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Use of Infrared Thermography in Determining Meat Quality

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Abstract

In recent years, meat quality has started to take an important place in food products. Situations such as stress, pain, and infection before slaughtering reduce the quality of the meat. There are many different techniques used in determining meat quality, these are include invasive and non-invasive techniques. Because of advances in technology, the popularity of non-invasive techniques has increased. Thermography can be considered as the most recent of these invasive techniques. Thermographic measurement can be made from different parts of the body such as skin and eye. Due to the minimal artifact formation, measurement of eye temperature is used more frequently than other areas of the body. This review is aimed to give information about the non-invasive detection of meat quality by infrared thermography.

Keywords: Artifact, eye, food, non-invasive, temperature.

Review article
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INTRODUCTION

Meat and meat products are the main sources of animal protein in human life. In addition to containing high-quality protein, meat contains vitamins, minerals, and other substances necessary for human health (Nurhayati et al. 2016). Advances in people's living standards have led to questioning the quality and safety of the food products that people consume. Chemical methods, mechanical methods, and inspection methods, which are among the traditional methods used in determining the quality of meat and meat products, have begun to leave their place to non-invasive methods because they are both times consuming and boring (Xiong et al. 2017). Non-invasive techniques recently used to determine meat quality include ultrasound imaging, dual-energy X-ray absorptiometry, tomographic imaging, optical imaging, magnetic resonance imaging, biopsy, and odor imaging (Scholz et al. 2015; Xiong et al. 2017).

Pre-slaughter stress

Stress is one of the most important factor affecting eating and meat quality. It has determined that the stress level before slaughter is directly related to the softness, aroma, juiciness, and shelf life of the meat (Warner et al. 2007). Many studies have reported that handling and stress applied to the animals before slaughter affect the meat quality by changing the pH and color, muscle glycogen, and rigor temperature of the meat (Hughes et al. 2014; Jorquera-Chavez et al. 2019).

Metabolic, behavioral, and physiological indicators can be used to determine stress in animals (Xiong et al. 2017). Metabolic indicators include the measurement of enzymes, hormones, and metabolites in blood plasma (Möstl and Palme, 2002). Behavioral indicators include lying, vocalization, and aggressive behaviors such as kicking (Rocha, 2016). Physiological indicators of stress are heart rate, heart rate variability, respiratory rate, body, and skin temperature (Mohr et al. 2002).

Measuring body temperature

Stimulation of the autonomic central nervous system increases metabolic activity and heat production by making changes in blood flow and peripheral vascular tone (Terrien et al. 2011). This situation causes an increase in body temperature. Previous studies have shown that body temperature in animals is directly related to stress (Stewart et al. 2005; Pighin et al. 2014). Body temperature can be measured with invasive and non-invasive ways; each has its own advantages and disadvantages. Although there are different anatomical sites used for measuring body temperature in animals, the most widely used region is the rectum (Giannetto et al. 2020). However, rectal measurement of body temperature is time-consuming and difficult in aggressive animals (Piccione et al. 2009). Therefore, temperature-detected microchips have been used in recent years (Jorquera-Chavez et al. 2019). These microchips can be implanted to the rumen, reticulum, ear, skin, or stomach (Prendiville et al. 2003; Gonzalez-Rivas et al. 2018; Jorquera-Chavez et al. 2019; Giannetto et al. 2020). However, since these microchips can migrate to other parts of the body, it may cause accidents, especially when eating meats offered for consumption (Pizzuti and Mirabelli, 2016).

Infrared thermography is a technique that measure the temperature of an object without any contact. In nowadays, this technology is using for the detection of body temperature in animals (Tattersall, 2016). So many anatomical locations have been used to detect body temperature in animals with infrared thermography such as skin and eye.

The previous studies have shown that the technique is highly correlated with animal core body temperature (George et al. 2014). Although the measurement of skin temperature is easier than the measurement of face temperature, the presence of residues, hair, feces, and water on the skin causes artifacts results by changing the temperature values (Weschenfelder et al. 2013). Because the eye is close to the brain and there is no possibility of artifacts that may occur in these areas, it is a suitable location for measuring the body's core temperature (Horcada et al. 2020). However, it has been stated that in commercial settings eye temperature becomes difficult to measure as animals shake their heads constantly (Rocha et al. 2019).

Relationships between ocular temperature and other biomarkers

Eye temperature is strongly correlated with meat color and pH. It has been reported that eye temperature is elevated when the meat color is darker than a score of 3. During stressful events, the animal body starts to use glycogen from the muscles. As a result of glycogen deficiency during slaughter, an insufficient amount of lactic acid will be produced and the pH of the meat will remain high, leading to dark, firm, and dry meat (Devine et al., 2006; Gregory, 2008). Since Creatine kinase and Aspartate transaminase have a direct relationship with muscle damage and physical stress (Mpakama et al. 2014; Tvarijonaviciute et al. 2017), eye temperature may increase.

CONCLUSION

The assessment of eye temperature in cattle is directly related to meat quality, as it may be a sign of pre-slaughter stress and pain in animals. This makes thermography a suitable technique for evaluating both meat quality and animal welfare.

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Value-added Fish Products

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Abstract

Fish industry is rising day by day, resulting in various by-products. By-products obtained from fish are highly beneficial and attracting the customers both from national and international market now a days. The various by-products obtained from the different portions like skin, bones, head, and viscera of fish. Different protein recovery methods are used to obtain hydrolysates that have properties of antioxidants, emulsifiers, gelatins agents etc. Fish protein hydrolysates (FPH) is also used as fish-based fertilizers for animal feed. Fermented products include fish sauce or fish oil. Surimi is a protein paste obtained from fish meat, having high nutritional value. Collagen and gelatins are derived from fish skin and bones and are being used in cosmetic, pharmaceutical and meat business. Enhanced consumption of fish waste materials as food not only lessens numerous environmental issues but it also helps in production various value-added products. Thus, it is essential to maximize the production of fish by products. In this review, various fish by-products and their benefits are discussed.

Keywords: Fish by-product, surimi, hydrolysates, collagen, fermentation

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INTRODUCTION

From last few decades, fish industry is growing constantly and producing a significant number of by-products. The by-products obtained from this industry include fish head, skin, bones thrones and viscera. Some portion is preceded for animal feed as protein source, collagen and fish oil production. Moreover, biofuels, fertilizers and some high value bioactive commands can also be obtained. Fermentation process which is customarily used for increasing shelf life of fish also develops bacterial metabolites of interest. Application of fermentation to by-products, quality of protein hydrolysates oils and production of antioxidants can also be increased. This technology can be viewed as promising field of study, which is safe, effective, low energy expenditures and environmentally friendly, presents points of interest for future application.

Fish is valuable source of protein also contains important minerals and fatty acids, has good nutrient digestibility and wholesomeness to satisfy customer's expectations (Nordvi et al., 2007). The provision of fish products constantly will extend the range of health promoting fish-based products and provide a viable option to upgrade the low value species and waste generated by fish processing industry.

Recovery methods of fish proteins

Most commonly used techniques for protein recuperation from processed fish by-products, have been used since last many years. All methods principally either degrade the peptide bonds of protein to release peptides, amino acids or isolate protein by changing its pH. An effective way of producing bioactive peptides or protein hydrolysates that recognize the native protein and subsequently recovering the peptides and amino acids by applying enzymatic, chemical or fermentation processes (Li-Chan, 2015). Recovery method techniques used for fish proteins depend on factors like cost, quality and time. Elimination of insoluble from FPH, produced during recuperation process, is carried out by hydrolysis using either centrifugation or microfiltration. This process of elimination continues until refined color of hydrolysate is attained (Pasupuleki and Braun, 2010).

Enzymatic hydrolysis

Proteolytic hydrolysis is a promising technique to recover hydrolysate from fish protein. Following method is performed by the use of endogenous enzymes and exogenous enzymes. It is a partially hydrolysis and properties of native proteins can be upgraded (Althouse et al., 2018). The enzymes commonly involved Alcalase,, Protamex α-chymotrypsin, Neutrase, Flavourzyme, pepsin, trypsin, pancreatin, bromelain, Pronase E, Orientase, thermolysin,, papain, Validase, Protease A amano, Protease N amano, and cryotin F (Chalamaiah et al., 2012). Processing conditions for example recommended temperature and pH must be in accordance to achieve the ideal action of the enzymes including hydrolysis time which is a main determinant in the production of hydroxylates with activity of antioxidant (Samaranayaka and Li-Chan, 2011). Numerous researches stated that peptides or hydrolysates obtained via proteolytic hydrolysis are source of antioxidants (Yarnpakdee et al., 2015; Jemil et al., 2014).

Chemical Hydrolysis

This technique of hydrolysates recuperation process includes either acid or alkaline results in the breakdown of fish protein into peptide fragments of various sizes and free amino acids. It is generally simple and relatively inexpensive method. In this process, sulfuric acid and hydrochloric acid are utilized at high temperature and pressure (Kristinsson and Rasco, 2000). Hydrolysates obtained using acid hydrolysis process is highly soluble but may be bitter with poor quality and nutritional values (Chobert et al., 1996).

Fermentation hydrolysis

Biochemical process such as fermentation process uses microbes and their metabolites to breakdown the fish proteins. Fermentation hydrolysis commonly uses lactic acid bacteria which produce lactic acid as finishing product of fermentation. Fermentation has been used since early history to preserve the food from microbial spoilage. However, it has become a great concern for health researchers, as it increases the level of bioactive compounds in food products as well as decrease anti-nutrient factors through hydrolysis. The one of the major benefits to consume fermented products is the ingestion of living microorganism together with food, some of them act as probiotics which improve the digestion and intestinal health and inhibit the colonization of pathogenic bacteria in the gut.

Physicochemical problems linked with fish proteins

Protein hydrolysates are made up of amino acids and peptides resulting from partial hydrolysis (Schaafsma, 2009). Local proteins improve their physicochemical and sensory functions via hydrolysis (Kristinsson and Rasco, 2000). Bioactive peptides are originated from fish protein hydrolysates (Chalamaiah et al., 2012). Marine products demand and consumption is increasing, resulting in increased fish processing byproducts or waste. Leftover by-products can be used for specialty foods with values such as vegetable manure; livestock feed and fish oil, which can be effectively separated. Huge amounts of byproducts coming from fish processing can cause alarming elimination problems in industrial and developing countries (Villamil et al., 2017).

The proteins from fish muscle are more vulnerable to denaturation. This technique and processing situations are serious issues in achieving the desired quality of hydration. Recovery methods for hydrolysate depend on the kind of fish and processing (Kristinsson and Ingadottir, 2006). The unpleasant flavor of hydrolysates is a key task in the development of hydrolysate products. This process is multiplied by peptide bonds that are 100% of the total number of peptide bonds in the native protein (Pasupuleki and Braun, 2010)

Fish and fish products have physiochemical properties that may vary in sensory attributes. Clearly, the hydrolysis of the protein results in better functions such as emulsification, solubility, water holding capacity and gelation (Foh et al., 2011). The level of hydrolysis which quantifies the degree of protein debasement is a limiting factor for the procedure (Quist et al., 2009. The strategy for proteolytic change of food proteins to enhance the tastefulness and timeframe of realistic usability of the accessible protein asset goes once more into the old occasions (Taha and Ibrahim, 2007). Relatively, protein hydrolysis forms peptides with enhanced valuable and tactile properties than their local proteins (Cheison and Wan, 2003).

Be that as it may, the FPH and disengages are messed with as they experience the different preparing steps. To an ever-increasing extent, mass and huge scope creations of FPH have been obliged by taste surrenders and the financial results that accompany hydrolysates. Despite the fact that the unpleasant taste related with FPH is as yet not completely comprehended, it is estimated to be brought about by the presentation of the hydrophobic tail of the amino corrosive during protein collapse. The strategy for hydrolysis altogether influences the nature, arrangement, and physico-synthetic characteristics that describe the hydrolysate. Catalyst hydrolysis has been the broadly utilized strategy for protein hydrolysis (Hou et al., 2017). The various properties of FPH, for example, water holding limit, solvency, emulsion capacity, frothing limit, and gelling capacity, could be used in various food definitions (Chalamaiah et al., 2012).

