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Comparison of polyphenol content between laboratory processed Laphet and China and Myanmar tea (*Camellia sinensis)* products

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ABSTRACT

Green tea, black tea and laphet (fermented pickled tea leaves) from china and Myanmar were extracted using hot water extraction method and their phenol compounds were compared. The total polyphenol content (TPC) was determined spectrophotometrically, while the phenolic compounds were determined by HPLC. Laphet contained more than twice as much polyphenol than fresh tea leaves. Chinese green tea showed higher TPC than Myanmar green tea. However, Myanmar black tea had higher TPC than Chinese black tea. For laphet, no substantial difference was observed. In the qualitative analysis by HPLC method, laphet had higher phenolic compounds (EGC, C, CA and EC), but lower EGCG and ECG compared to fresh tea leaves. Chinese green contained higher EGC, CA and EGCG. But C, EC and EGC were higher in Myanmar green tea. In black tea comparison, all phenolic contents of Chinese black tea were lower than Myanmar black tea. Like in spectrophotometric method, HPLC analysis showed no difference in laphet results, According to these findings, Chinese green tea could give more health benefits than other tea products, because Chinese green tea had the highest polyphenol contents.

Keywords: Laphet, green tea, black tea, China, Myanmar.

Introduction

Tea, a beverage prepared from processed fresh leaves (two leaves and a bud) of Camellia *sinensis* plant that belongs to the family Theaceae, is the second most consumed beverage in the world (Namita *et al*., 2012). It surpasses coffee, beer, wine and carbonated soft drinks. There has been increased economic and social interest of tea. As such, its consumption has become part of many people daily routine as a beverage as well as a therapeutic drink. (Namita *et al*., 2012; Cabrera *et al*., 2006). In China, tea has been cultivated and consumed for more than 2000 years (Li, 1983), now produced in over 20 provinces, where there are proper climate, sufficient humidity, adequate sunshine and fertile soil. There are three main varieties that characterize tea species namely; *Camellia sinensis* var. *sinensis* (*L*.) O. Kuntz (Chinese tea), *Camellia sinensis* var. *assamica* (Masters) with big leaf and *Camellia.assamica* var. *lasiocalyx* (Planchon ex Watt) with the intermediate leaf size (Sealy, 1958; Hara, 2001; Ji *et al.*, 2011).

Depending on the manufacturing process, teas are classified into three major types (Cabrera *et al*., 2006: (1) “Non-fermented” (green tea); is produced by drying and steaming the fresh leaves). This process enables the leaves to keep their original green color and retain most natural substances like polyphenols and chlorophyll contained within the leaves. This kind of tea is produced all over China and is the most popular category of tea. (2) “Semi-fermented” (oolong tea); is produced by subjecting the fresh tea leaves to a partial fermentation stage before drying. The partial fermentation imparts to it the characteristics of both green and black teas. Its taste is more similar to green tea than black tea, but has a less "grassy" flavor than green tea. The three major oolong-tea producing areas are on the southeast coast of China e.g. Fujian, Guangdong and Taiwan. (3) “Fully fermented” (black tea, red tea and *pu-erh* or *puer tea*). In these types of teas, fresh tea leaves undergo a post-harvest fermentation stage before drying and steaming, giving them a strong flavor and dark color. Fermentation of black tea is due to an oxidation catalyzed by polyphenol oxidase, and that of *pu-erh* tea is attained by using microorganisms over years of fermentation and pressing, giving it a unique earthly flavor. In comparison to other tea categories, the “fully fermented” tea has a longer lasting flavor and highest concentration of caffeine. This is the most popular form of tea in south Asia and Europe.

However, worldwide, 80% of the tea consumed is black tea. It is the most popular in Europe, North America and North Africa (except Morocco), whereas green tea is drunk throughout Asia. Oolong tea is popular in China and Taiwan. Approximately, black tea makes 76-78% on world production and consumption, 20-22% is green tea and <2% is oolong tea (Cabrera et al., 2003).

Apart from these three major teas, Myanmar, a country in South – East Asia, has another type of tea called *Laphet*. Laphet is fermented pickled tea leaves, which are rather eaten than processed into a drink. Myanmar is one of the countries that have an eating habit of tea leaves. Because if good climate and soil, Myanmar produces one of the best tea in the world. And also called “Myanmar Tea”, is one of the inorganic teas because no chemical fertilizer and pesticide are used during its cultivation (Hlaing, 2008). In Myanmar traditional, word like: *“Of all flesh, pork is the best”, “Of all fruit, mango is the best”, “Of all the leaves,*

Laphet (fermented pickled tea leaves) is the best" are commonly heard.

Laphet tea is a popular and important in Myanmar culture, as well as the drinking of tea. It is served during official functions and traditional ceremonies like including: Judicial affairs; engagement, wedding and funeral ceremonies among others. It is known as "Lord leaves" or "Lord Medicine" because of its usefulness for health. It is believed that eating tea increases life for as long as 120 years (Hlaing, 2008). The consumption style of Laphet has mainly two forms. First style is *A-Hlu-Laphet*, mainly served in ceremonies. In this style, Laphet and other ingredients such as fried green beans, fried special lab beans, fried garlic, roasted sesame seeds, fried peanuts, put separately in traditional shallow lacquer ware dish called "Laphet ohk" and people eat as they like. In the second style, popularly among Myanmar women, tea leaves are served as salad, in which they mix all ingredients (Laphet and some salt, oil, lemon juice and soya sauce) together.

It is widely reported that tea contains substantial amount of bioactive compounds such as flavanoids. Catechins dominate in green tea and theaflavins and thearubigins predominate in black tea (Nshimiyimana and He, 2010). These kinds of tea flavonoids are thought to have the strongest chemopreventitive effect in animal models at the concentration usually consumed by humans. The catechin present in green tea is commonly called polyphenols. Tea leaves, especially green tea, has been found to be very rich in human health related catechins and gallic acids, which include (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epicatechin (EC). (Anesini *et al*. 2008; Cabrera *et al.*, 2003; Wu and Yu, 2006). Black tea contains lower concentrations of these catechins than green tea. Green, Black and Oolong tea are also good sources of other compounds such as flavanoids, amino acid, vitamins (C, E and K), caffeine, theophylline and theobromine and polysaccharides among many others.

