at larger areas and these are the major energy source in the whole world than any other cereal crop. In developing countries wheat, rice and maize are the major constitutes of the entire diet of poor people. In Pakistan wheat (*Triticumaestivum*L.) is main food crop and used as staple food in country. In agricultural policies wheat it occupies a central position. In agriculture wheat contributes 12.5 percent to the value added and 2.9 % contribute to GDP. Its annual production was about 18.47 million tons and it is cultivated on an area of 8 million hectares in 2007-2008. At village stage, the storage space contains mud containers, metal containers, concrete rooms, jute hand bags and wood made bins. Open storage by putting bagged grain on plinth is also practiced by the whole sellers and for the protection it is covered with tarpaulin. Post-harvest losses are quite substantial because of poor drying and storage facilities **(Baloch and Irshad, 1986)**.
In Pakistan, maize is the third most important cereal crop after wheat and rice and is used as a staple food for humans, as feed for livestock and as raw material for industry. During 2006, it was planted on 1030 thousand hectares in Pakistan, with total production of 3560 thousand tons and having an average yield of 3.458 tons ha-1 **(Khan *et al*., 2009)**. Maize accounts for 4.8% of the total cropped area and 3.5% of the value of agricultural output of Pakistan. It is planted on an estimated area of 0.9 million hectare with an annual production of 1.3 million tones. The bulk (97%) of the total production come from two major provinces (NWFP and Punjab), NWFP, accounting for 57% of the total area and 68% of total production **(PARC, 2007)**.
World collections of maize comprise about 12,000 accessions that are represented in 256 races, of which about 30 are in the process of extermination. Genetic erosion and habitat destruction by modern agriculture has increased the importance of germplasm characterization of plant materials **(Carvalho *et al*., 2004)**. Maize and wheat are a multipurpose crop, providing food and fuel for human beings, feed for animals, poultry and livestock. Its grains have great nutritional value and are used as raw material for manufacturing many industrial products **(Afzal *et al*., 2009)**. Its grains are important for the production of oil, starch and glucose **(Niaz and Dawar, 2009)**. Moreover, food composition data is important in nutritional planning and provides data for epidemiological studies **(Ali *et al*., 2008).** However, there is limited information about the nutritional composition of the different commercial corn and wheat products which are manufactured in Pakistan. The present study aims to investigate, proximate composition, mineral and tocophero composition of the different commercial corn and wheat products which are manufactured in Punjab province of Pakistan.

MATERIALS AND METHODS

Moisture content

The moisture content in the fortified whole wheat flour samples was determined in an air forced draft oven (Memmert) by following the method described in AACC [2000].

Ash content

Each flour sample was tested for ash content using muffle furnace (NEY M-525) by following the procedure outlined in AACC [2000].

Fat content

The crude fat content in whole wheat flour samples was estimated using petroleum ether as a solvent in Soxhlet apparatus (Sox. Tec System H T- 2, 1045) according to the procedure given in AACC [2000].

Protein content

The nitrogen content in flour samples was determined by Kjeldahl's method as described in AACC [2000]. The nitrogen percentage was determined as:

$$Nitrogen\ \% = \frac{\text{Vol. of 0.1 N H2SO4 used} \times 0.0014 \times 250}{\text{Weight of sample (g)} \times \text{Vol. of diluted sample used}}\ x\ 100$$

The protein percentage was calculated by multiplying % nitrogen with a factor 5.7.

Crude fiber

The crude fiber of corn products were determined as mentioned in AACC Method No. 32-10. Moisture free and fat free sample was used for this study.

Nitrogen Free Extract (NFE)

Nitrogen free extract was calculated by subtracting the percentages of moisture, protein, fat, fiber and ash from 100 as follows:

NFE = 100 - (moisture % + crude protein % + crude fat % + crude fiber % + ash %)

Mineral composition:

Mineral content was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) **(Zand *et al*., 2011)**.

Sample digestion

Each sample was mixed and homogenized using a domestic blender and three independent replicates of 0.5 g were weighed prior to adding 10 mL of concentrated H_2SO_4 (overnight) then added 1 mL H_2O_2 and digest it on the hot plate at 150 °C until white fumes comes out and transparent solution will obtained. Remove the solution and cool it. Then diluted upto 50mL vol. flask and filtered the solution with the help of whatman No.1 filter paper.