Fermented fish products

Numerous countries across the world use fermented fish as a conventional food, particularly in Asia, Europe and Africa as well. As fish owing short shelf-life, the human wanted from the day first to increase their shelf life. Among the ferments products the utmost one is the fish sauce. The by-products are gained as an outcome of fish handing out at an industrial level from aquaculture. Such consequences signify about 60.00 percent of entire fish mass afterwards industrial processing, though it varies rendering to species (Ideia et al., 2019). A huge variety of superior component can be obtained and may be employed for human ingesting (Zamora-sillero et al., 2018; Ideia et al., 2019). As previously stated, by-products can characterize a major fraction of fish total weight, with the upsurge in global fish request, the by-products likewise rise.

For example, fish by-products are largely employed for production of oil and animal feedstuff (Vazquez et al., 2019). As it is recognized, fish are an ironic basis of long chain v -3 fatty acids, being liver the key tissue wherever lipids are accrued, while they might be too originating in other wastes like the skin or head (Rai et al., 2010). One of the furthermost extensive use for fish waste is the animal feed production (Mo et al., 2018). For this aim, protein hydrolysates gained from fish wastes are used, as it is the valuable means for healthier adaptation of the proteins, in order to obtain an improved animal daily protein intake (Saadaoui et al., 2019). In count, the other practices for fish wastes are the production of biofuel, while not as common as production of oil or animal feed, are likewise a significant share of the industrial movement assigned to the fish by-products (Cadavid-Rodriguez et al., 2019; Radziemska et al., 2018). Lastly, one more significant use of fish waste is for obtaining enzymes. The chief group of aquatic enzymes is proteases, which have an imperative profitable practice. These mostly comprise pepsin and trypsin, while collagenase, elastase, and chymotrypsin are also available (Derouiche et al., 2019). The usage of enzymes is vital in a huge figure of industrial applications like food technology (Saranya et al., 2018).

Fermentation is a harmless method, biologically friendly and profitable, which permits gaining an extensive variation of composites, including bioactive peptides. Currently, this technique is being used to get the peptides by the use of bases or acids to encourage protein hydrolysis, mostly due to its cost effectiveness (Suresh et al., 2018, Ramirez et al., 2013), so it may be considered beneficial implementation in nutritional class of fish goods that are known as main source of protein in feed (Ozyurt et al., 2019). Via fermentation, superiority of oil obtained from fish by-products rises in contrast with action with formic acid (Ozyurt et al., 2018).

Hydrolysates have numerous functional properties primarily solubility, emulsifying and foaming attributes, fat binding and water holding capacity. Molecular weight and structure may affect these functions, playing a vital role in their utilization as nutritional supplements, gelling agents and emulsifiers (Tahergorabi et al., 2015).

Surimi

Myofibril proteins formed a blend known as surimi and this protein could be acquired from flesh of fish. Fish washed with salt solution to clear away sarcoplasmic proteins and preserved with the incorporation of cry preservatives. Revamp fishery merchandise are contrived from minced muscle, that is used to a procedure of jellification. In subject to enhance the gel making properties, some specific methods and binding agents like transglutaminase, could be selected for this process (Uresti et al., 2004). A Japanese term used for stable myofibrillar protein paste of fish meat known as surimi, having extra ordinary nutritive value and capability of gel formation (Moreno et al., 2016). However, generally made from fish species, fresh water species of fish have been recognized as desirable substrates for surimi production due to general reduction in worldwide harvest of aquatic fish and persistent development in fish framing. The aquatic business uses white muscle for processing of surimi, and fish wastes that alleged for 60 to 70% of the weight of fish are commonly thrown out as waste, which may causes environmental impediments (Torres et al., 2007). As economical sources of rich protein, these wastes could be converted into value added biological active hydrolysates, peptides and additionally, consumed in food systems as nutritional, physiological and protein functionality modifiers (Nikoo and Benjakul, 2015).

For economical seafood production washed fish mince or surimi is a good source. Unwashed fish mince also a good economical source having nutritional prime importance containing water soluble vitamins, lipids and minerals, economically low in price and enhanced protein yield. Magnusdottir (1995) elaborated higher yield in fish mince are specifically significant nowadays as many conventional fish resources around the globe lead to decrease. Surimi can be used directly in several systems of food and in a physically or chemically changed form to generate nutritional and functional products (Babbitt, 1986).

Surimi has some exceptionally extraordinary traits, like gel foaming, emulsifying and water binding characteristics. The higher amount of myofibril protein empowers the product to form a gel upon heating to form an elastic texture (Lanier, 1986). Under low constant storage temperature, surimi at frozen temperature can be stored up to 1-2 years without any major alterations in functional characteristics (Bertak and Karahadian, 1995). Commercial blocks of surimi are made with the label “good if used within 2 years”. Park and Lin, 2005 explained because of its peculiar properties, surimi is considered a raw material for kamaboko processing (Holmquist et al., 1984) and surimi products with several types and spice of crab meat (Verrez et al., 1992), lobster meat (Moskowitz and Porretta, 2002), and shrimp meat. Moreover, its application has been prolonged further shellfish analogs. It includes fish nuggets, frankfurters, and fish patties (Buck and Fafard, 1985; Destura and Haard, 1999).

Nowadays, fish industry has raised demands for consumption of all raw materials. Regrettably, seafood processors using only 15-30% of the harvest for producing fish fillets and surimi (Wendel et al., 2002). Enhanced consumption of fish fillet or improve quality of surimi by-product as food not only solve several environmental problems (Martin, 1992), but it is also being used for the production of value added fish products (Hoke et al., 2000).

Properties of Surimi

Functional characteristics of proteins display their capability to form gels and holding oil or water or examine visually as whiter. These contain solubility, water absorption and binding capacity, gelatin, viscosity, swelling capacity, emulsifying capacity. Among all, the crucial functional feature in surimi is the gelling feature (Marsili, 1993). Gelation is thermo irrevocable (Chang and Regenstein, 1997). Fish myofibril protein, which is the main culprit for gelation, can form a strong and elastic gel upon heating (Lanier, 1994). Fish myofibril proteins may be heated to high temperatures without give up gel strength or water holding capacity. The functional feature of surimi can majorly afflicted through the biological traits of the fish such as their species (Pipatsattayanuwong, 1995) seasonality, sexual puberty, rigor and some external factors such as (Park and Lin, 2005) harvesting and onboard handling, processing water (Lin and Park, 1997), time, temperature (Suvanich et al., 2000) washing cycle (Chand and Regenstein, 1997), pH, (Thawornchinsombut, 2004), salinity, and functional ingredients (Lian et al., 2000).

Oily/red fish fleshed can be utilized to prepared mince or surimi. Although, the quality of surimi changes with respect to the whiteness, trimethylamine oxide (TMAO), and fat constituents of the fish. To make surimi with storage strength, few steps must be followed to contradict the belongings of the heme proteins and oil (Putro, 1989). Heme proteins like myoglobin and hemoglobin, is responsible for the red coloration of dark muscle. Moreover, oxidation of fat muscles in dark muscles is helped by these heme proteins, which causes a rancid odor. Antioxidants or vacuum packaging can be utilized to avoid from lipid oxidation in flesh of the fish (Chen, 2002). The functional characteristics of surimi are affected by fish freshness. It is possible due to freshness affects the biochemical traits of fish muscle. Freshness of this fish is mainly dependent on temperature or on time. Freshness of fish is also be affected by situations of harvesting and approach used for capturing of fish, such as weather situations at sea, salt uptake, length and size of tow and temperature of fish after capturing, and on-board approach of handling and vessel storage situations

For fish capturing, time and temperature are the crucial factors to determine the functional characteristics of surimi. Several proteolysis changes could be occurring in the final products, surimi, if prolonged the duration of storage or processing time and raised the temperature of storage and processing procedure. Therefore, to prepare surimi with healthy functional characteristics, fish must be processed immediately on capturing or kept at 0 ℃ when holding is compulsory.

Collagen and Gelatins

Fish by-product speak to almost 30% of fish fillet handling waste. These are created as an outcome of the arrangement of various fish items. Collagen is the basic protein found in the skin and bones of creatures and gelatins are their debasement items. Bones and skin are consequently a rich wellspring of collagen and gelatin, and a few investigations have been done to get collagens from wastage including bones, fins, skin and scales of various species of fishes, and spineless creatures, that in any case would be discarded (Morimura et al., 2002; Senaratne et al., 2006). The collagen yield got from various sources extended from 36% - 54% (Nagai and Suzuki, 2000). The collagen possible uses include: consumable housings for the meat businesses, beauty care products (as it has great saturating properties) (Swatschek et al., 2002) and biomedical or pharmaceutical applications, which incorporate creation of wound and cut dressings, vitreous inserts or bearers for sedate conveyance (Takeshi and Suzuki, 2000).

A few studies likewise show that collagen might display high enemy of radical action (Morimura et al., 2002). Fish gelatins might likewise be utilized as emulsifiers in various food items (Surh et al., 2006) since it has been seen that gelatin from fish can balance out emulsions, tolerably stable to bead conglomeration and creaming, significantly subsequent to being exposed to changes in temperature, salt concentration and pH.

CONCLUSION

Investigations on the re-utilization of fish by-products can add to increment modern development by maintainable create mint. As appeared here, aging may give a subsequent life to angle side-effects from angling or aquaculture to acquire bioactive peptides, biocides, additives, cancer prevention agents or different items. In this way, fish waste could be a significant wellspring of mixes important to food or pharmaceutical industry. This is a valuable procedure for fish squander valorization, along these lines assisting monetarily and limiting the natural effect, and adding to the maintainability of fish creation. Moreover, it ought to be noticed the possible utilization of aging as a perfect innovation for the creation of these mixes of intrigue.

The principle issues to be confronted when attempting to actualize and implement the board quantifies in fishery exercises are those identified with the framework required both on board vessels and shoreward. As most of the fish species are highly perishable, quick handling is required. This prompts the need to build up and actualize conventions of side-effect detachment, classification and capacity, just as recommendations for preservation or pre-preparing options whenever the situation allows, either ready or ashore, in order keeping up the materials in the proper handling conditions. A portion of the principle impediments in actualizing the innovation to acquire benefits from disposes of and by-get are the accompanying:

- Scant interest in latest advancements applied to disposes of and by-catch to acquire advanced included worth items.
- Restricted storerooms of trawlers and inclination of augmenting stockpiling of species with high business esteem, instead of those disposes of or by-get, which as of now have a lower showcase cost.
- Lack of a worldwide approach system and serious enactment with respect to disposes of and by-get.

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Revelation and Determinationof 3-Monochloropropane-1,2-Diol (3-Mcpd) in Bread from Modern Bakeries in Khartoum State, Sudan

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Abstract

This study was carried out for the revelation and determinationof 3-Monochloropropan-1, 2 diol (MCPD) in bread from modern bakeries in Khartoum state, Sudan. Twenty five samples (25) of bread were collected from different modern bakeries of Khartoum, Khartoum Bahari and Omdurman, they include, round bread (13) samples and long bread (12) samples. The moisture content and oil content of samples were determined according to the AOAC method and the quantity of 3-MCPD by Gas Chromatography Mass Spectrometry (GC-MS) was investigated. 3-MCPD exposure for individual by body weight per day for adults and children was estimated. Moisture content in round bread ranged from 31.7% to 35.7%, and in long bread from 28.2% to 33.6%. Oil content in round bread ranged from 0.7955% to 0.9894% and oil content in long bread from 0.7684% to 1.005%. 3-MCPD was detected in round bread and long bread with a concentration of 0.15493-1.05873ppm to 0.11334-0.59644ppm, respectively, which were lower than that of European Commission Scientific Committee for Food Standardization. Exposure of adults and children to 3- MCPD upon consumption of bread is estimated to be (0.015446-0.031277µg/kg)-(0.002397-0.014118µg/kg)-(0.018039-0.036528µg/kg) and (0.002799-0.016488µg/kg) for adult and children, respectively. These exposure levels do not constitute a health risk.

Keywords: 3-Monochloropropan-1, 2 diol, round bread, long bread, Gas Chromatography Mass Spectrometry

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INTRODUCTION

3-monochloropropane-1, 2-diol (3-MCPD) esters are food preparation contaminants found in vegetable oils and foodstuffs (Crews et al. 2013).The European commission scientific committee for food established maximum tolerable daily intake (TDL) for 3-MCPD of 2ug/kg of body weight per day, and the lowest observed effect at 0.1ug/kg body weight per day. Amounts of 3-MCPD measured in a bakery fat and/or retail soft dough biscuit were 1285 and 632ug/kg and consistent with amount reported previously, and the concentration of free 3-MCPD in bread samples was at interval <9-54.5ug/kg (Dolezal, et al. 2009). Hamlet et al. (2004), estimated 3-MCPD-esters in long bread most elevated levels were found in areas of the bread that accomplished the most noteworthy temperature, i.e the crust, and level expanded from 60-160ug/kg when the bread was long more than 40-120 seconds. The most elevated level of 3-MCPD-esters (6100ug/kg-1) was found in French fries (Svejkovska, et al. 2004).