The chemical structures of polyphenols, gallic acid theophylline and caffeine and are shown in Figure 1.

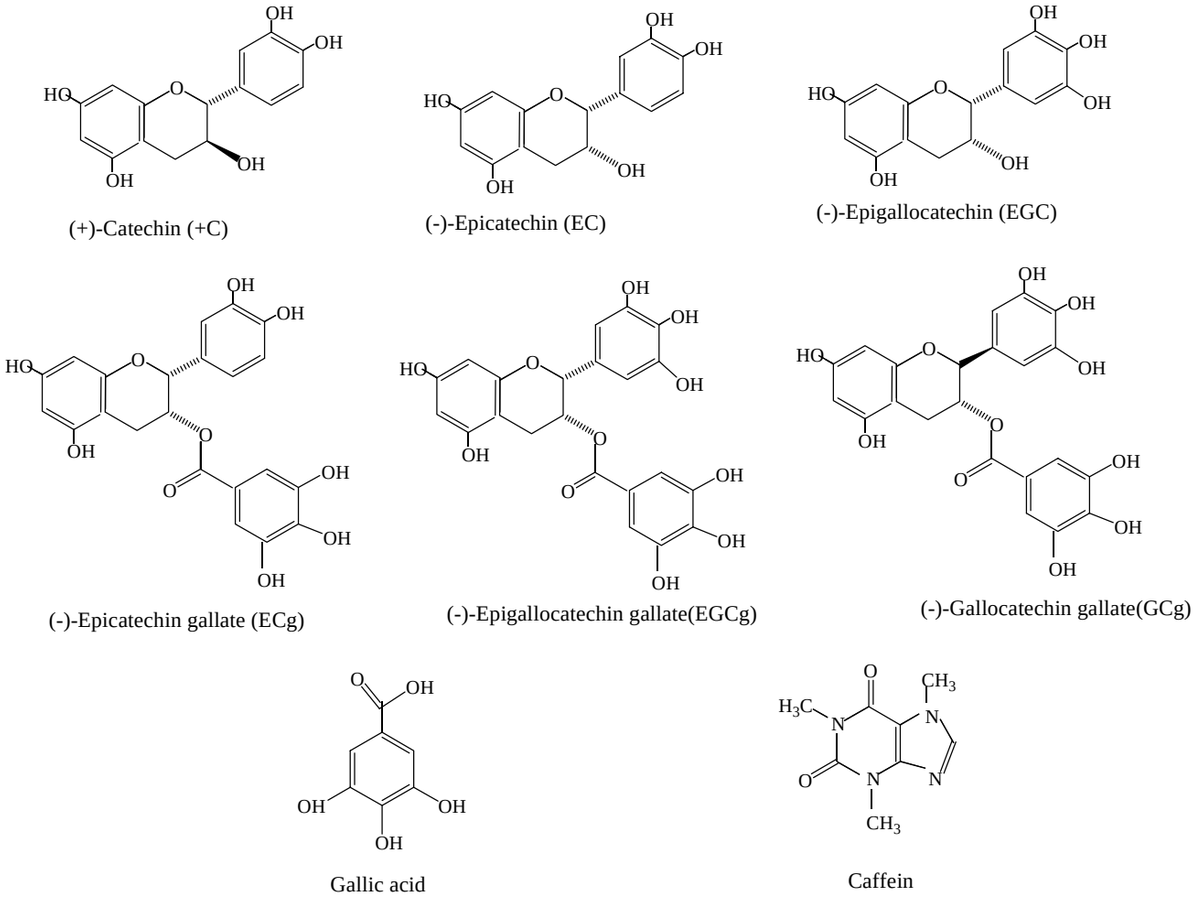

Figure1: Chemical structure of tea polyphenols, gallic acid and caffeine.

To the authors' knowledge, there is no reported Laphet produced in China on the market, let alone from Chinese tea varieties. This study therefore, aimed at processing Laphet from Chinese tea and analyzes its phenolic components in comparison with those in green tea and black tea from China and Myanmar, and

Myanmar Laphet. The tea species from these countries (*Camellia sinensis*) are same but their climates, processing technologies and environmental conditions differ in one way or another. As such, the study will provide information on the relationship between polyphenol compounds and processing condition which include cultivation.

MATERIALS AND METHODS

Procurement of chemicals

Freshly harvested tea leaves were obtained from Suzhou, China. Processed black tea and green tea were purchased from local markets in Myanmar and China. Chinese Laphet was processed from in the Laboratory of Food Safety and Quality Control, Jiangnan University, Wuxi, China. Myanmar Laphet was purchased from Myanmar Local market. Standards were obtained from Sangon Biotech (Shanghai) Co., Ltd and Shanghai Sunny Biotech CO., Ltd, China. All other chemicals were of analytical grade. Distilled or deionized water was used in all laboratory processed.

Laphet processing

The technology of Laphet processing was adopted from Pindaya town, Taunggyi district, Myanmar. Pindaya is famous for Laphet producing in Myanmar and the method of Laphet processing for Laboratory scale and Industrial scale are same, only different in the amount of fresh tea leaves used.

In this process, the freshly harvested tea leaves (two most tender leaves and a bud per branch) were boiled in distilled water for 5-8 minutes for enzymatic deactivation. The water was then completely removed (watering out). At this stage, complete removal of water is very important because the amount of water remaining determines the shelf life of the product. After watering out, the leaves were rolled and quickly sealed in a plastic bag (to avoid aeration), and kept at room temperature. The fermentation starts after keeping in plastic bag. After 12 hours, tea leaves were rolled again, pressed with some weights, and allowed to ferment again for 14 days, at room temperature. After the 2 weeks, the tea leaves turned to Laphet and were sealed in plastic bags and stored at -4 °C until further analysis. The processing steps are summarized in figure 2.