Analysis by Inductivity Coupled Plasma–Optical Emission Spectrometer (ICP–OES)

9 mL digested samples were quantitatively analyzed using an Inductivity Coupled Plasma–Optical Emission Spectrometer. Working solution of certain concentration are run to see the performance and linearity behavior of instrument. We calculate the concentration by using formulae:
Conc. in sample = Conc. × Dilution Factor / Weight of sample

Tocopherol analysis

Materials

For the analysis, oil was extracted from commercial corn and wheat products. Standard of alpha-tocopherolwas purchased from Aldrich (Steinheim, Germany), Sigmma and Gamma tocopherols were from Sigma (St. Louis, USA). All solvents were of HPLC grade.

Sample Preparation

The sample preparation and analysis of Tocopherol in corn and wheat products were performed as described in method **(Swiglo and Sikorska, 2004).** Sample of corn and wheat products were weighed (0.0400 to 0.1200 g) and dissolved in 1mL of 2-propanol. The selection of 2-Propanol was chosen because it allows solubility of oils and it is miscible with all solvents used in chromatography. Thus, no additional sample treatment is necessary. Vortexed-mixed samples were directly injected onto HPLC column. Stock and working solutions of tocopherols were also prepared in 2-propanol. Sample and standard solutions were prepared directly before analysis.Care was taken to exclude air and light exposure of sample and standard solutions throughout the analytical procedure.

HPLC analysis of tocopherols

All HPLC analyses of tocopherols were performed at room temperature on Shimadzu LC-10A high performance liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with Discovery supelco C18 (250mm × 4.6 mm, 5µm, MA, USA). For determination of tocopherols in oils, a mobile phase consisting of 50% of acetonitrile (solvent A) and 50% of methanol (solvent B) was used with the flow rate 1.5 mL min-1. Injection volume was 20 µl. The eluate was detected using a fluorescence detector (RF-530) set at emission wavelength of 325 nm with an excitation at 295 nm. Tocopherols were identified by comparing their retention times with those of corresponding standards and by spiking of samples with appropriate standard.

RESULTS AND DISSUSIONS

Proximate compostion corn and wheat products

The mean values for moisture content of different corn and wheat samples have been shown in the Table 1. The moisture content of different products of wheat ranged from the 3.40-28.75%. Among the products higher moisture content (28.75%) was noted in the bread followed by the (12.08%) in the white flour, (11.20%) in wheat flour and (3.40%) in the biscuit. Similar results for the moisture content in the wheat samples were obtained by **(Parveen, 2007; Rehman, 2011)**. While the result pertaining to the moisture content of commercial corn samples have been given in Table 1. Among these samples higher moisture content was determined in corn flour (9.50%) followed by the air popped popcorn(9.0%), oil popped popcorn (2.82%) and corn flakes(2.75%). The result of this study are in close agreement with the finding of **(Padovani *et al*., 2007; Akram *et al*., 2011)**. The ash content in the wheat samples varied from 0.32-0.90%. The result showed that maximum ash content was found in the bread (0.90 %). Lowest was observed inwhite flour (0.32 %). Mean values of all samples of corn are presented in the Table 1. The highest value of ash (2.70 %) was determined in oil popped popcorn followed by the corn flakes (1.90%), Air popped popcorn (1.72%) and corn flour (0.40%). The crude fat content in the wheat samples varied from 1.32-21.52%. Among the samples higher fat content (21.52 %) was noted in the biscuit followed by the (6.09 %) bread, (1.67 %) in wheat flour and (1.32 %) in the white flour. Among corn samples higher crude fat content was determined in oil popped popcorn (26%) followed by the air popped popcorn (3.9 %), corn flour (1.06%) and corn flakes(0.75 %). Highest amount of crude fat is determined in the oil popped popcorn because these popcorn are fried in the oil. . The crude fiber content in the wheat products varied from 0.25-1.14%. The result showed that maximum crude fiber content was found in the wheat flour (1.14 %). Lowest was observed in biscuit (0.25 %). Crude fiber content for the different corn products were significantly different from each other The highest value of crude fiber (4.06 %) was determined in the air popped popcorn followed by the oil popped popcorn (3.40%), corn flakes. (3.00 %) and corn flour (2.30%).The crude protein content in the wheat samples varied from 7.75-11.54%. Among the products higher protein content (11.54 %) was noted in the white flour followed by the (10.71 %) wheat flour, (9.21 %) in biscuitsand (7.75 %) in bread. Among corn products higher crude protein content was determined in oil popped popcorn (8.74%) followed by the air popped popcorn (8.67 %), corn flakes (6.26%) and corn flour(0.940 %). The mean values for NFE content of different wheat products have been shown in the Table 1 The NFE content in the wheat samples varied from 55.96-74.54%. The minimum mean value of NFE was found in bread (55.96%) followed by the biscuit (65.02%), white flour (74.20 %) and wheat flour (74.54 %).