In addition, domestic processing can create generous increments in the 3-MCPD substance of bread or cheddar (Breilling – utzmann et al. 2003). Stauf, et al. (2020) evaluated the content and formation of free and bound monochloropropanediol and bound glycidol in some bakery products include short bread. Their analytical screening revealed, that free 3-MCPD can be quantified in products with low water and fat content, which are produced using high baking temperatures. A few investigations on the component of the 3-MCPD arrangement have been performed (Hamlet et al., 2003; Muller, et al. 2005), and demonstrated that it is framed from glycerol or acylglycerols and chloride particles in heat prepared foods that contain fat with low water activity as expressed by Delezal, et al. (2009). In spite of the fact that the general degrees of 3-MCPD in baking items are moderately low, the elevated level of utilization of bread, and its extra development from longing, demonstrate that this staple food alone can be a critical dietary source of 3-MCPD (Breilling- Utzmann, et al. 2003).

Heat processing of lipids with added sodium chloride occurring 3-MCPD in the present of acid hydrolyzed vegetable protein, hydrolysis of 3-MCPD esters by lipases as can occur in baked bread (Baer, et al. 2010). Hamlet, et al. (2004), measured 3-MCPD-esters in long bread, highest levels were found in regions of the bread that attained the highest temperature, i.e the crust, and level increased from 60-160ug/kg1when the bread was long over 40-120 seconds, the highest level of 3-MCPD-esters (6100ug/kg-1) was found in a sample of French fries Svejkovska, et al. (2004). Belkova et al. (2020) investigated the influence of dough composition on 3-MCPD esters, formation during bread toasting. They found that, toasting of bread for 2.5 min considerably enhanced the formation of acrylamide and 3-MCPD esters. 3-MCPD was detected and quantified by direct, and indirect methods e.g. derivatization and GC-MS analysis, DGF method C-VI 18 10, and SGS 3-in-1. In Sudan there is no data about the 3-MCPD in bread, although, Sudan consumes a large amount of bread and vegetable oils. And consequently detection and quantification of 3-MCPD in modern bakeries bread is important to evaluate the extent of potential health risk. The study aims to provide information about detection and quantification of 3-MCPD in modern bakeries bread in Khartoum state, Sudan, and assessment of the exposure levels of the population.

MATERIAL and METHODS

Samples collection

Twenty five samples of round and long bread were collected randomly from various bakeries of Sudan capital state (Khartoum, Khartoum Bahri and Omdurman).The samples were divided into two groups, (12 long breads and 13 round breads), 12 long breads were collected from different areas of Khartoum, 4 samples were collected from various bakeries Khartoum (Sharwani, Burri, Manshiya and Jabelawlya), 4 samples were collected from various bakeries of Khartoum Bahri (Dar alssam, Mazad, Danagla and Margania) and four sample were collected from Omdurman (Almulazmeen, Shuhada, Fetihaab and Shigla) and the second group is 13 samples round breads were collected from bakeries of Khartoum, Khartoum Bahari and Omdurman. Divided into the 5, 4, and 4 samples, respectively. Five samples from bakeries of Khartoum (Duyum east, Duyum west, Hila Jadida and Alsajana), four samples from bakeries of Khartoum Bahri (Kadaru, Al-uzba, Kobar, and Kafuri) and four samples from Omdurman bakeries (Salha, Jaddain North, Jaddain South, and Libya market).

Reagents and Chemicals for 3-MCPD

NaCl (p.A), phenyl boronic acid (≥98%, PBA), acetone, hexane, methyl tert-butyl ether (MTBE), methanol, and ethyl acetate (all suprasolv for GC) as well as CH_3COOH and H_2SO_4 96%, were obtained from VWR (Damstadt, Germany). CH_3NaO (25% w/v in methanol), 3-monochloropropane-1, 2-diol (98%), and 3-methoxypropane-1, 2-diol (98%) were bought from Sigma Aldrich (Weinherin, Germany). A solution of 50 µL of H_2SO_4 in 5 mL of CH_3OH was arranged along these lines for change of glycidyl esters (CH_3OH / H_2SO_4). 1, 2-dipalmitoyl 3-chloropropane and glycidyl palmitate were bought from Campro Scientific (Berlin, Germany). A NaCl solution (NaCl solution 20%) of 200 g/L was prepared in deionized water. The Derivatization reagent PBA was prepared by dissolving 5 g of PBA in 19 mL of acetone and 1.0 mL of deionized water.

Equipments

Glass wool, Soxhlet, Mortar Pestle, Sensitive Balance, Vortex mixer (2500 rpm, 30 s), Micro pipette, Beakers, Volumetric flask And Gas Chromatography-Mass Spectrometry (GCMS) from Japan, Shimadzu Company.

Methods

Determination of moisture content

Sample was weighted and then registered weigh of wet samples, dried at temperature (105ºC) in oven for 6 hours and moisture content was determined according to AOAC method (2000).

Extraction and determination of oil

About 200g of dried bread was used to extract the oil following the AOCS Am 2-93 method (AOCS 2017) using Soxhlet apparatus. The extracted oil from all the samples, was kept in plastic bottles (polyethylene), and stored in freezer at temperature (-18ºC), until analyzed.

Analysis of 3-MCPD using PBA derivatization

Investigations were performed by Breitling-Utzmann, et al. (2004). The subjective and quantitative examination of the sample was done by utilizing GM/MS procedure model (GC/MS-QP2010-Ultra) from Japan, Shimadzu Company, capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). The sample was infused by utilizing split mode, helium as the bearer gas went with stream rate 1.20 ml/min, the temperature program was begun from 100ºC with rate 20ºC/min to 160ºC hold for 1.0 moment then the rate was changed to 5ºC/moment to 180ºC, at last, the rate was changed to 30ºC/minutes arriving at 300 as definite temperature degree, the infusion port temperature was 320ºC, the particle source temperature was 230ºC and the interface temperature was 300ºC. The example was broke down by utilizing SIM mode choosing m/z 91, 147, 196, the all out run time was 15 minutes, and results were recorded. At that point the measure of 3-MCPD was processed. Three tests of each sample were made.

Concentration of 3-MCPD exposure assessment for adults and children

For estimation exposure of individual to concentration of 3-MCPD at specified point in a day, twenty breads were give (4-6g) oil, average weight of round bread (65g) and long bread (55g). Individual consumption per day in Sudan about 10 breads, for adults 18-20+ years was estimated, and 6 bread for children 14-15 years. Body weight for deterministic exposure estimates based on the WHO/JEFCA.(2007), simulated diets, mean body weight for adults 68.03kg and children 47.67kg. Concentration of 3-MCPD body weight per day for individual were determined (Vannoort and Thomson 2005).

RESULTS and DISCUSSION

Moisture content for round and long bread from the different bakeries

Table 1 reports the moisture content of round bread (RB) and long bread (LB) collected from different bakeries of Khartoum state (Khartoum, Khartoum Bahri and Omdurman), the ID number from 1-9 represents moisture content of bread collected from Omdurman, while ID number from 10-17 idealizes moisture content of bread collected from Khartoum and ID number from 18-25 represents moisture content of bread collected from Omdurman .There was no significant difference between all bread collected from different areas of Khartoum state. Lower moisture content in RB were 31.7%, while medial moisture content in round bread were 33.56% and higher moisture content in round bread were (35.8%). While the lower moisture content in long bread were 27.2%, medium moisture content in long bread was 31.02%, and higher moisture content in long bread were 33.6%. From this information moisture content in round bread higher than moisture content in long bread.

Table 1. Moisture and oil contents of round and long bread

ID	Samples	Moisture content (%)	Oil Content (%)
1.	Modern LB	31.9	0.768
2.	Modern LB	30.8	0.9465
3.	Modern LB	28.4	0.998

4.	Modern LB	33.1	0.9955
5.	Modern RB	34.4	0.7955
6.	Modern RB	34.1	0.9894
7.	Modern RB	33.4	0.9305
8.	Modern RB	33.7	0.9855
9.	Modern RB	31.7	0.907
10.	Modern RB	31.8	0.900
11.	Modern RB	33.4	0.9905
12.	Modern LB	33.6	0.7805
13.	Modern LB	31.9	0.8305
14.	Modern LB	28.8	0.9435
15.	Modern LB	28.2	1.005
16.	Modern RB	35.5	0.894
17.	Modern RB	35.7	0.8945
18.	Modern RB	33.6	0.905
19.	Modern RB	32.9	0.975
20.	Modern RB	33.3	0.9105
21.	Modern RB	32.8	0.944
22.	Modern LB	27.2	0.823
23.	Modern LB	32.8	0.833
24.	Modern LB	32.7	0.8305
25.	Modern LB	32.7	0.8785

Oil content for round and long bread from the different bakeries

Table 1 shows the oil contents of round bread (RB) and long bread (LB) collected from different bakeries of Khartoum state (Khartoum, Khartoum Bahri and Omdurman), the ID number from 1-9 represents oil content of bread collected from Omdurman, while ID number from 10-17 idealizes oil content of bread collected from Khartoum and ID number from 18-25 represents oil content of bread collected from Omdurman. There was no significant difference between all bread collected from different areas of Khartoum state. Lower oil content in round bread (RB) was found to be 0.7955%, while medial oil content in round bread was 0.92% and higher oil content in round bread were 0.995%.

While the lower oil content in long bread as 0.894%, medial oil content in long bread were 0.894%, and higher oil content in long bread were 1.005%. And consequently, oil content in round bread was higher than oil content in long bread.

DISCUSSION

Table 4 shows concentration of 3-MCPD in round bread oil, ranged from 0.15493 - 1.05873ppm, but find two samples were not detected, the main reason due to heat treatment with short time, food processing, and moisture content. Because, the concentration of 3-MCPD increased by temperature with time increased. And traditional of oil processing and less amount of sodium chloride were used. Dimensions (length, width, and thickness) with moisture content leads to increased of the concentration of 3-MCPD (if the moisture content and the length, width was high, bread needs long time with high temperature to matured). Lower concentration levels of 3-MCPD esters in round bread oil were 0,15493ppm, average concentration levels were (0.49825ppm), and higher concentration level was 1,05873ppm.

Table 2.shows concentration of 3-MCPD (ppm) in round bread oil

ID	Sample	Concentration of 3-MCPD (ppm) of oil	Concentration of 3-MCPD mg/kg of bread
1	S5/RB	0.52279	0.00188
2	S6/ RB	0.15493	0.00055
3	S7/RB	0.31573	0.00114
4	S8/RB	0.36274	0.00131
5	S9/ RB	0.34149	0.00123
6	S10/ RB	0.58253	0.00210
7	S11/ RB	0.28591	0.00103
8	S12/RB	1.05873	0.00382
9	S13/ RB	0.96438	0.00348
10	S18/RB	0.32869	0.00118
11	S19/ RB	0.23414	0.00084
12	S24/RB	N.D	ND
13	S25/RB	N.D	ND

S= Sample. R=Round. T=Long. B= bread

Table 2 shows concentration of 3-MCPD esters in long bread oil, was generally formed low, ranging from 0.11334ppm –0.667ppm.lower concentration of 3-MCPD esters were found in long bread oils, 0.11334ppm, average concentration level was 0.346472ppm, and higher concentration level was 0.66749ppm. From these concentrations result obtained it is consider no large difference between them, but find two sample were no detected, the main reason for difference quantity of 3-MCPD in long bread oil is due to the heat treatment processing, moisture content, oil content and type of oils, (refining, partially and traditional oil) used, bread color, and its dimensions (length width and thickness). Comparison between the quantity of 3-MCPD in long bread oil and round bread, showed that, the quantity of 3-MCPD esters in round bread oil was higher than the quantity in long bread oil. Because the average quantity of 3-MCPD in round bread oil were 0.49825ppm and average in long bread oil was 0.346472ppm, while higher concentration of 3-MCPD in round bread oil was 1.05873ppm and the higher concentration of 3-MCPD in long bread oil was 0.66749ppm and

lower concentration of 3-MCPD in round bread oil was 0.15493ppm and the lowest concentration in long bread oil was 0.11334ppm.