Sample extraction

Tea sample (1 g) was extracted in a beaker with 107 g of boiling distilled water extracted for 4 minutes under magnetic (Chen and Ho, 2007). The mixture was then filtered by using cotton wool to remove the tea powder. The extract was left to cool down at room temperature. The beaker with tea extract was weighed and net weight bought to 100 g. The excess tea infusion was stored at -20 °C for other analyses.

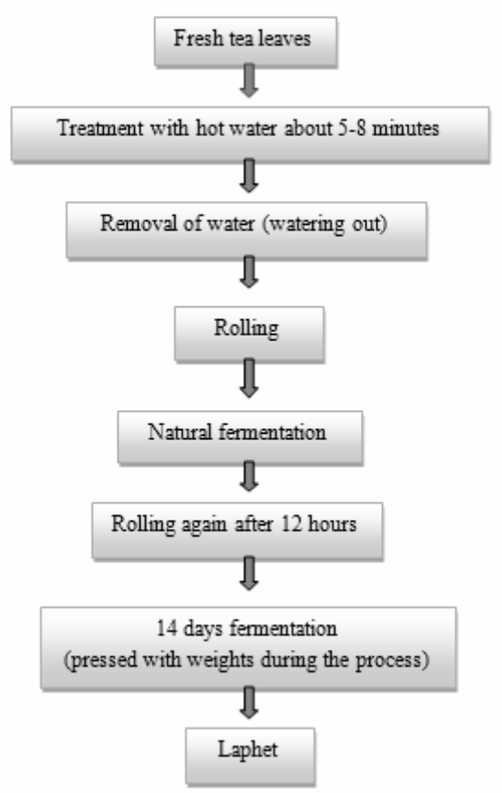

Figure 2: Stages involved in the processing Laphet

Total polyphenol content (tpc) analysis

Total polyphenol content (TPC) was determined by Folin-Ciocalteu reagent method as described by Chen and Ho (2007). Saturated sodium carbonate was prepared and stored. Working solutions of Gallic acid standards (25 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm) were prepared freshly each time at room temperature. Then, a mixture of 0.5 ml of Gallic acid or 1:10 diluted sample, 4.5 ml of distilled water, 0.2 ml of Folin reagent and 0.5 ml of saturated sodium carbonate were prepared for standard curve and sample analysis. Samples were allowed to fix for at least 1 h at room temperature. The T-1900 UV spectrophotometer was used to determine the absorbance of each sample at 725 nm using 1 cm^3 cuvette. The results were determined by calibration curve using garlic acid as standard. All measurements were carried out in triplicate. Final results were expressed as Gallic acid equivalent (GE).

Determination of catechins contents by HPLC

High liquid performance chromatography (HPLC) (Aglient 1100, China series and C18 column) was used for the identification of catechins in tea samples. The HPLC analysis was performed using mobile phase consisted of 2% acetic acid in water (v/v) (solvent A) and acetonitrile (solvent B). The total running time was 1 h. After 10 minutes, solvent B was increased from 8-12% then to 35% over additional 50 minutes and then back to the starting ration over an additional 5 minutes (Chen. and Ho, 2007). Gallic acid(GA), epicatechin

(EC), caffeine (CA), Catechin (C), epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin gallate (ECG) were identified by comparing their retention time to that of standards.

Statistical analysis

One-way analysis of variance (ANOVA) was carried out using IBM SPSS statistics 19 to obtain statistical mean values and standard variations for each data.

RESULT AND DISCUSSION

Analysis of total polyphenol contents

In the spectrophotometric results, a straight-line standard curve was obtained in the range of 25-200 µM Gallic acid, characterized by a correlation coefficient of 0.9933. The calibration equations for tea polyphenols was $y = 0.0042x - 0.0257$ $R^2 = 0.9933$

The content of total polyphenol in laboratory processed Laphet was determines at the start, after 7 days fermentation, and at the completion the process (end product). At the start of the process, the fresh tea leaves contained 28.75±0.88 mg/g TPC. Then, it increased to 32.17±0.53 mg/g and 62.562.5±1.95 mg/g after 7 and 14 days of fermentation. These results show the changes in TPC that took place at various processing stages of Laphet processing. During the first week, the fermentation rate and the rate of polyphenol change were slow. But later, towards the end of the processing stages, the rates of both fermentation and polyphenol content increased quickly. The increase may be associated with the concentration of compound after the pressing, which extract a substantial amount of water from the leaves.

According to the results, Laphet showed high TPC. Polyphenols are beneficial for human health. Studies have shown that tea polyphenol has the activity of the inhibition of skin tumor genesis and EGCG inhibit tumor initiation and promotion by chemical carcinogens and UV light (Yang *et al.,* 1993). Caffeine and EGCG cause reduction of lung tumor incidence (Xu *et al.,* 1992). Tea consumption reduced the risk of Lung cancer in male cigarette smokers (Mu et al., 2003). It was reported that green tea drinking decreased liver cancer 78% among alcohol drinkers and 43% among cigarette smokers because of tea polyphenols and pigments in tea. Tea polyphenols had an effect on many other health problems such as cancer, urinary bladder tumor, cardiovascular diseases, diabetes, etcetera (Khan *et al*., 2007). This shows that Laphet has a potential in reducing many of these health problems due to its high composition of TPC.