Mineral composition

Iron content for the different wheat products which include T_1, T_2, T_3, and T_4 was ranged from 9.59 to 51.21 mg/kg. The highest value of iron (51.21 mg/kg) was determined in the T_1 followed by the T_3 (36.42 mg/kg), T_4 (28.75 mg/kg) and T_2 (9.59 mg/kg). The results were comparable with the finding of **Padovani*et al.* (2007).** They compared proximate, mineral and vitamin composition of common foods. Kadam*et al*. (2012) also found the similar results. They determined the iron content in whole wheat flour. Three elements group after milling showed major decrease in the concentration, i.e., Fe, Mg and Zn. Aleurone layer particularly abundant with these metals. Iron content was determined in the different corn samples which include S_1, S_2, S_3 and S_4. The highest value of iron (41.06 mg/kg) was determined in S_2 followed by the S_3 (27.90mg/kg), S_4 (26.70 mg/ kg) and S_1 (11.1 mg/kg). Calcium content for the different wheat samples was ranged from 38.49 to 355.20 mg/kg. The highest value of calcium (355.2 mg/kg) was determined in T_1 followed T_4 (230 mg/kg), T_2 (180.50 mg/kg), T_3 (38.49 mg/kg). Calcium content for the different corn products were ranged from 50 to 560 mg/kg. The highest value of calcium (560 mg/kg) was determined in S_2 followed by S_3 (100.21 mg/kg), S_4 (98 mg/kg) and S_1 (50 mg/kg). Zinc content for the different wheat samples was ranged from 7.89 to26.35 mg/kg. The highest value of calcium (26.35 mg/kg) was determined in T_3 followed by T_1 (17.32 mg/kg), T_2 (8.40 mg/kg), T_4 (7.89 mg/kg) . Zinc content for the different corn samples were ranged from 1.90 to 32.40 mg/kg. The highest value of calcium (32.40 mg/kg) was determined in S_4 followed by S_3 (25.40 mg/kg), S_1 (7.22 mg/kg) and S_2 (1.90 mg/kg). Potassium content for the different wheat samples was ranged from 990 to2468 mg/kg. The highest value of potassium (2468 mg/kg) was determined in T_4 followed by T_1 (1810.6 mg/kg), T_2 (1511 mg/kg), and T_3 (990 mg/kg). Potassium content for the different corn samples which include S_1, S_2, S_3 and S_4. The highest value of potassium (2946 mg/kg) was determined in S_4 followed by S_3 (2210 mg/kg), S_1 (1620.5 mg/kg) and S_2 (820 mg/kg). Magnesium content for the different wheat products was ranged from 106.5-1512 mg/kg. The highest value of Magnesium (1512 mg/kg) was determined in T_3 followed by T_1 (943.80mg/kg), T_2 (310 mg/kg), and T_4 (106.4 mg/kg). **Kadam *et al*., 2012).** Magnesium content for the different corn samples which include S_1, S_2, S_3 and S_4. Range of magnesium content was from 79 to 1310 mg/kg. The highest value of Magnesium (1310 mg/kg) was determined in S_4 followed by S_3 (1060 mg/kg), S_2 (505 mg/kg) and S_1 (400 mg/kg).