The main differences between concentrations of 3-MCPD in round bread and long bread, were mainly due to the heat treatment processing, moisture content, oil content and type of oils, (refined, partially refined and traditional oil) used, bread color ,and dimensions (length width and thickness).the temperature ,time ,length, width, moisture content and oil content in round bread oil were (250ºC 7-8 minutes, 11-13cm, 4-5cm, 33.56%, and 0.924%), respectively, and brownish colour and the temperature, time ,length, width, moisture content and oil content in long bread oil were (250ºC, 6-7 minutes, 20-25cm, 3-4cm, 31.02%, and 0.894%), respectively, and brownish colour. Comparison with previous studies (Dolezal, et al. (2005), and Divinova, et al. (2007), carried out for concentration of 3-MCPD in bread were formed to be ranged between 1.56-23.6mg/kg was higher than concentrations of 3-MCPD was found in round bread oil and long bread oil that ranged (0.11334-0.66749ppm) (0.000346-0.002039mg/kg) and (0.15493-1.05873ppm) (0.000559-0.003823mg/kg), respectively. While Kertisova, et al. (2009), reported values of concentration for bound 3-MCPD esters in bread crumb ranged from 1.56 to 21.13mg/kg of fat and concentration of 3-MCPD esters in bread crust ranged from 2.82 to 3.60 mg/kg of fat. Concentrations of 3-MCPD were found in round bread fat and long bread oil ranged 0.11334 to 0.66749ppm (i.e 0.000346-0.002039mg/kg) and from 0.15493 to 1.05873ppm (i.e 0.000559-0.003823mg/kg), respectively, is lowest than that reported.

Hamlet, et al. (2004), analyzed 3-MCPD-esters in long bread and, highest levels were found in regions of the bread that attained the highest temperature, i.e the crust , and level increased from 60-160ug/kg-1 when the bread was long over 40-120 seconds ,the highest level of 3-MCPD-esters (6100ug/kg-1) concentration of 3-MCPD were found in round bread fat and long brad oil range (0.113340.66749ppm) (i.e 0.000346-0.002039mg/kg) and (i.e 0.15493-1.05873ppm) (0.0005590.003823mg/kg), respectively, were increased by temperature and time increase.

Table 3. Concentration of 3-MCPD (ppm), in long bread oil

ID	Sample	Concentration of3-MCPD(ppm)ofoil	Concentration of 3-MCPD mg/kg of bread
1	S1/Long Bread	0.56378	0.00172
2	S2/LongBread	0.37503	0.00114
3	S3/Long Bread	0.20270	0.00061
4	S4/LongBread	0.26577	0.00081
14	S14/LongBread	0.66749	0.00203
15	S15/Long Bread	0.59644	0.00182
16	S16/Long Bread	0.21776	0.00066
17	S17/Long Bread	0.29495	0.00090
20	S20/LongBread	0.16746	0.00051
21	S21/LongBread	N.D	ND
22	S22/LongBread	N.D	ND
23	S23/LongBread	0.11334	0.00034

Lower Concentration of 3-MCPD exposure for adults from round bread were (0.005345µg/kg) body weight per day, higher concentration were (0.036528µg/kg) body weight per day, and medium concentration of 3-MCPD exposure for Adults were (0.016171µg/kg).

Table 4. Estimated quantity of 3-MCPD (µg\kg) for body weight per day exposure for individual adults (Round bread oil).

ID	Name	Mean Adult Age	Body Weight	3-MCPD\µg\kg exposure
1	S5\ RB	18-20	68.03	0.01803
2	S6\ RB	18-20	68.03	0.00534
3	S7\ RB	18-20	68.03	0.01093
4	S8\RB	18-20	68.03	0.01258
5	S9\ RB	18-20	68.03	0.01178
6	S10\ RB	18-20	68.03	0.02009
7	S11\ RB	18-20	68.03	0.00988
8	S12\RB	18-20	68.03	0.03652
9	S13\ RB	18-20	68.03	0.03327
10	S18\RB	18-20	68.03	0.01134
11	S19\ RB	18-20	68.03	0.00807
12	S24\RB	18-20	68.03	ND
13	S25\RB	18-20	68.03	ND

Mean estimated quantity of 3-MCPD exposure for Adults = 0.016171µg\kg.

Table 5. Estimation of concentration of 3-MCPD (µg/kg) exposure for adults from long bread**.**

ID	Name	Body Weight	Mean Adult Age	3-MCPD\µg\kg exposure
1	S1\ TB	68.03	18-20	0.01392
2	S2\TB	68.03	18-20	0.00926
3	S3\ TB	68.03	18-20	0.00565
4	S4\TB	68.03	18-20	0.00656
14	S14\TB	68.03	18-20	0.01648
15	S15\ TB	68.03	18-20	0.01473
16	S16\ TB	68.03	18-20	0.00537
17	S17\ TB	68.03	18-20	0.00728

20	S20\TB	68.03	18-20	0.00413
21	S21\TB	68.03	18-20	ND
22	S22\TB	68.03	18-20	ND
23	S23\TB	68.03	18-20	0.00279

LB: long breadMeanestimatedquantityof3-MCPDexposureforadults=0.0086234µg\kg

Lower Concentration of 3-MCPD exposure for adults from long bread were0.002799µg/kg body weight per day, higher concentration were 0.016488µg/kg body weight per day, and mean concentration of 3-MCPD exposure for adults were 0.0086234µg/kg.

Table 6 shows estimation of concentration of 3-MCPD (µg/kg) exposure for children from round bread.

Table 6. shows estimated quantity of 3-MCPD (µg\kg) for body weight per day exposure for individual children, (Round bread oil).

ID	Name	Mean kids Age	Body Weight	3-MCPD\µg\kg exposure
1	S5\ RB	14-15	47.67	0.01544
2	S6\ RB	14-15	47.67	0.00457
3	S7\ RB	14-15	47.67	0.00935
4	S8\RB	14-15	47.67	0.01077
5	S9\ RB	14-15	47.67	0.01008
6	S10\ RB	14-15	47.67	0.01720
7	S11\ RB	14-15	47.67	0.00846
8	S12\RB	14-15	47.67	0.03127
9	S13\ RB	14-15	47.67	0.02849
10	S18\RB	14-15	47.67	0.00971
11	S19\ RB	14-15	47.67	0.00691
12	S24\RB	14-15	47.67	ND
13	S25\RB	14-15	47.67	ND

RB: Round bread. Meanestimatedquantityof3-MCPDexposureforchildren=0.013846µg\kg

Lower concentration of 3-MCPD exposure for adults from round bread were 0.004577µg/kg body weight per day, higher concentration were 0.031277µg/kg body weight per day, and mean concentration of 3-MCPD exposure for adults were 0.013846µg/kg.

Table 7. Estimation concentration of 3-MCPD (µg/kg) exposure for children age (14-15) years

ID	Name	Mean Kids Age	Body Weight	3-MCPD\µg\kg exposure

1	S1\ TB	14-15	47.67	0.01192
2	S2/LB	14-15	47.67	0.00793
3	S3/ LB	14-15	47.67	0.00468
4	S4/LB	14-15	47.67	0.00562
14	S14/LB	14-15	47.67	0.01411
15	S15/ LB	14-15	47.67	0.01261
16	S16/LB	14-15	47.67	0.00460
17	S17/LB	14-15	47.67	0.00623
20	S20\TB	14-15	47.67	0.00354
21	S21\TB	14-15	47.67	ND
22	S22\TB	14-15	47.67	ND
23	S23\TB	14-15	47.67	0.00239

TB: Long bread   Mean estimated quantity of 3-MCPD exposure for children = 0.00736µg/kg

from long bread. Lower concentration of 3-MCPD exposure for children from long bread were 0.002397µg/kg body weight per day, higher concentration were 0.014118µg/kg body weight per day, and mean concentration of 3-MCPD exposure for adults were 0.007368µg/kg. Concentration of 3-MCPD exposure for children from round bread higher than concentration of 3-MCPD exposure for children from long bread. Consequently, concentration of (3-MCPD) boy weight per day exposure for children from round and long bread were lower than concentration of (3-MCPD) body weight per day exposure for adults. Because, concentration of 3-MCPD body weight per day exposure increase by weight decrease and concentration of 3-MCPD (µg/kg) exposure decrease by weight increase.

Comparison with the international standard of 3-MCPD, results of international studies of 3-MCPD by The European commission's scientific committee for food established range tolerable daily (0.1µg/kg - 2µg/kg) body weight per day. Concentration of free 3-MCPD in round bread for adults WAS formed ranged to be between (0.15493-1.05873ppm), (i.e 0.005345-0.036538µg/kg), body weight per day, while concentration of 3-MCPD in long bread oil for adults range between (0.11334ppm – 0.667ppm), (i.e 0.002799-0.016488µg/kg) body weight per day, while concentration of 3-MCPD in round bread for children range between (0.15493-1.05873ppm), (i.e 0.004577-0.031277µg/kg), body weight per day, and concentration of 3-MCPD in long bread oil for children ranged between(0.11334ppm – 0.667ppm), (i.e 0.002397-0.014118µg/kg) body weight per day. The concentration of 3-MCPD were found in both round and long bread fat is lower than standard of European commission's scientific committee for food.

Comparison with studies of Karsulinova et al (2007), Dolezal et al., (2005), and Divinova et al., (2007) for the amount of 3-MCPD in bread range between (1.56-23.6mg/kg) of fat (i.e

5.7-84,9ug/kg) is higher than amount of 3-MCPD in round bread and long bread oil were found. WHO/JECFA (2007) was reported average dietary exposures of the general population from a wide range of foods, related products, ranged from 0.02 to 0.7 ug/kg (bw) per day, and these for consumers at the high percentage (95th), including young children ranged from 0.06 to 2.3 ug/kg bw per day.

While concentration of 3-MCPD in round bread for children range between (0.0045770.031277µg/kg), body weight per day, and the percentage of 3-MCPD in long brad oil for children range between (0.002397-0.014118µg/kg) body weight per day were found. While Hwang et al, (2009), reported value for mean intake level of 3-MCPD in the Republic of Korea was estimated in the range from 0.0009–0.0026ug/kg bw per day and at the 95th percentile of consumption was 0.005 ug/kg bw per day. And You et al (2008) reported the levels of 3-MCPD in a wide range of food items and estimated their dietary exposure for secondary school students in Hong Kong and they estimated the average exposure to be 0,063-0,150ug/kg bw per day. While that for high consumers was 0.152-0.300 ug/kg bw per day. While average MCPD exposure for children from long and round bread were (0.007368-0.013846 µg/kg), respectively, and Mean estimated quantity of 3-MCPD exposure for Adults from long and round bread were (0.0086234-0.016171µg/kg), respectively.

CONCLUSION

3-MCPD was detected in round and long bakery bread, and it was found with concentrations of 0.154931-0.5873ppm to 0.11334-0.59644ppm, respectively, which were lower than that of European Commission Scientific Committee for Food Standardization. Exposure of adults and children to 3-MCPD upon consumption of modern bakeries bread does not constitute a health hazard.

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Conflict of interest statement

There is no conflict of interest pertaining to this study.

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Impact of Storage Time on the Content of Kefir

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Abstract

Kefir is assumed as a functional beverage out of its probiotic content and its consumption has been increasing continually worldwide for few decades. This study was conducted to monitor the effect of storage time on the pH, acidity, total dry matter and total free amino acid content of kefir at cold storage (+4°C) for 7 days. The characteristics of kefir were analysed daily to observe the changes occurring during the storage. Total free amino acid content measurements were achieved via spectrophotometric method. The pH values of kefir inclined to decrease from 4.25 to 4.02 steadily during the storage. The acidity contents of kefir were within the range of 0.77- 0.92 g lactic acid/100 mL and have shown fluctuations in the storage duration. Levels of total dry matter augmented until the middle of the storage time and then showed fluctuations. While the highest amounts of total free amino acids were detected at the second day of storage, total free amino acid values were varying between 0.0214 and 0.0431 g/100 mL (as leucine equivalent) and storage caused significant differences on this trait of kefir.