The TPC in laboratory processed Laphet was compared to the industrial processed tea samples (green tea, black tea and laphet from China and Myanmar) and the results are presented in figure 3. The Chinese green tea showed higher polyphenol content (204.58 mg/g) than Myanmar green tea (152.5 mg/g). But Myanmar black tea contained more than twice as much higher TPC than that of Chinese black tea. The values obtained from both Laphets were similar (62.25 mg/g for Chinese and 69.25 mg/g). These results showed that green tea still had the highest TPC compared to black tea and Laphet. Nevertheless, Chinese tea, Myanmar black tea and Laphet, surpasses their comparable samples

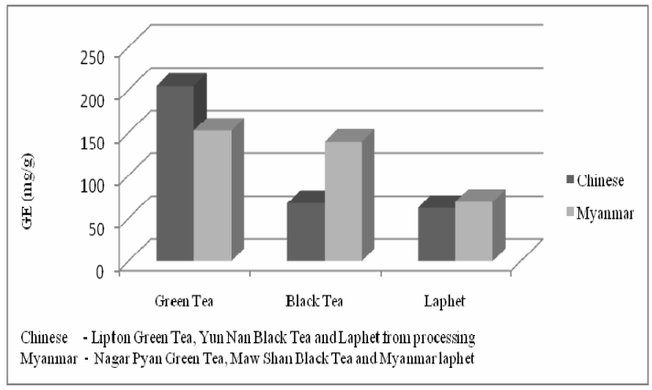

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Figure 3. Total polyphenol contents in different tea products of China and Myanmar

Determination of catechins contents by HPLC

HPLC analyzing was very important to identify the major polyphenols in tea and the quantity of individual tea polyphenol content. After optimization, the active compounds were identified in Laphet processing after extraction according to their retention time, Gallic acid (GA), 3.973 min; epigallocatechin (EGC), 6.358 min; Catechin (C), 7.366 min; Caffeine (CA), 8.123 min; epicatechin (EC), 9.558 min; epigallocatechin gallate (EGCG), 10.075 min; epicatechin gallate (ECG), 14.663 min. In the analyzing between the processing steps, EGC and C were not detectable in fresh tea leaves and EGCG was not detected in Laphet during processing. At the end of production, Laphet had all catechin detected. It had the maximum rate of the caffeine and EGCG was the lowest. However, after the fermentation, EGC content was lower in Laphet than Fresh tea leaves. Laphet had EGC of 6.78 ± 0.03 mg/g, 0.57±0.03 mg/g C of, 9.66 ±0.39 mg/g CA, 3.13 ±0.15 mg/g EC, 0.24 ±0.01 mg/g EGCG and ECG of 0.19 ± 0.01 mg/g.

Polyphenolic compounds of tea products from China and Myanmar by HPLC

The results of polyphenolic compounds of tea products from the two countries are shown in figure 4. In general, the catechin composition of Chinese green tea was higher than Myanmar tea. EGC, CA and EGCG were higher in Chinese green tea, while CEC and ECG were lower compared to Myanmar green tea. The EGC and EGCG were not detected in Chinese black tea but were found in Myanmar black tea. CA was higher in Chinese black tea than Myanmar black tea. The Myanmar black tea had indicated higher EGC, EGCG and ECG than Chinese black tea but slightly lower C

and EC. There was a similarity in all the polyphenolic compounds between the two Laphet samples, except ECG, which was not detected in Myanmar Laphet. Myanmar Laphet had a slightly higher amount than Chinese Laphet. But EC was found in double amount in Chinese laphet than Myanmar laphet.

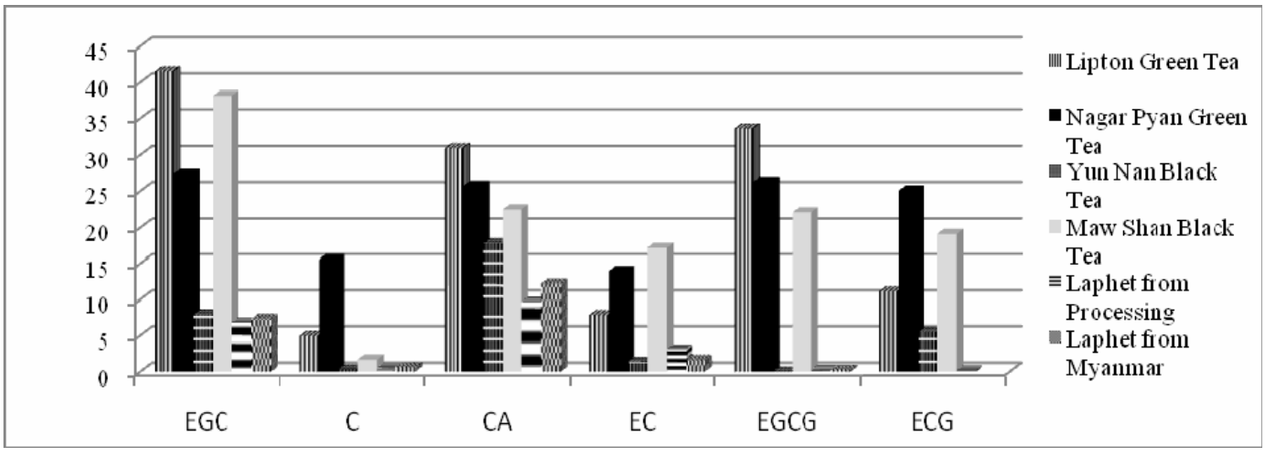

Figure 4: Polyphenolic compounds of tea products from China and Myanmar. Epigallocatechin - EGC, Catechin - C, Caffeine - CA, epicatechin- EC, epigallocatechin gallate - EGCG, epicatechin gallate - ECG.

CONCLUSION

Chinese tea variety can be used to produce Laphet with polyphenolic compounds similar to that of Myanmar Laphet. The study clearly showed that environmental and cultivation conditions have no significant effect on the polyphenolic composition of the product. Although the experimental Laphet was prepared at laboratory level, the processing technique used was almost the same as that of industrial scale.

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Effect of cooking on nutritional composition of Mushroom (*pleurotus Florida l*)

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ABSTRACT

The proximate composition of raw and boiled mushroom cultivar (*Pleurotus florida*) of Pakistan was studied in order to assess their role in human nutrition. Mushrooms contain appreciable amount of protein and fiber contents. The fat content of raw mushrooms was found higher than boiled mushrooms. The crude protein and ash contents were higher in caps of mushroom plant than mycelia. The protein contents of mushrooms were much higher than cereals and comparable to that of food legumes. Its nutritional value can be further enhanced when used in combination with meat and dairy products.