Tocopherol analysis

Comparison of mean of wheat samples which include T_1, T_2, T_3 and T_4 showed that higher amount of Alpha-tocopherol exists in T_4 (7.135 mg/100g) followed by

T_1 (4.21 mg/100g), T_2 (3.67 mg/100g) and T_3 (1.52 mg/100g) as shown in the Table 4.30. The finding of present study are in line with the finding of **(Piironen *et al*., 1986).** Even if cereal tocols vary in dependence on genotype and location. They reported that the richest source is wheat germ. The germ contains lipids, vitamins, and minerals. The lipids mostly consist of linolenic acid (2.3 Wt. %), linoleic (55 Wt. %) and oleic (1 8 Wt. %) (Kulp and Ponte, 2000).Tocopherol is the major fat soluble vitamin which is present in the germ. Corn products which include S_1, S_2, S_3 and S_4. Among these samples Alpha-tocopherol content was ranged from 0.3385 to5.885 mg/100g. The highest value of alpha-tocopherol (5.885 mg/100g) was determined S_3 followed by S_2 (3.59 mg/100g), S_4 (1.70 mg/100g) and S_1 (0.3385 mg/100g) . Gamma-tocopherol content for the different wheat products was ranged from 0.0002 to 2.81 mg/100g. The highest value of Gamma-tocopherol (2.62 mg/100g) was determined in T_3 followed by T_4 (1.197 mg/100g), T_2 (0.430 mg/100g) and T_1 (0.000212 mg/100g). The analysis of varience of gamma- tocopherol content in different samples of wheat are shown in the Table 4.33. Our results are also in good agreement with the finding of **(Piironen *et al*., 1986)**. Even if cereal tocols vary in dependence on genotype and location. They determined the tocopherol and tocotrienols in cereals from Finland. Our results are also supported by **Panfili*et al.* (2003)**. **Nielsen and Hansen (2008)** reported that gamma tocopherol is in traces in wheat flour. Corn samples which include S_1, S_2, S_3 and S_4. Among these samples Gamma-tocopherol content was ranged from 10.33 to 0.565 mg/100g. The highest value of Gamma-tocopherol (9.665 mg/100g) was determined in S_4 followed by S_2 (8.255 mg/100g), S_3 (2.77 mg/100g) and S_1 (0.5285 mg/100g). **Swiglo and Sikorska (2004)** also found the similar results. They determined that Gamma-tocopherol in corn oil is more than the alpha and sigma tocopherol. **Akram (2011)** determined the tocopherol contents of commercially available corn products. Our result are in line with the finding of **Akram (2011). Piironen*et al.* (1986)** determined the tocopherol and tocotrienols in cereals from Finland. Our results are also in good agreement with the finding of **Piironen *et al.* (1986).** Comparison of mean of wheat samples which include T_1, T_2, T_3 and T_4 showed that higher amount of Sigma-tocopherol exists in T_4 (0.6455 mg/100g) followed by T_3 (0.3965 mg/100g), T_2 (0.0888 mg/100g) and T_1 (0.00194 mg/100g). Our results are also in good agreement with the finding of **(Piironen *et al*., 1986).** They determined the sigma tocopherol from different cereal products which are in close agreement with our finding. **Nielsen and Hansen (2008)** reported that gamma tocopherol is in traces in wheat flour. Our results are aslo supported by **Panfili *et al.* (2003).** Sigma-tocopherol content for the different corn samples was ranged from 0.0144-1.685 mg/100g. The highest value of sigma-tocopherol (1.685 mg/100g) was determined in S_4 followed by S_2 (1.225 mg/100g), S_3 (0.3135 mg/100g) and S_1 (0.0144 mg/100g). Our results are also in good agreement with the finding of **(Piironen *et al*., 1986).** They determined the tocopherol and tocotrienols in cereals from Finland. Our results are also comparable with finding of **Akram (2011)**. They determined the nutritive quality and aflatoxins contents of commercially available corn products.