Keywords: Kefir, fermented milk beverage, functional beverage, storage, content

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INTRODUCTION

Kefir is a probiotic fermented dairy product assumed as functional drink that is originated from Caucasus mountains of Russia and has an important role in the Mediterranean diet which is known in different names (such as kephir, knapan, kiaphur, kepi, kefer, kippi, knapon, kefyr) but mostly called "kefir" (Atalar, 2019; Rattray & O'Connell, 2011). It is produced as a result of both acidic and alcoholic fermentations, therefore it has a tart-acidic flavour with little alcohol content (about 0.5%) and creamy sparkling texture (Izquierdo-González et al., 2019). The production of kefir is traditionally based on the addition of kefir grains, yellowy tiny cauliflower-like shaped particles composed from principally lactic acid bacteria and yeasts, and sometimes acetic acid bacteria and moulds embedded in a complex polysaccharide, to cow milk (Wang, et al., 2021). However, as kefir grains from different origins contains different microorganisms, the standard production with kefir grains is hard. Herewith, in the industrial production, kefir is generally manufactured by starter cultures. The limiting factors with grains in commercial production can be listed as: contamination of grains, separation of kefir grains from the final product (Sarıca, 2019). Regarding the above-mentioned reasons, industrially, kefir is manufactured by the addition of lyophilized starter culture (combination of some bacteria and yeasts) to milk (Delgado-Fernández et al., 2019).

Consumption and popularity of kefir is constantly increasing worldwide because it has many health benefits such as lowering lactose content of milk, antimicrobial, antioxidative, anticarcinogenic, and therapeutic effects, and it also improves the absorption of nutrients, supports immune system, regulates digestion system, increases lifetime, lowers cholesterol and helps hormonal changes in the human body (De Oliveira Leite et al., 2013; John & Deeseenthum, 2015). Even though its consumption was limited with Middle East, Russia and Eastern Europe in the past, nowadays it is consumed in the most European, and Nordic countries and the United Kingdom (Delgado-Fernández et al., 2019). The first industrial level kefir production was made in Russia at the beginning of 1930s and the technique applied for kefir production today again was developed by a Russian research institute. In Turkey, industrial kefir production started in the middle of 1980s and the production was fluctuating, but after 2004 the production and consumption of kefir started to increase steadily (Alagöz Kabakcı et al., 2020; Sarıca, 2019). The characteristic of kefir changes depending on the type of milk, the starter culture used, and the conditions of fermentation process (Aiello et al., 2020, Atalar, 2019). Though kefir is generally produced from cow milk, it can also be produced from some other animal milks (goat, sheep, mare, and buffalo) or plant milks (soy, peanut, rice, coconut and almond) and lastly whey can be used for kefir production. Additionally, more recently, water kefir also has been produced (Atalar, 2019; Vimercati et al., 2020). In spite of its health benefits, kefir is not preferred by some consumers due to its acidic taste. Considering this, kefir with different flavours have been manufactured to make kefir drinking more desirable (De Roos, & De Vuyst, 2018).

Kefir is a fermented milk beverage that is produced by lactic acid and ethyl alcohol fermentation. As a result of fermentation process, lactose and proteins are degraded to less complex substances, and some vitamins and other bioactive substances like phenolic compounds are synthetized by microorganisms. The main products of the fermentation are lactic acid, ethanol and CO_2 while the secondary products are acetic acid, antibiotics, bacteriocins, amino acids and aromatic compounds such as diacetyl, acetaldehyde and acetoin (Liu et al., 2005; Sarıca, 2019). Lactic acid formed via fermentation and bioactive peptides produced by protein degradation underlie the antioxidant and antimicrobial activities of kefir (Shiby & Mishra, 2013). The vitamin (such as vitamin B_1, B_2, B_6, and B_{12},) essential amino acids and mineral content of kefir make it attractive for health-aware consumers (Alagöz Kabakcı et al., 2020).

The composition and nutritional value of kefir alters according to the composition of milk, production method of kefir (with kefir grains or starter culture) and finally the time and conditions of the fermentation process. Although kefir can be manufactured from many types of milks in different ways, the most preferred industrial production type is cow milk fermentation by starter culture. Nowadays, related with consumer demand for production kefir at homes, kefir culture is also sold in the stores. The studies in the literature have not studied the changes in kefir content daily, therefore this study aimed to provide a close look to the changes in kefir content during the cold storage period. In this study, some chemical properties of kefir produced by starter culture obtained from a countrywide market chain monitored daily during the storage (7 days at +4°C). Even though the shelf life of kefir has been specified differently, the most appropriate duration is generally given as 7 days, therefore this study focused on the changes of content and pH values of kefir for 7 days of storage.

MATERIALS and METHODS

Materials

Pasteurised milk was bought from the stores in İzmir, Turkey. Kefir starter culture in powder form was obtained from Doğadan Bizim Gıda ve Süt Ürünleri Ind.Trade. Co.Ltd. (İstanbul/Turkey) which is sold in a countrywide market chain.

Chemicals

L-leucine and ethanol were supplied from Sigma (St. Louis, MO, USA); phenolphthalein and glacial acetic acid were from Pancreac (Barselona, Spain); hydrochloric acid (HCI), sodium hydroxide (NaOH) and ninhydrin were from Merck (Darmstadt, Germany).

Production of Kefir

Kefir was produced in plain form independently twice and analysed during 7 days of storage. The analyses carried out for 7 days as the most suggested time for kefir consumption is the first 7 days. To produce kefir, pasteurised milk (2 L) was heated to 90°C for 5 min and subsequently cooled to 25 °C. After that, kefir culture (2 g) was added to the milk and fermented in an incubator at 25°C for 48 hours and then stored at +4°C. For each analysis, two samples have been taken from each replicate at the 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th days of fermentation, and total dry matter, total titratable acidity, pH value and total free amino acid content has been determined for each sampling time. All experiments were repeated two times.

Measurement of pH Values

The pH values of samples were detected at approximately 15°C via the immersion of a digital pH meter (Isolab Laborgeräte GmbH, Germany), which adjusted with pH=4 and 7 standard buffer solutions, to kefir samples (Kebede et al., 2007).

Determination of Total Dry Matter Content

Total dry matter content of kefir samples were estimated gravimetrically by drying the samples in the oven at 105°C until obtain a constant weight and the results were given as % (w/w).

Estimation of Titratable Acidity

Total titratable acidities were performed via titration of samples with 0.1 N NaOH by addition of phenolphthalein to the sample mixture and results were expressed as % of lactic acid (Gül et al., 2015).

Determination of Total Free Amino Acid Content

Quantification of total free amino acid content of samples were achieved following the procedure of Folkertsma & Fox (1992) with slight modifications. 30 mL of 0.1 M HCI added to 30 mL of kefir sample and homogenized. The final blend was mixed by using a magnetic stirrer for 15 min, then allowed to stand at 40°C (1 hour), and then centrifuged (Hettich Universal 320 R, Tuttlingen, Germany) at 460*g* (30 min). Pursuant to filtration of the aqueous phase, 0.3 mL of supernatant was diluted to 2 mL by using ultrapure water, then 4 mL of Cd-ninhydrin reagent (0.8 g ninhydrin + 80 mL of ethanol (HPLC grade) + 10 mL of glacial acetic acid + 1 mL of Cd solution (1 g/mL) added and the resulting solution was vortex-mixed. After keeping it at 84°C (5 min), the mixture was cooled, centrifuged and the absorbance values were recorded at 507 nm by using a spectrometer (Agilent Cary 60 UV-Visible, Santa Clara, CA, USA).

To increase the reliability of the experiment, two additional blank solutions were used. The first solution was prepared by following the same procedure with the exception of addition 4 mL of Cd reagent without ninydrin and this solution was utilized for prevention of colour errors related with samples. The second blank solution was prepared with the same procedure by replacing 4 mL of ultrapure water in place of sample. The results were represented as leucine equivalent (g/100 ml kefir) by using calibration graph prepared with L-leucine as standard.

Statistical Evaluation of the Results

The findings of all experiments were reported as mean values and standard deviations of 4 measurements. The obtained data were compared by means of SPSS 20.00 statistical package program (IBM Corp., New York, USA). ANOVA test was applied to detect the differences and Duncan's multiple range test ($P<0.05$) was used to detect the degree of significance between the storage days, and finally Pearson's correlation test was performed to find out the relationship between pH, acidity, total dry matter and total free amino acid values of kefir samples ($P<0.05$).

RESULTS and DISCUSSION

The changes of pH values in kefir during 7 days of storage have been given in Fig. 1. The pH values of kefir tended to decrease continually with increasing storage time and were in the range of 4.02-4.35 (Table 1). The highest pH decline was observed between the 5th and 6th days of storage. Öner et al. (2010) determined the pH of kefir samples produced from cow milk with kefir grains and starter culture, and reported that the pH values of kefir with starter culture was 4.47 at the 0th day and 4.48 at the 7th day of storage while there were no significant difference due to the storage time. Similarly, Yüksel-Bilsel, & Şahin-Yeşilçubuk (2019) reported pH values of kefir as 4.26, 4.21, and 4.13 at the 0th, 5th, and 10th days of storage, respectively. Contrary to our results, Alagöz Kabakcı et al. (2020) indicated the pH values of kefir as 4.31 at the 0th day and 4.38 at the 12th day of storage which were rising with time. Regarding these studies, it can be concluded that the type of milk, type of starter culture and fermentation conditions affects the pH changes of kefir during storage.

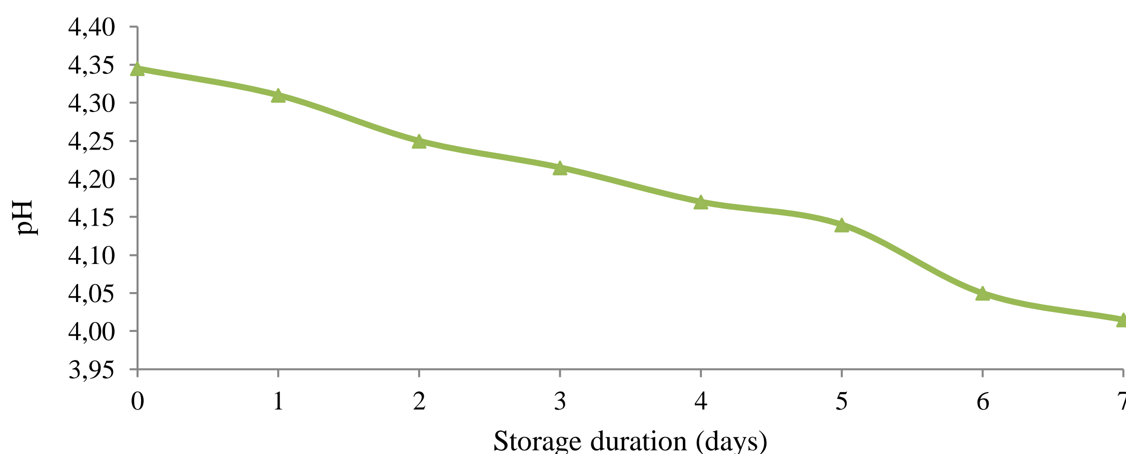

Figure 1. pH values of kefir during the storage at +4°C for 7 days

Total titratable acidities of kefir variations during the storage have been illustrated in Fig. 2. The acidity showed a downward tendency until the 5th day and then the values increased gradually. As can be seen from Fig. 2, acidity values of kefir were showing a fluctuating change during the storage. The acidity of kefir was changing between 0.77 and 0.92 g lactic acid/ 100, and significant changes (P<0.05) were recorded between the beginning and the end of the storage time. Sarıca (2019) pointed out that the acidity values of kefir remained the same between the 1st and 7th days of storage while Alagöz Kabakcı et al. (2020) and Yüksel-Bilsel, & Şahin-Yeşilçubuk (2019) notified steadily increments in the acidity of kefir during storage.