Key Words: Pleurotus florida, mushroom, cooking, composition, nutrition.

Introduction

Mushroom cultivation is widely practiced in many countries of the world, to fulfill the requirements of protein food for human beings. These require less space, less care, less equipment and expenditure for cultivation, than most of the plants and animals. The protein value of mushroom is double than cabbage, potatoes and asparagus, four times to that of tomatoes and carrot and six times to that of oranges. Mushrooms are source of niacin and riboflavin. They serve as a good source of trypsin enzyme, and are rich in iron, copper, calcium, potassium, vitamin D, and folic acid. They have a long history of use in traditional Chinese medicine to promote good health and vitality and increasing body's adaptive abilities (Alam and Raza, 2001).

Mushrooms are good source of protein, vitamins and minerals (Khan *et al*., 1981) and have many uses both as food and medicine (Alice & Kustudia, 2004).Mushrooms are known to produce many kind of bioactive compounds, generally linked with mycelial cell wall, that help in enhancing the immune capacity to fight against carcinogens (Ramesh and Pattar, 2010). The total phenol contents showed that major antioxidants components ranged from 9.55 to 16.8 mg/g in different mushrooms (Kumari *et. al*., 2011)

In the developed countries, mushrooms have become one of the most important of all the horticultural crops. The production of mushrooms is increasing rapidly throughout the world, which is available all the year round and is used in many kinds of table dishes. Total commercial mushroom production worldwide has increased more than 21 times in 35 years, from about 350,000 tons in 1965 to about 7.5 million tons in 2000 (Boa, 2004). The world production value of edible mushrooms in 2001 was estimated at around US$ 23 billion, which exceeds the value of many agricultural products (Chang, 1999). Their cultivation on extensive scale can help to solve many problems of global importance such as protein shortage and resource mobilization. In advanced countries mushroom cultivation is a multi million dollar industry. This on one hand will produce nutritional vegetables to consumers and on the other hand will offer job opportunities for the unemployed educated youth as well as to become a source of additional income for house hold women, small farmers and landless communities. However, there is lack of information about mushroom varieties grown in Pakistan. Therefore the present study was under taken to evaluate the nutritional composition of raw and cooked mushroom in order to evaluate the effect of cooking on mushroom (*Pleurotus florida*) grown in Pakistan.

MATERIALS AND METHODS

A commonly consumed mushroom species *Pleurotus florida* was collected from plant pathology laboratory of *Khyber* Pakhtunkhwa *Agricultural* University, Peshawar. Samples were divided into two portions i.e. strips and caps. The caps and strips of mushroom were analyzed separately. All the samples were oven dried and powdered in a stainless steel grinder to pass through 35 mm mesh sieve. The samples were then stored in airtight clean bottles at 4 to 5 ºC in a refrigerator for chemical analysis.

Proximate Analysis: The powdered samples of mushrooms were analyzed for their proximate composition. The proximate analysis of mushroom was carried out for moisture content, total ash, crude fat, crude fiber, crude protein and nitrogen free extract (NFE) according to AOAC (2005). All the chemicals used were of analytical grade. All analysis was done in triplicate.

Sensory evaluation: Samples were evaluated by a panel of six judges for sensory characteristics like color, taste, flavor, texture and overall acceptability as described by Meilgaard *et. al*. (2007). Scoring was done according to 9-Point-Hedonic Scale.

RESULTS AND DISCUSSION

Proximate Analysis of mushroom: The proximate composition of fruiting bodies and mycelia of raw mushroom *(Pleurotus florida)* is presented in Table 1 and boiled mushroom in Table 2. The crude protein content of the fruiting and mycelia of raw and boiled mushroom variety, *Pleurotus florida* ranged from 24.84 % to 27.21%, respectively. The crude protein contents of raw mushrooms were higher than boiled mushrooms. Mattila *et. al*. (2002) also observed similar results. The cap of both mushrooms had higher protein contents than their mycelia. Similarly crude fat contents in the fruiting bodies and mycelia of the raw and boiled mushroom variety vary from 0.35 to 1.35 %, the highest being in the fruiting body of raw mushroom. The crude fiber content of the raw and boiled mushroom variety varied from 8.48 to 9.30%, the highest being in the mycelia of raw mushrooms. The fruiting body of raw mushrooms contained 7.60 % ash that was higher than that of fruiting body of boiled mushrooms. It was concluded that caps of mushrooms contain more nutrients than mycelia. These results are in line with those of Verma *et. al.* (1987) and also coincide with the results of Adejumo and Awosanya (2005) and Mshandete and Joyce (2007).

Variations were found in the carbohydrates content from 51.39 to 56.03 % in two samples. The proximate composition results of mushroom variety are in line with those of Shah *et. al.* (1997). These results suggest that like other foods, mushrooms can contribute a large amount of protein, fiber and water in human diet. The protein contents of mushroom were higher than cereal grain, wheat and maize (Saxena and Singh, 1984). It was however comparable with that of food legumes such as peas (Jabeen *et. al.,* 1988) and lentil (Khalil and Varanani, 1996).