Table 1 Proximate composition of commercial corn and wheat products

Treatment	Moisture	Ash	Fat	Fiber	Protein	NFE
Wheat flour	11.20^{b}	0.74^{ab}	1.67^{c}	1.14^{a}	10.71^{b}	74.54^{a}
White flour	12.08^{b}	0.32^{c}	1.32^{c}	0.46^{b}	11.54^{a}	74.20^{a}
Bread	28.75^{a}	0.90^{a}	6.09^{b}	0.55^{b}	7.75^{d}	55.96^{c}
Biscuits	3.40^{c}	0.60^{b}	21.52^{a}	0.250^{a}	9.21^{c}	65.02^{b}
Corn flour	9.50^{a}	0.40^{c}	1.06^{c}	2.30^{d}	0.940^{c}	85.80^{a}
Corn flakes	2.75^{c}	1.90^{b}	0.75^{c}	3.0^{c}	6.26^{b}	85.34^{a}
Oil popped popcorn	2.82^{c}	2.70^{a}	26.0^{a}	3.40^{b}	8.74^{a}	56.34^{c}
Air popped pop corn	9.00^{a}	1.72^{b}	3.9^{b}	4.06^{a}	8.67^{a}	72.64^{b}

Note: Values in each row having the same letters are not significantly different (p>0.05). Values in each column having the superscript values are not significantly different (p>0.05).

Table 2 Mineral composition of commercial corn and wheat products (mg/kg)

Treatment	Iron	Calcium	Zinc	Potassium	Magnesium
Wheat flour	51.21^{a}	355.20^{a}	17.32^{b}	1810.6^{b}	943.80^{b}
White flour	9.56^{d}	180.50^{c}	8.40^{c}	1511^{c}	310^{c}
Bread	36.42^{b}	38.49^{d}	26.35^{a}	990^{d}	1512^{a}
Biscuits	28.75^{c}	230^{b}	7.89^{c}	2468^{a}	106.40^{d}
Corn flour	11.11^{c}	50.0^{b}	7.22^{c}	1620.5^{c}	400^{d}
Corn flakes	41.06^{a}	560^{a}	1.90^{d}	820^{d}	505^{c}
Oil popped popcorn	27.90^{b}	100.21^{b}	25.40^{b}	2210^{b}	1060^{b}
Air popped pop corn	26.70^{b}	98^{b}	32.40^{a}	2946^{a}	1310^{a}

Note: Values in each row having the same letters are not significantly different (p>0.05).Values in each column having the superscript values are not significantly different (p>0.05).

Table 3 Tocopherol composition of commercial corn and wheat products (mg/100g)

Treatment	Alpha-Tocopherol	Gamma-Tocopherol	SigmmaTocopherol
Wheat flour	4.21^{b}	0.000212^{d}	0.00212^{c}
White flour	3.67^{b}	0.430^{c}	0.0888^{c}
Bread	1.52^{c}	2.625^{a}	0.3965^{b}
Biscuits	7.135^{a}	1.197^{b}	0.6455^{a}
Corn flour	0.3385^{d}	0.5285^{c}	0.0144^{c}
Corn flakes	3.59^{b}	8.255^{a}	1.225^{b}
Oil popped popcorn	5.885^{a}	2.77^{b}	0.3135^{c}
Air popped pop corn	1.70^{c}	9.665^{a}	1.685^{a}

Note: Values in each row having the same letters are not significantly different (p>0.05). Values in each column having the superscript values are not significantly different (p>0.05).

CONCLUSION

The data indicate that commercial corn and wheat products vary greatly in term of protein, fats and crude fiber contents. The variability observed in carbohydrates, protein, fats, ash content, crude fiber and moisture content is both genetic and environmental which may influence the individual chemical composition. These results will be useful to know about the nutritional properties of commercial corn and wheat products and Food composition data is important in designing strategies for nutritional planning and provides data for epidemiological studies.

REFERENCES

AACC. (2000). Approved Methods of the American Association of Cereal Chemists. American Association of Cereal Chemists, Inc; St Paul, Minnesota, USA. http://dx.doi.org/10.1002/star.19890411114

Afzal, M., Nazir, Z., Bashir, M.H.B.S. & Khan,B.S (2009). Analysis of host plant resistance in some genotypes of maize against Chilopartellus (swinhoe) (pyralidae: lepidoptera). *Pakistan. Journal of Botany,* 41, 421-428.

Akram, S. 2011. *Assessment of nutritive quality and aflatoxin content of commercially available corn product,*MSc. (Hons.) Thesis.Dept. Chem. Biochem. Univ. Agri. Faisalabad, Pakistan.