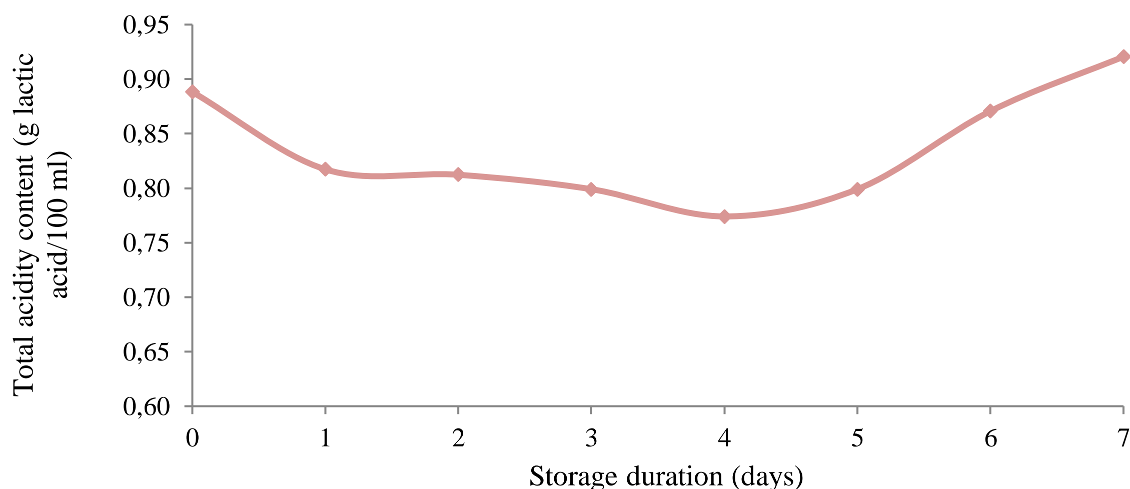

Figure 2. The alteration of total dry matter content with the increasing time at +4°C storage

The observations of total dry matter content of kefir for 7 days of storage have been exhibited in Fig. 3. According to the findings of the study, total dry matter content interval of kefir values were 10.41 (6th day) and 11.11 g/100 g (0th day). Storage time had variable effects on the total dry matter content of kefir and no significant differences (P>0.05) were detected between the values at the beginning and the end of storage (Table 1). The total dry matter contents of the current study were slightly different than the intervals stated by Atalar (2019) and Öner et al. (2010) and this variation is thought to be related with the characteristic of milk and the activity of starter cultures used in the production. Eventually, while the correlations between total dry matter and acidity, and total free amino acids were not statistically significant (P>0.05), the correlations of total dry matter and pH were positive significant (r= 0.738, P<0.05).

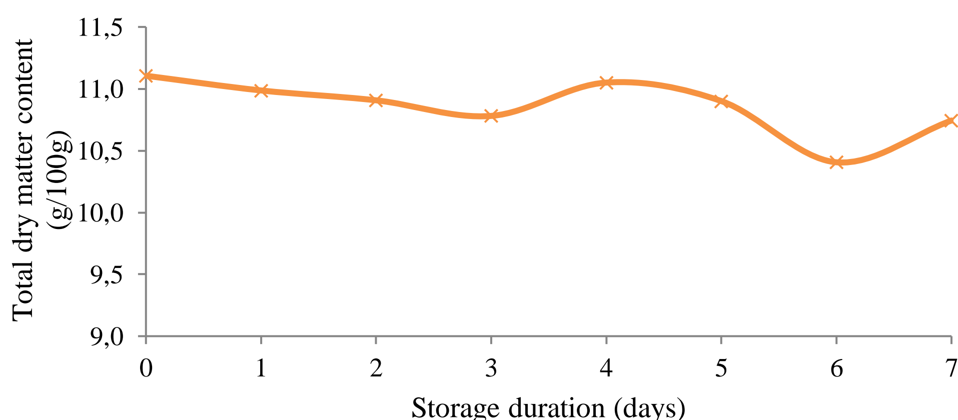

Figure 3. The effect of time on the total dry matter content of kefir during the cold storage (+4°C)

Free amino acids are nutritious components which are produced by proteolysis reaction and have noteworthy role in aroma formation, but they can also cause formation of several undesired toxic compounds. Thereof, determination of total free amino acid content of food products is of importance because free amino acids are precursors of some toxic compounds e.g. biogenic amines, acrylamide, nitrosamines and heterocyclic amines (Akan & Özdestan Ocak, 2019; De Mey at al., 2014; Keşkekoğlu & Üren, 2014; Lingnert et al., 2002).

During the storage of kefir, the fluctuations in the amounts of kefir have been displayed in Fig. 4. Related with the high protein content of milk and fermentation, total amino acid content of kefir samples showed increments and decrements during the 7 days of storage due to the actions of active microorganisms at the storage temperature (+4°C).

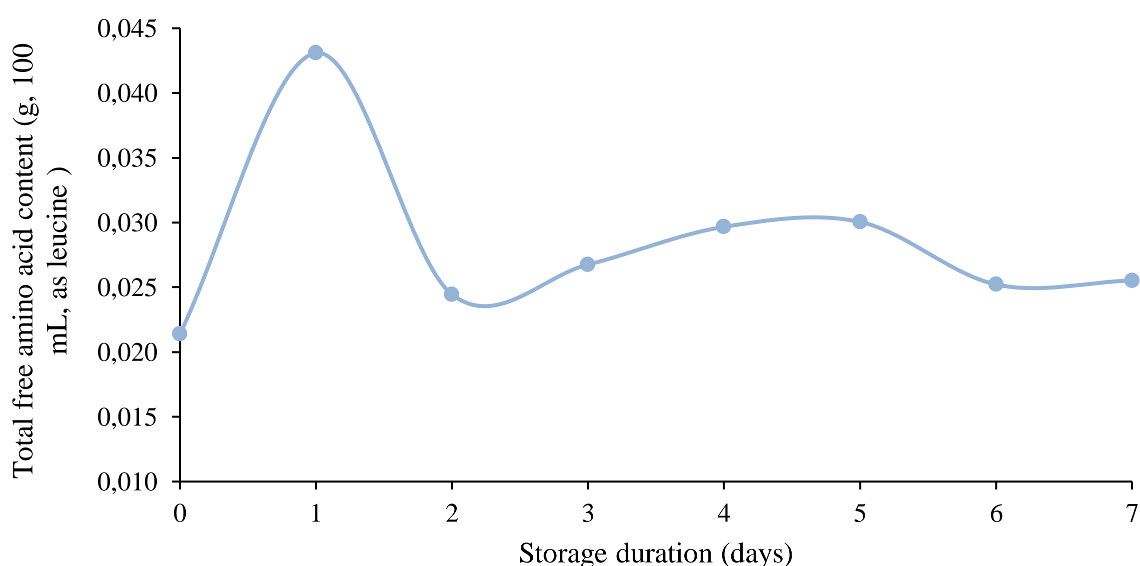

Figure 4. Changes of total free amino acid content during 7 days of storage at +4°C

The levels of total free amino acids of kefir were in the range of 0.0214 and 0.0431 g/100 mL (as leucine equivalent) which were compatible with the results (0.0070-0.0206 g leucine/100 mL) of Özdestan & Üren (2010). Contradictory with the current research, total free amino acid content of kefir reported as 0.00063 and 0.00072 g/100 g by Güler et al. (2016), and as 0.00057 g/100 g by Simova et al. (2006).

The variation in the total free amino acid content of kefir is thought to be originated from the type and composition of milk and the duration of fermentation. Özdestan and Üren (2010) found significant correlations between the amounts of total free amino acid and total biogenic amine of different kefir samples. In line with this idea, total free amino acid content of kefir also might be an indicator of total biogenic amine content.

Table 1. The alterations in the content and pH values of kefir during the storage at +4°C

Storage duration (days)	Total dry matter (g/100 g)	pH	Acidity (g lactic acid/100 mL)	Total free amino acids (g/100 mL, as leucine equivalent)
0	11.11±0.09[a]	4.35±0.01[a]	0.89±0.01[a,b]	0.0214±0.0003[d]
1	10.99±0.11[a,b]	4.31±0.01[a,b]	0.82±0.01[c]	0.0431±0.0007[a]
2	10.91±0.01[a,b]	4.25±0.00[b,c]	0.81±0.01[c,d]	0.0245±0.0005[c,d]
3	10.78±0.02[a,b]	4.22±0.01[c,d]	0.80±0.01[c,d]	0.0268±0.0010[b,c]
4	11.05±0.36[a]	4.17±0.01[d]	0.77±0.01[d]	0.0297±0.0025[b]
5	10.90±0.03[a,b]	4.14±0.06[d]	0.80±0.02[c,d]	0.0300±0.0002[b]
6	10.41±0.24[b]	4.05±0.01[e]	0.87±0.01[b]	0.0252±0.0005[c]
7	10.74±0.08[a,b]	4.02±0.00[e]	0.92±0.01[a]	0.0255±0.0003[c]

a-e; Expresses that dissimilar letters within the same column shows significant differences (P<0.05).

CONCLUSION

Kefir is a functional beverage that has been popular for few decades. The demand for kefir consumption is increasing day by day as it has many health benefits and therapeutic effects. Nowadays, preparation of food products at homes is an increasing trend because consumers think that production foods and beverages at home conditions is more hygienic, nutritious and healthy. Due to the request of consumers, nowadays kefir starter culture is sold at stores and consumers can produce kefir at home easily. This study has been conducted to determine the chemical traits of kefir prepared with starter culture obtained from a countrywide market chain. The investigation disclosed that the pH values and content of kefir changes daily at +4°C cold storage while acidity, total dry matter and total free amino acid content values were showing fluctuations.

Conflict of Interest Statement

The author indicates that there is no personal or commercial relationship that could influence the results of the study reported in this article.

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Study of Physicochemical Properties and Antioxidant Content of Mango (*Mangifera indica* L.) Fruit

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Abstract

The objectives of this study were to determine the physicochemical properties and antioxidant composition of four mango varieties (Tommy Atkins, Apple, Keitt, and Kent) grown in Ethiopia and to compare their composition to previously reported results on mangoes grown in other parts of the world. The Keitt variety had the highest fruit weight, fruit length, fruit diameter, and juice volume content. The pH, TSS, TA, and TSS/TA and proximate composition (moisture, ash, fat, fiber, and protein) showed significant differences among the varieties at $p \leq 0.05$. Minerals such as Na, Mg, K, Ca, Fe and Zn were also evaluated and potassium had the highest concentration among the minerals with the grand mean of 267.44 mg/100 g. Varieties also differed in antioxidant content such as total carotenoids and vitamin C with values ranging from 0.6 to 4.8 µg/g and 14.2 to 36.4 mg/100 g, respectively. Apple, Kent and Keitt mango varieties are good sources of vitamin C. The four mango varieties had similar physicochemical properties and antioxidant content compared to mangoes grown in other countries.

Keywords: acidity, mango, total carotenoids, vitamin C

Research article
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INTRODUCTION

Mango (*Mangifera indica L.*) is one of the most important tropical fruits commercialized and consumed worldwide. The fruit may be used fresh or processed and possesses an attractive color and distinct taste and aroma (Singh et al., 2000). It is a nutritionally rich fruit that provides health benefits to humans. Mango is not only delicious but is also rich in prebiotic dietary fiber, vitamins, minerals, antioxidant compounds such as polyphenols and carotenoids. Mango also possesses medicinal properties, and is a very good source of both vitamins A and C (Ajila et al*.,* 2007; Altemimi et al., 2017; Alsaad et al*.,* 2019). The physicochemical properties of mango varieties are varied across the world due to different factors such as maturity and ripening stage, type of variety (Othman and Mbogo, 2009), cultivation practices (Hofman, 1995), climatic conditions (Léchaudel and Joas, 2006), ripeness at harvest (Jacobi et al*.,* 1995; Lalel et al*.,* 2003), and postharvest storage and treatment (Hofman et al., 1997; Nunes et al*.,* 2007).

Nowadays, there are four varieties of mango widely distributed throughout Ethiopia with the help of the Melkassa Agricultural Research Center. The Center has been doing research on the improvement of fruit productivity and its quality. There are a number of varieties in the research stations which are available but the most widely distributed and most consumed varieties are Kent, Keitt, Apple, and Tommy Atkins. Physiochemical properties and sensory profiles of mango varieties are very important quality parameters for promoting mango export in a highly competitive international market. Quality traits have been studied in almost all major mango-producing countries around the world. However, in Ethiopia, information is lacking on mango quality. Therefore, in view of the importance of mango fruit and the abundance of mango studies worldwide, the present research was carried out to assess the different mineral contents, physiochemical and antioxidant properties of four mango varieties at ripeness.

MATERIAL and METHOD

Study area

Studies were conducted in the Food Science and Nutrition research laboratory of the Melkassa Agricultural Research Center, one of the research centers of the Ethiopian Institute of Agricultural Research. The Center is located in the Ethiopian rift valley, 117 km away from Addis Ababa in the southeast direction located at 8°24'N and 39°12'E and an altitude of 1550 m. The mean minimum and maximum temperature of the environment are 13.8°C and 28.6°C, respectively. The Center receives a mean of total annual rainfall of 825.9 mm with erratic distribution, having a high coefficient of variation. The soil contains volcanic ash but is mainly sandy loam with a pH range of 6-8.