Table 1: Proximate composition of Pleurotus florida mushroom (Raw)

Parameters	Pleurotus Florida mushroom (Raw)	
	Fruiting Body	Mycelia
Moisture	90.72 %	86.62 %
Protein	27.21 %	26.08 %
Fat	1.35 %	0.40 %
Fiber	8.71 %	9.30 %
Ash	7.60 %	6.19 %
Nitrogen free extract (NFE)	52.50 %	56.03

Table 2: Proximate composition of Pleurotus florida mushroom (Boiled)

Parameters	Pleurotus florida mushroom (Boiled).	
	Fruiting Body	Mycelia
Moisture	92.00 %	89.00%
Protein	26.53 %	24.84 %
Fat	1.22%	0.35%
Fiber	8.48 %	9.17 %
Ash	7.53 %	6.28 %
Nitrogen free extract (NFE)	51.39 %	55.35

Sensory evaluation of mushroom: Sensory evaluation of boiled mushroom was done by a panel of six judges for sensory characteristics like color, taste, flavor, texture and overall acceptability. Mycelia of boiled mushrooms got higher scores for all the sensory characteristics than the fruiting body of boiled mushroom. The highest scores were assigned for color and flavor of mycelia. Overall acceptability of mycelia got higher scores (7) as compared to fruiting body (6) as given in Table 3.

Table 3: Sensory evaluation of Pleurotus Florida mushroom (Boiled)

Samples	Fruiting Body	Mycelia
Color	7.0	8.0
Taste	6.0	7.0
Flavor	7.0	8.0
Texture	6.0	7.0
Overall acceptability	6.0	7.0

1= Extremely dislike, 2 = Strongly dislike, 3 = Moderate dislike, 4 = Slight dislike,
5 = Neutral, 6 = Slight like, 7 = Moderate like, 8 = Strongly Like, 9 = Extremely like

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Physical properties, sensory attributes and consumer preference of fruit leather

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ABSTRACT

A study was conducted to prepare fruit leathers from three different fruit pulps *Such as* apple, mango, and guava. All fruit leathers were then analyzed for their organoleptic characteristics such as color, flavor, texture, sweetness and overall acceptability during storage period of three months. The results revealed that all leather remained unchanged during storage period and has shown a remarkable ranking even after storage period. It was also observed during the study that all fruit leather had identical color, flavor and sweetness which cannot be compared with one another. However overall mean values represent high score for mango leather followed by guava and then apple respectively.

Key words: Fruits, Leather, sensory, storage, apple

INTRODUCTION

Fruit leathers are dehydrated fruit based products. They are a tasty, chewy, dried fruit product. Fruit leathers are made by pouring pureed fruit onto a flat surface for drying. When dried, the fruit is pulled from the surface and rolled. It gets the name "leather" from the fact that when the pureed fruit is dried, it is shiny and has the texture of leather. Fruit leather generally lasts quite a long time in this state and does not require refrigeration. The popularity of the fruit leather has increased significantly in the last 10 years because many view these snacks are more healthful than other confections because it is produced from fruit to which vitamins (particularly vitamin C) has been added.

The advantages of making our own fruit leathers are to use less sugar and to mix fruit flavors. For the diabetic adult or child, fruit leathers made without sugar are a healthy choice for snacks or desserts. Individual fruit leathers should contain the amount of fruit allowed for the fruit exchange. Directions follow for making fruit leathers. Fresh, frozen or drained canned fruit can be used. Drying removes the moisture from the fruit leather so that bacteria, yeasts and molds cannot grow and spoil the fruit leather. It also slows down the action of enzymes, but does not inactivate them. Because drying removes moisture, the food becomes smaller and lighter in weight. Fresh mangoes are highly perishable, and therefore, they are often processed to extend
Shelf-life and facilitate exportation.

The king of the fruits," mango fruit is one of the most popular, nutritionally rich fruits with unique flavor, fragrance, taste, and heath promoting qualities making it a common ingredient in new functional foods often labeled “super fruits." Mangoes are a rich source of vitamin C, vitamin A, and dietary fiber , and they contain vitamins E and K, thiamin, riboflavin, and niacin.
A major limitation on the exportation of fresh mango is its short shelf-life. Mangoes are subjected to chilling injury during storage, but increasing storage temperatures leads to rapid decay in fruit quality (Mohammed and Brecht 2002; Nair and Singh 2009). Therefore, fresh mango is often processed to facilitate exportation and to preserve the fruit paste in its season. A number of products made from ripe mangoes are available on the international market, including canned mango, mango purée, mango juice, dried mango, mango leather, and mango jam.

An apple a day is perhaps one of the most delicious prescriptions ever made. Apples are a powerful source of antioxidants, including polyphenols, flavonoids, and vitamin C, as well as good source of fiber, and potassium. There are only 47 calories in an average sized apple. The secret behind the super antioxidant capacity of the apple is its skin. The apple skin alone provides two to six times the antioxidant activity of the apple flesh alone. So it is important to eat the skin to obtain the full health benefits of apples.

Guava is another tropical fruit rich in nutrition. With its unique flavor, taste, and health-promoting qualities, the fruit easily fits in the new functional foods category, often called “super fruits. Guava-fruit is an excellent source of antioxidant **vitamin-C**. Scientific studies shown that regular consumption of fruits rich in vitamin C helps the body develop resistance against infectious agents and scavenge cancer causing harmful free radicals from the body. Further, the vitamin is required for collagen synthesis within the body. Collagen is the main structural

protein in the human body required for maintaining the integrity of blood vessels, skin, organs, and bones.

MATERIALS AND METHODS

Fresh fruits of guava, mango, and apple were purchased from local market. They were thoroughly washed, apples were peeled, cored and cut into small pieces, similarly guavas were cored, while mangoes were destoned and pulp has been obtained. . After thoroughly cleaning peeling and coring of fruits all the fruits were cut in to two or four pieces. Guava, apple, and mango leather were prepared separately by adding 20% of sugar, 0.2% of citric acid, and 0.1% of sodium benzoate to 80% of their fruit pulp. It was then boiled, cooled and spread on trays oiled with cooking oil. It was then cooled to 60C for 8 hours before packaging for analysis. After making the fruit leathers they were rolled in plastic wraps and kept in air tight containers. The leathers were stored at room temperature for further study.

Sensory evaluation

All fruit leathers were analyzed for color, texture, flavor, sweetness, and over all acceptability by a panel of 10 judges by 9 point Hedonic scale as described by Larmond (1977) starting from extremely dislike to extremely like.