Ali, M., Ali,W., Ahmed,S. & I. Ullah.(2008). Mineral Composition, Quality and Physico chemical Parameters of the Local Tallow of Pakistan. *Pakistan Journal of Nutrition*, 7, 717-720. http://dx.doi.org/10.3923/pjn.2008.717.720 .

Baloch, U.K., Irshad, M. (1986). Post-harvest research on food grains in Pakistan (A Review). *Pakistan Agriculture Research Council*, 2, 45-46.

Carvalho, V.P., Ruas, C.F., Ferreira, J.M., Moreira, R.M.P. & Ruas, P.M.(2004).Genetic diversity among maize (*Zea mays* L.) landraces assessed by RAPD markers.*Genetics and Molecular Biology.* 27,228-236. http://dx.doi.org/10.1590/s1415-47572004000200017 .

Kadam, M.L., Salve, M.L., Mehrajfatema,Z.M. & More.S.G.(2012). Development and evaluation of composite flour for missi roti /chapatti. *Journal of Food Processing Technology*. 3, 122-125. http://dx.doi.org/10.4172/2157-7110.1000134

Khan, A., Jan, M.T., Marwat, K.B. & Arif, M. (2009). Organic and inorganic nitrogen treatments effects on plant and yield attributes of maize in a different tillage systems. *Pakistan Journal of Botany.* 41, 99-108.

Kulp, K., and Ponte, J.G. (2000). *Handbook of cereal science &technology*. New York, NY: Marcel Dekker. pp. 431-438.

Niaz, I., Dawar, S.(2009).Detection of seed borne mycoflora in maize (Zea mays L.). *Pakistan Journal of Botany.* 41,443-451.

Nielsen, M.M., Hansen, A. (2008). Rapid High-Performance Liquid Chromatography Determination of Tocopherols and Tocotrienols in Cereals. *Cereal Chemistry*. 85, 248–251. http://dx.doi.org/10.1094/cchem-85-2-0248 .

Padovani, R.M., Lima, D.M., Fernando, A.B.C., & Rodriguez-Amaya,D.B. (2007). Comparison of proximate, mineral and vitamin composition of common Brazilian and US foods. *Journal of Food Composition and Analysis.* 20, 733–738. http://dx.doi.org/10.1016/j.jfca.2007.03.006 .

Panfili, G., Fratianni, A., & M. Irano, M. (2003). Normal phase high-performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals. *Journal of Agricultural and Food Chemistry.* 51,3940-3944. http://dx.doi.org/10.1021/jf030009v .

PARC(2007).Status paper on maize, sorghum and millet.www.parc.gov.pk/1SubDivisions/NARCCSI/ CSI/StatusPaper.doc <http://www.parc.gov.pk/1Sub Divisions/NARCCSI/CSI/StatusPaper.doc>.

Parveen, S. (2007). *Baking quality of wheat flour cookies supplemented with fiber from different sources.* MSc. (Hons.) Thesis. Dept. Food Sci. Tech. Univ. Agri. Faisalabad, Pakistan.

Piironen, V., Syvaoja, E.L., Varo, P., Salminen, K., & Koivistoinen, P. (1986). Tocopherol and tocotrienols in cereals from Finland. *Cereal chemistry*. 63, 78-81. http://dx.doi.org/10.1021/jf00070a038 .

Rehman, Z., (2011). Effect of supplementation of hydrocolloids and potato flour on the functional characteristics and shelf life of bread. MSc. (Hons.) Thesis. Dept. Food Sci. Tech. Univ. Agri. Faisalabad, Pakistan.

Steel, R.G.D., Torrie, J.H., and Dicky, D.A., (1997). Principles and Procedures of Statistics. A Biometrical Approach. (3rd Edition). McGraw Hill Book Co. Inc., New York.

Swiglo, A.G. and Sikorska, E. (2004). Simple reversed-phase liquid chromatography method for determination of tocopherols in edible plant oils. *Journal of Chromatogry Analysis*. 1048,195–198. http://dx.doi.org/10.1016/j.chroma.2004.07.051 .

Zand, N., Chowdhry,B.Z., Zotor,F.B., Wray,D.S., Amuna, P., and Pullen, F.S.(2011). Essential and trace elements content of commercial infant foods in the UK. *Food Chemistry*. 128,123-128. http://dx.doi.org/10.1016/j.foodchem.2011.03.005 .