Sample collection

Four mango varieties (Apple, Tommy Atkins, Keitt, and Kent) were used for this study. The fruits were cultivated in horticultural research stations at the Melkassa Agricultural Research Center. The samples were collected from ten different mango trees for each variety and the mango skin color was found to be pink with red, red with some yellow and deep red.

The fruits were free from mechanical damage, insect infestation, disease, and physiological deterioration. The samples were stored at room temperature of 25-30°C for 5 d during which ripening and subjective observation of softness, peel color (Crisosto, 1994) and TSS value were monitored.

Sample preparation

Ripe samples of mango were subjected to sample preparations with the aid of a clean sharp knife. The peel and seed (kernel) of the fruit were removed and the pulp was homogenized. The mango puree was lyophilized, stored at –20°C and protected from light until analysis of antioxidants. The fresh fleshes were processed into juice to measure some parameters. The tests were performed in triplicate except for the mineral content.

Methods

The physical parameters of the fruit such as the color of skin and flesh, fruit weight, fruit diameter, fruit length, and juice volume were measured by a color chart, analytical balance, digital caliper and graduated cylinder, respectively.

pH

pH was measured with an electronic pH tester (HI 98106 Champ®, Hanna Instruments, Woonsocket, RI, USA).

TSS (total soluble solids)

TSS was determined using a hand-held refractometer (Model 9099, Atago Co., Ltd., Tokyo, Japan). A drop of homogenized mango pulp was placed on the prism of the refractometer (previously calibrated), the lid closed and TSS read from the digital scale at 20 ± 1°C. The results were expressed in °Brix.

TA (titratable acidity)

0.01 M NaOH was titrated against 10 mL of filtered juice using phenolphthalein indicator. The end of the titration was indicated by a change in color of the sample to pink. The amount of acid in mg/100g was calculated using the following equation (Horwitz, 2000):

$$\text{Titratable acidity} = (0.01 * 0.0064 * T * 10 * 1000)/(\text{Ft} * \text{S}) \quad (1)$$

Where 0.01 is the molarity of NaOH used; 0.0064 represents the conversion factor for citric acid since it is main acid present in mango, T is the titer value, Ft is the quantity of filtrate used, S is the sample amount, 10 is the dilution factor, and 1000 is the conversion to mg/100g.

Moisture determination

Moisture was determined using an oven drying method (Horwitz, 2000). Mango flesh (2 g) was transferred to a dried and tared dish. Sample containing dishes were placed in the drying oven and dried for 1 h at 130°C or until constant weight was attained. The dried samples were removed from the drying oven and then cooled in desiccators at room temperature. Moisture (%) was calculated using the following equation:

$$\text{Moisture (\%)} = ((W1 - W2) * 100)/SW \quad (2)$$

Where W1 is the weight of dish and fresh sample, W2 is the weight of dry sample and dish, SW is the sample weight.

Ash determination

Four grams of lyophilized mango flour was placed into a clean crucible of predetermined weight. The sample containing crucible was placed in a muffle furnace and heated to 550ºC. The samples were ignited until the powder turned a light gray or until constant weight was obtained (Horwitz, 2000). Ash (%) was calculated using the following equation:

$$\text{Ash (\%)} = ((W1 - W2) * 100)/SW \quad (3)$$

Where W1 is the weight of ash + crucible after ashing, W2 is the weight of empty crucible, SW is the sample weight.

Crude fat determination

Crude fat was determined using a previously described method (Horwitz, 2000). Mango flour (2 g) was placed into a previously prepared extraction thimble. The sample was extracted with petroleum ether (b.p. 35-60°C) for 4 h. The last traces of the solvent were evaporated in an oven at 103ºC for 30 min. The dried flasks containing fat were cooled in desiccators and then reweighed. Crude fat was calculated using the following equation:

$$\text{Crude Fat (\%)} = ((Wf - W) * 100)/SW \quad (4)$$

Where Wf is the weight of the flask and fat, W is the weight of flask, SW is the sample weight.

Crude fiber determination

Two grams of sample was transferred into a one-liter beaker. The sample was digested on a hot plate for 1 h with a sequential digestion with 2.5 M H_2SO_4 followed by an equal volume of 2.5 M NaOH. Sample filtering was aided by the addition of a small portion of ethanol. The precipitate was quantitatively transferred to a porcelain crucible and dried with an oven at 100ºC until constant weight was obtained. The crucible contanining precipitate was cooled and weighed (W1). The crucible containing precipitate was incinerated at 600ºC for 3 h in a muffle furnace. The crucible containing ash was removed, cooled and weighed (W2) (Horwitz, 2000). Fiber (%) was calculated using the following equation:

$$\text{Fiber (\%)} = ((W1 - W2) * 100)/SW \quad (5)$$

Where W1 is the weight of crucible and sample before ashing, W2 represents the weight of crucible containing ash, SW is the weight of the sample.

Protein determination

The test was performed by the Kjeldahl method of Horwitz (2000). Mango flour sample (0.5 g) was weighed in a 50 mL Kjeldahl flask followed by the addition of 8 mL of concentrated H_2SO_4 with 5 g of (copper and potassium sulfate) mixture catalyst. Samples were digested until

pure colorless solution observed. Digested samples were distilled and the distilled vapor gas (ammonia) was collected in a conical flask containing 25 mL of 2% boric acid solution containing mixed indicator. The distilled sample was titrated against 0.1 N HCl until a pink color persisted. Crude protein was calculated using the following equation:

$$\text{Crude protien} = ((a * b * 14 * 6.25) * 100)/w \quad (6)$$

Where a is the normality of the acid, b represents the volume of standard acid used (mL), corrected for the blank (i.e., the sample titer minus the blank titer), w is the sample weight (g), and 6.25 is the conversion factor for protein from % nitrogen.

Total carbohydrates

The total carbohydrate content was determined by the difference (the measured protein, fat, ash and moisture was subtracted from 100%). (Pearson, 1976).

$$\text{TC} (\%) = 100 - \{\text{Moisture} (\%) + \text{Protein} (\%) + \text{Fat} (\%) + \text{Ash} (\%)\}. \quad (7)$$

Gross food energy was estimated by the following equation (Edeoga et al., 2003):

$$\text{FE} \left(\frac{Kcal}{g}\right) = (\%\text{TC} - \%\text{CF}) * 4 + (\%\text{TF} * 9) + (\%\text{CP} * 4) \quad (8)$$

Where FE is the food energy, TC is the total carbohydrate content, CF is the crude fiber, TF is the total fat and CP is the crude protein.

Total carotenoids

Total carotenoids was performed spectrophotometrically using the method described by Rodriguez-Amaya and Kimura (2004). Lyophilized samples of mango (5 g) were ground with cold acetone with a mortar and pestle until the residue became colorless and then vacuum-filtered using a Büchner funnel. The extract was partitioned with petroleum ether, then each fraction was washed with distilled water for complete acetone removal. The extracts were made up to a volume of 50 mL with petroleum ether. All of the procedures were performed in dim light. The extracted carotenoids were collected and measured at 450 nm using a UV spectrophotometer. Total carotenoids were calculated with the following equation:

$$\text{Total carotenoids} (\mu g/g) = (A * \text{volume (mL)} * 10000)/ \text{A1cm1\%} * \text{sample weight (g)}) \quad (9)$$

Where A is the absorbance, volume is the total volume of extract (50), A1cm1% is the absorption coefficient of β-carotene in petroleum ether.

Ascorbic acid (vitamin C) determination

Ascorbic acid was determined spectrophotometrically using trichloroacetic acid as extraction chemical (Horwitz, 2000). Vitamin C was calculated using the following equation:

$$\text{Vitamin C (mg/100g)} = (\text{As} - \text{Ab}) * 10/([\text{A10}\mu\text{g Std} - \text{Ab}] \quad (10)$$

Where As is the sample absorbance, Ab represents absorbance of blank, $A_{10\mu g\ Std}$ is the absorbance of 10 µg AA standard.

Analysis of the mineral composition

Mango powder (0.5 g) was digested with nitric acid (HNO_3) and perchloric acid ($HClO_4$). The digested sample was filtered and made up to 100 mL in a volumetric flask. An atomic absorption spectrophotometer was used to determine all of the minerals using appropriate lamps (Horwitz, 2000).

Statistical analysis

Statistical analysis of the data was carried out using analysis of variance (ANOVA) technique of completely randomized design (CRD) and all pair-wise comparisons test whereas the least significant difference test was used for comparison of the treatment means at $p \leq 0.05$.

RESULTS and DISCUSSION

Physical properties of mango fruit

Physical properties of mango such as skin and flesh color, fruit weight, fruit diameters, fruit length and juice volume content of the pulp were evaluated. Mango flesh color is an important indicator of maturity and ripeness. Most of the mango varieties develop orange and yellow pigments in the flesh with maturity and ripening but changes in skin color are not always correlated with maturity and ripeness. During ripening peel color may change from green to yellow or deep orange depending on the cultivar, or may remain green. Likewise, changes in the firmness of the skin is another indicator of maturity and ripeness. The three physical parameters, fruit weight, fruit length, and juice volume showed significant differences among tested varieties of mango at $p \leq 0.05$ while fruit width was not significantly different. The fruit weight ranged from 433.5 to 727.3 g. In fruit weight, the Keitt variety was significantly heavier ($p \leq 0.05$) than the other varieties.

The length of the fruit ranged from 93.2 and 137.3 mm with Keitt having the longest length. The extracted juice volume of mango varieties varied from 316.5 to 540.0 mL. The Keitt variety had significantly higher juice volume than the other varieties. Keitt had the highest fruit weight, fruit length and juice volume while Apple had the lowest values (Table 1). Fruits which have high juice volume are appreciated by consumers and food processors. The weight and length of the mango varieties studied are consistent with the previous study of Rodríquez Pleguezuelo et al. (2012) who found that the average weight and length of nine mango varieties ranged from 143 to 792 g and 72.1 to 133.8 mm, respectively.

Rodríquez Pleguezuelo et al. (2012) also found that the mango fruit diameter ranged from 67.9 to 98.9 mm which is similar to our findings. In most cases the fruit weight is not a determining factor for quality but medium-sized fruits are more appreciated by consumers. The size and weight variation observed among the varieties might be due to different growing conditions and genetic variability.

Physicochemical characteristics

pH values were significantly different among the varieties ($p \leq 0.05$) with their values ranging from 3.86 to 4.73. Among the four varieties, Apple (3.86) was the most acidic while Tommy Atkins (4.73) was the least acidic (Table 2). pH of the fruit pulp plays an important role in flavor as well as preservation (Okoth et al., 2013). Fruit with lowest pH value are preserved longer than the fruit with higher pH value. This study is in agreement with Kansci et al. (2008) who found that the pH values of four mango varieties varied from 3.91 to 4.35. In this study the pH of Tommy Atkins was 4.73; this result was similar to that reported by Rodríquez Pleguezuelo et al. (2012) for Tommy Atkins (4.9).

Titratable acidity is presented in terms of citric acid concentration since it is the major organic acid present in mango fruit (Ueda et al., 2000). The Apple variety had significantly higher titratable acidity (6.40 g/L) than the other varieties. Titratable acidity (citric acid content) of the four mango varieties ranged from 3.48 to 6.40 g/L.

Total soluble solids (TSS) (°Bx) of the four mango varieties were significantly different at $p \leq 0.05$. Kent had the highest TSS (18.97°Bx), followed by Apple (18.07°Bx) while Keitt had the lowest (13.60°Bx). TSS in fruit is an index used to determine fruit maturity and is a strong indicator of harvesting time. Previous studies on mango have reported different TSS which may be due to genetic variations and varied climatic conditions. However, the results of this study are comparable to those of Othman and Mbogo (2009) and Rodríquez Pleguezuelo et al. (2012) who reported that TSS ranged from 14.5 to 30.1°Bx and 15.7 to 20.0°Bx, respectively. Kansci et al. (2008) also found that the total simple sugar content of mango pulp varied between 9.43 and 15.16°Bx (using hot 80% ethanol for extraction). Mango pulp with higher sugar content is good for food processing because they require the addition of less sugar. Kent may have advantages due to its high TSS.