RESULTS & DISCUSSIONS

Fruit leather from three different fruits such as guava, apple and mango were prepared. All leathers were then analyzed by a panel of 10 judges by 9 point Hedonic scale at zero interval i.e. fresh, and then after each month for a total period of three months.

The data pertaining to apple leather is represented in table 1.The data for color, flavor ,texture, and overall acceptability has been in the range of 6 to 7,while mean values during whole interval is about 6.1,which represents a stable product at the end of storage period.

Our results are in agreement with Demarchi *et al. (2010)* who evaluated the influence of pretreatment on final product structure as well as the effect of hot air drying on color and antioxidant retention in apple leather with and without preservative agents. They concluded that losses of antioxidant activity are more dependent on drying temperature than on drying time.

These results are also in agreement with Quientero-Ruize and Giner. (2010) analyzed apple leather quality for formulations with and without preservatives agents over a short period of six months at room temperature.

The results are in agreement with Che Man *et al*. (1992) who prepared sapota leather having shelf life of three months. Similar results were given by Irwandi *et al.* (1998) produced 12 week stable leather from a formulation including sucrose and sorbic acid. The table (2) shows organoleptic scoring for mango leather .Table shows a stable product during whole storage period, however the mean values for mango leather has remained highest followed by guava leather followed by apple leather *i.e.* 6.9.

Similar research has been conducted by Gujar and Khana (2002), indicated that sucrose resulted in the highest acceptability in mango leather. These results strengthen the efficiency of using cane sugar as preservative material for mango slices. Table.3 shows sensory results for guava leather,the mean value during whole storage period is 6.5,while overall results has been constant during storage. Similar research has been conducted by Vijayanan *et al.* (2000) compared conventionally prepared leather with guava leather; Color, texture; flavor and sensory acceptability were analyzed during storage. Both products maintained a high acceptability after 90 days at 27C.

CONCLUSIONS

The advantages of making our own fruit leathers are to use less sugar and to mix fruit flavors. For the diabetic adult or child, fruit leathers made without sugar are a healthy choice for snacks or desserts. Individual fruit leathers should contain the amount of fruit allowed for the fruit exchange. Directions follow for making fruit leathers. Fresh, frozen or drained canned fruit can be used. Drying removes the moisture from the fruit leather so that bacteria, yeasts and molds cannot grow and spoil the fruit leather. It also slows down the action of enzymes, but does not inactivate them. Because drying removes moisture, the food becomes smaller and lighter in weight. Care should be taken not to keep any plastic wrap from touching the sides of the dehydrator.

Honey is the most recommended sweeteners for fruit leather, always start from 2 tablespoon, then we should add additional spoons gradually until it reaches desired sweetness aGranulated sugar is not a good option as it may crystallize during storage, making the leather, brittle. Lemon juice can be to help brighten the flavor of the fruit. Also for color lightening we can add ascorbic acid. The optimum temperature for drying food is 140° F. If higher temperatures are used, the fruit leather may "case harden"; that is cook and harden on the outside while trapping moisture on the inside. The fruit leather will eventually mold when moisture equilibrates during storage. Thus, the drying process should never be hurried by raising the drying temperature.

Table 1: Effect of storage on sensory attributes of apple leather

Fruit	Color	Flavor	Texture	Sweetness	Overall acceptability	Mean
Fresh	6	7	6	5	6	6
1st month	6	6	7	7	7	6.6
2nd month	6	6	6	6	6	6
3rd month	5	6	6	6	6	5.8
Mean	5.75	6.25	6.25	6	6.25	6.1

Table 2: Effect of storage on sensory attributes of mango leather

Fruit	Color	Flavor	Texture	Sweetness	Overall accept ability	Mean
Fresh	8	8	6	7	7	7.2
1st month	8	7	7	7	7	7.2
2nd month	7	7	7	7	7	7
3rd month	6	6	7	6	6	6.2
Mean	7.25	7	6.75	6.75	6.75	6.9

Table 3: Effect of storage on sensory attributes of guava leather

Fruit	Color	Flavor	Texture	Sweetness	Overall accept-ability	Mean
Fresh	7	7	7	6	7	6.8
1st month	7	7	6	6	6	6.4
2nd month	6	6	6	7	7	6.4
3rd month	7	6	7	6	6	6.4
Mean	7	6.5	6.5	6.25	6.5	6.5

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Preservation of mango pulp of fruit from Rusitu Valley, Chimanimani in Zimbabwe

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ABSTRACT

Rusitu Valley in Zimbabwe produces a lot of mango fruit most of which is lost due to its perishable nature. The fruit is processed into pulp for preparation of juice and jam. The major problem is that the pulp has a short shelf life due to microbial degradation. The objective of the work was to find an effective preservation method for pulp from the fruit. The pulp was divided into samples which were treated with chemical preservatives and untreated ones. Treated samples were preserved with sodium metabisulphite, potassium sorbate, and citric acid followed by pasteurization at 80 to 90°C. One sample, C_0, was not treated with chemical preservatives and was not pasteurized. Total soluble solids, pH, colour and odour were measured within 12 days. The pH of treated pulp samples decreased with time. The pH of C_0 and C_1 which were not chemically preserved rapidly decreased within 3 to 4 days. The total soluble solids content of samples S_2 and S_3 was constant, but decreased with time for the rest of the samples. Unpasteurized pulp retained the yellow colour while heat treated samples turned yellowish brown. Moulds colonies were observed and stale odours were detected in samples C_0 and C_1. Samples S_1, S_2 and S_4 which were not pasteurized had an urgent odour. Pasteurized samples S_3, S_5 and S_6 had a cooked mango odour which was not astringent. Sodium metabisulphite, citric acid, potassium sorbate and pasteurization increased the shelf life and stabilized the pH of the pulp.

Key words: Mango, preservation, pH, Total soluble solids, Hurdle concept

INTRODUCTION

Mango (Mangifera indica, L.) is one of the highly priced fruit in the tropics. In this context, mango is known as an appreciable fruit due to its pleasant aroma and flavour, whose nutritional value presents high calories and vitamin contents, among others (Moraes et al., 2010; Sharma et al., 2009).