The TSS/TA of Kent (49.62) was significantly higher ($p \leq 0.05$) than the other varieties while Apple (28.45) had a significantly lower TSS/TA than the other varieties. TSS/TA indicates the degree of sweetness of the fruit, giving information about the flavor, whether sweet or sour or a balance of the two. This ratio is one of the most used methods of evaluating taste, being more representative than the isolated measures of sugars or acidity. Fruit with a higher TSS/TA suggests good quality whereas lower TSS/TA fruit indicates lower quality (Rodríquez Pleguezuelo et al., 2012).

Proximate compositions of mango

Moisture, ash, protein, fiber and fat content of mango are presented in Table 3. All parameters showed significant differences among the varieties at $p \leq 0.05$. Tommy Atkins (83.62%) had the highest moisture while Keitt (79.48%) had the lowest moisture. Fruit with high moisture content has low dry matter.

Moisture content of mango ranged from 79.48 to 83.62%. Fruit with a higher moisture content has a shorter shelf life. In general, fruits and vegetables deteriorate within a short period of time due to their high moisture content. On the other hand, the edible pulp of fruit with high moisture content can be used for juice production.

Dry matter content ranged from 16.38 to 20.52%. Keitt (20.52%) had the highest dry matter content followed by Kent (18.61%) whereas Tommy Atkins (16.38%) had the lowest dry matter content. Results were comparable to the reports of Kansci et al. (2008) and Saranwong et al. (2002) who had reported that dry matter content of mango ranged from 13.30 to 17.28% and 16.89 to 19.22%, respectively. Low dry matter content of fruit has a negative impact on nutrition and shelf life (Kansci et al., 2008). Mango fruit with a higher dry matter content such as Keitt could be best for mango-based food products. The ash content of the tested mango varieties ranged from 0.31 to 0.57% with significant variations occurring among the varieties (Table 3). Our results are in accord with those reported by Kansci et al. (2008) who reported ash content ranging from 0.32 to 0.49%. Keitt (0.57%) had the highest ash content while Apple (0.31%) had the lowest. The fat and protein content ranged from 0.14 to 0.47% and 0.29 to 0.56%, respectively. Keitt (0.47%) and Tommy Atkins (0.42%) had the highest fat content. Fat content found in this study was comparable to the results of Kansci et al. (2008) who reported ranges of 0.17 to 0.33 g/100 g FW. There were significant differences in protein content among the varieties ($p \leq 0.05$). The protein content noted in this study was similar to that reported by Kansci et al. (2008) who found protein content ranging from 0.16 and 0.24 g/100 g FW.

The crude fiber content of the four mango varieties ranged from 0.37 to 0.79% and significant differences were observed at $p \leq 0.05$. Fiber has interesting properties, such as water and oil holding capacity (WHC and OHC, respectively; useful in products that require hydration), yield improvement, and modification of texture and viscosity (Elleuch et al., 2011). The fiber content of mango offers the potential for its use in bakery products (Vergara-Valencia et al., 2007). Keitt (18.9%, 80.7 Kcal/g) had the highest carbohydrate and energy content while Tommy Atkins (15.3%, 62.9 Kcal/g) had the lowest (Table 3). Significant differences were observed among the varieties at $p \leq 0.05$. Many studies have shown that fruits and vegetables are not good sources of protein and fat and this study also showed that mango has a low protein and fat content (Table 3).

Antioxidants

Antioxidants are substances capable of preventing oxidative damage caused by free radicals (Flora, 2009). They include polyphenols, carotenoids, and vitamins which gives health-promoting properties to mango due to their antioxidant activities (Dorta et al., 2012; Sogi et al., 2012; Siddiq et al., 2013). Mango is considered to be a good source of dietary antioxidants such as ascorbic acid, carotenoids and phenolic compounds (Schieber et al., 2000). In this study, mango antioxidants such as carotenoids and vitamin C were analyzed.

Carotenoids are bioactive substances with powerful antioxidant activity. They have a role in the enhancement of immune response and reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataracts, and macular degeneration.

In most cultivars of mango fruit (*Mangifera indica L.*), β-carotene accounts for more than half of the total carotenoid content (Chen et al., 2004) and it substantially contributes to provitamin A supply in tropical and subtropical countries. The total carotenoid content of mango fruit ranged from 0.6 to 4.8 μg/g with significant differences among the varieties at $p \leq$

0.05 (Table 4). Apple (4.8 µg/g) had the highest carotenoid content followed by Keitt (3.2 µg/g) while Kent (0.6 µg/g) had the lowest content. Previous reports on mango have shown different total carotenoid content which may be due to differences in storage time and ripening stage (Ellong et al., 2015). Rocha Ribeiro et al. (2007) reported that the total carotenoid content of mango fruit ranged from 1.91 to 2.63 mg/100 g while Haque et al. (2015) found that the total carotenoid content varied from 94.22 to 444.66 µg/100 g.

Vitamin C is an essential human diet component, required for scurvy prevention, required for the biosynthesis of collagen, L-carnitine, and certain neurotransmitters, improves inorganic iron absorption, inhibits nitrosamine formation, and contributes to immune defense. Ascorbic acid acts as an antioxidant and therefore offers some protection against oxidative stress-related diseases (Diplock et al., 1998). Keitt (36.4 mg/100 g) had the highest vitamin C content while Tommy Atkins (14.2 mg/100 g) had the lowest concentration (Table 4). The tested varieties of mango had statistically different vitamin C content at $p \leq 0.05$. Previous studies reported wide variations in vitamin C content in mango. Rocha Ribeiro et al. (2007) reported that vitamin C content ranged from 9.79 to 77.71 mg/100 g, Carvalho et al. (2004) found ranges from 31.7 to 56.7 mg/100 g while Vinci et al. (1995) reported 25.3 mg/100 g in mango. Our study found vitamin C content of a similar magnitude to these reports. Other studies reported lower vitamin C content with an average value of 15.97 mg/100 g (Tommy Atkins) and 9.1 to 16.8 mg/100 g, respectively (Sogi et al., 2012; Sulaiman and Ooi, 2012). Apple, Kent and Keitt varieties met the minimum vitamin C requirement of 15 mg/100 g recommended by EU/WHO for fruit groups (Ellong et al., 2015). Differences in total carotenoid content and vitamin C content variations compared to previous studies can be attributed to genotypic variation, and preharvest factors including climatic conditions, agricultural practices, and ripening stage (Lee and Kader, 2000). This research work also verified previous information that mangoes are a good source of antioxidants.

Mineral composition of mango

Potassium was the most abundant electrolyte in mango with the maximum value of 369.25 mg/100 g. Keitt had the highest potassium and magnesium content whereas Kent had the highest sodium and iron content. Potassium and sodium content ranged from 218.97 to 369.25 mg/100 g and 0.36 to 13.32 mg/100 g, respectively. Magnesium, calcium, and iron content ranged from 8.07 to 14.28 mg/100 g, 6.44 to 9.29 mg/100 g and 0.78 to 2.55 mg/100 g, respectively. Zinc was not detected in Kent and Keitt whereas Tommy Atkins and Apple had values of 0.96 and 0.39 mg/100 g, respectively (Table 5).

CONCLUSIONS

Four varieties of mango were evaluated for their physicochemical properties and they showed significant variations at $p \leq 0.05$. This study provided evidence that the physicochemical properties and antioxidant contents of mangoes grown in Ethiopia were not different from mangoes grown in other countries. Apple, Kent, and Keitt are good sources of vitamin C with moderate total carotenoid content. Keitt had the highest antioxidant and nutritional value of the tested varieties. Mango fruit has the potential to be used as a source of functional ingredients and natural antioxidants to the food industry.

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Table 1. Physical properties of mango fruit.

Variety	skin color	flesh color	Wt. (g)	F_d (mm)	L (mm)	J_v (mL)
Apple	Yellow with red	Yellow	$433.5^b \pm 99.5$	$91.2^a \pm 9.5$	$93.2^b \pm 7.1$	$316.5^b \pm 59.7$
Keitt	Pink with red	Golden-yellow	$727.3^a \pm 50.6$	$93\ 8^a \pm 3.6$	$137.3^a \pm 17.9$	$540.0^a \pm 13.9$
Kent	Yellow with red	Orange-red	$458.9^b \pm 62.9$	$96.7^a \pm 4.8$	$99.8^b \pm 5.2$	$368.2^b \pm 20.1$
Tommy Atkins	Red purple	Yellow orange	$466.9^b \pm 51.5$	$87.7^a \pm 6.9$	$108.6^b \pm 5.3$	$333.7^b \pm 25.6$
Mean			521.7	92.3	109.7	389.6
CV			13.2	7.1	9.4	8.9
LSD			106.4	10.1	15.9	53.4

Means with different superscripts are significantly different at $p \leq 0.05$; Wt. is the fruit weight, F_d represents fruit diameter, L is the fruit length, J_v is the juice volume.

Table 2. Results of pH, TSS (°Bx), TA (g/L) and TSS/TA.

Varieties	pH	TSS (°Bx)	TA (g/L)	TSS/TA
Apple	3.86 ± 0.06^d	18.07 ± 0.35^b	6.40 ± 0.64^a	28.46 ± 3.40^c
Keitt	4.00 ± 0.01^c	13.60 ± 0.26^d	3.48 ± 0.39^b	39.44 ± 4.56^b
Kent	4.29 ± 0.01^b	18.97 ± 0.29^a	3.84 ± 0.32^b	49.62 ± 4.15^a
Tommy Atkins	4.73 ± 0.02^a	15.03 ± 0.21^c	3.54 ± 0.16^b	42.51 ± 2.11^b
Grand Mean	4.22	16.42	4.31	40.01
CV	0.69	1.72	9.60	9.18
LSD	0.05	0.53	0.78	6.92

Means with different superscripts are significantly different at $p \leq 0.05$.
TA is the titratable acidity (citric acid), TSS /TA is the total soluble solids to titrable acidity ratio.

Table 3. Proximate composition of mango varieties.

Variety	Moisture (%)	Ash (%)	Fat (%)	Fiber (%)	Protein (%)	CHO(g/100g)	FE(Kcal/g)
Apple	82.49 ± 1.13^{ab}	0.31 ± 0.04^c	0.29 ± 0.02^b	0.68 ± 0.10^{ab}	0.31 ± 0.02^c	16.6 ± 1.1^{bc}	67.6 ± 4.2^{bc}
Keitt	79.48 ± 0.85^c	0.57 ± 0.07^a	0.47 ± 0.07^a	0.37 ± 0.06^c	0.56 ± 0.02^a	18.9 ± 0.8^a	80.7 ± 3.2^a
Kent	81.39 ± 1.17^b	0.44 ± 0.08^b	0.14 ± 0.04^c	0.62 ± 0.09^b	0.38 ± 0.02^b	17.6 ± 1.0^{ab}	70.9 ± 4.3^b
Tommy Atkins	83.62 ± 0.55^a	0.39 ± 0.05^{bc}	0.42 ± 0.05^a	0.79 ± 0.07^a	0.29 ± 0.01^c	15.3 ± 0.5^c	62.9 ± 2.3^c
Grand Mean	81.74	0.43	0.33	0.61	0.38	17.11	70.52
SEM	0.55	0.04	0.03	0.05	0.01	0.50	2.02
LSD	1.78	0.12	0.09	0.15	0.04	1.62	6.59

Means with different superscripts are significantly different at $p \leq 0.05$.
CHO is the total carbohydrate content, FE is the food energy.

Table 4. Total carotenoids and ascorbic acid concentrations of four mango varieties.

Variety	TC (µg/g)	AA (mg/100 g)
Apple	4.8 ± 0.2[a]	27.5 ± 2.9[c]
Keitt	3.2 ± 0.1[b]	36.4 ± 2.5[a]
Kent	0.6 ± 0.3[d]	32.8 ± 4.7[b]
Tommy Atkins	2.2 ± 0.3[c]	14.2 ± 3.6[d]
Grand Mean	2.7	27.7
SEM	0.1	1.0
LSD (p <0.05)	0.5	3.4

Means with different superscripts are significantly different at $p \leq 0.05$
TC is the total carotenoids, AA is ascorbic acid.

Table 5. The mineral composition of mango fruit (mg/100 g).

Variety	Na	Mg	K	Ca	Fe	Zn
Kent	13.32	9.76	257.80	6.44	2.55	ND
Keitt	0.36	14.28	369.25	8.99	0.78	ND
Tommy Atkins	3.47	8.07	218.97	7.75	1.72	0.96
Apple	3.94	9.08	223.75	9.29	1.09	0.39

ND is not detected.