The fruit is an emerging tropical export crop produced in about 90 countries in the world with a production of over 25.1 million tonnes (Durrani et al., 2012). The mango world market earns about 700 million dollars per year, and world production in 2007 and 2008 was superior to 30×10^6 tons whose world export was approximately 11 million tons (FAO, 2009). Asia is the main producer with 76.9% of the total world production, followed by America with 13.38%, Africa with 9% and less than 1% each for Europe and Oceania (Rathore et al., 2007). In terms of production by country, India accounts for almost half of the world production, followed by China (3 million tons), Pakistan (2.25 million tons), Mexico (1.5 million tons), and Thailand (1.35 million tons) (Indurao et al., 2011).

In Zimbabwe, the production of mango is mainly for consumption as fresh fruit. On average, Zimbabwe produces 170840 tonnes of mango annually (Government of Zimbabwe Agricultural Report, 2012). Most of the fruit is produced at subsistence level by rural communities who primarily use it for home consumption. Some growers sell their produce to local beverage industries and fruit and vegetable industries for export. Other farmers harvest ripe and semi-ripe fruit and deliver it to produce markets in urban centres. The fruit begin to ripen in November and the peak ripening months are December and January. From the end of January the yield of the fruit decreases and at the end of February the mango season ends. Producers incur losses of the fruit at harvesting and distribution due to short shelf life of the fruit. To minimize the losses, it is important to find methods of preserving the fruit or produce pulp which can be preserved.

The mango pulp can be preserved by aseptic packaging, quick freezing, canning, chemical means or a combination of two or more of these methods (Moraes et al., 2010; Younis et al., 2011). The experiments done here were aimed at identifying the most suitable techniques for preservation of mango pulp using available resources. Combinations of potassium sorbate, sodium metabisulphite, citric acid and heat treatment were used for preservation of the pulp of mango grown in

Chamanimani, Zimbabwe Chemical preservatives suppress microbial proliferation, reduce enzyme activity and block browning reactions resulting in increase in shelf life of the pulp and products made.

MATERIALS AND METHODS

Study area

The study was conducted in Rusitu Valley area in Chimanimani. Chimanimani is a district located in the south eastern part of Zimbabwe in Manicaland Province. The district covers an area of 3 353 km^2 and borders Chipinge, Mutare and Buhera (Chimanimani Business Trust Report, 2007). Agriculture constitutes the major economic activity within Manicaland, while forestry and tourism are other economic activities. Rusitu Valley is a growing area for a variety of fruits that include mangoes, bananas, pineapples, lemons, oranges and avocardo pears (Zimbabwe Opportunities Industrial Centre, 2008). The research was carried out at a factory owned by Rusitu Valley Jam Canners Cooperative. The factory is located at Koppa Business Centre, Rusitu Valley in Chimanimani district. The cooperative is made up of forty five women who dedicated their time and effort to processing of the local fruit into jam, juice and puree.

Collection of fruit and pulp extraction

Ripe mangoes of different varieties were brought by the community men and women to the factory. The fruits were washed with running tap water and then hand peeled. The peeled mangoes were passed through a stainless steel pulping machine with 5mm mesh where the juice was extracted from fresh fruit by pressing. The pulp was stored at room temperature of 25 to 30^0C in 100 litre plastic containers with air tight plastic lids.

Pulp preservation

Five hundred (500) millilitre samples of pulp were each treated with varying amounts of sodium metabisulphite and potassium sorbate followed by heat treatment at 80-90^0C for some of the samples as recorded in table 1(FAO, 1997). One sample, C_0 was not treated with

Table 1: Treatment of mango pulp of fruit from Chimanimani in Zimbabwe

Sample	Treatment
C_0	Unpasteurized sample, no heat treatment.
C_1	Pasteurized sample at 90^0C.
S_1	1000 ppm sodium metabisulphite, 500 ppm citric acid, no heat treatment
S_2	1000 ppm sodium metabisulphite, 500 ppm citric acid, 1000ppm potassium sorbate, no heat treatment
S_3	1000 ppm sodium metabisulphite, 500 ppm citric acid, 1000ppm potassium sorbate, pasteurization at 90^0C
S_4	1500 ppm sodium metabisulphite, 500 ppm citric acid, no heat treatment
S_5	1000 ppm sodium metabisulphite, 500 ppm citric acid, pasteurization at 90^0C
S_6	1500 ppm sodium metabisulphite, 500 ppm citric acid, pasteurization at 90^0C

Chemical preservatives or heat and was used as a control. Sample C_1 was pasteurized, but had no chemical preservatives added to it. Samples S_1, S_2, S_3, S_4, S_5 and S_6 were treated with chemical preservatives as outlined in table 1. After the treatments, subsequent measurement and observations made on the samples were made at room temperature over a period of 21 days.

Determination of total soluble solids

Total soluble solids were measured using a hand refractometer (Model ATAGO N-1E) and expressed in ^{0}Brix (Rababah et al., 2011). The instrument was calibrated using distilled water.

Determination of pH

The pH of the fruit pulp samples were determined using a pH meter (model WTW pH 340i 82362 Weilheim) at a temperature of 20 to 25°C. The pH meter was calibrated using pH 4.00 and 7.00 standard buffers.

Determination of colour and odour of pulp

Colour of the pulp was assessed by visual inspection while odour was evaluated by smelling.

RESULTS AND DISCUSSION

pH

Samples S_4 and S_6 were treated with sodium metabisulphite and citric acid. Sample S_4 was not pasteurized and had lower pH values than sample S_6 which was pasteurized. The unpasteurized sample S_4 was likely to have a higher microbial load that fermented sugars in the pulp to form alcohols and acids which lowered the pH. Samples S_1 and S_5 were treated with sodium metabisulphite and citric acid. Sample S_1 which did not undergo heat treatment had a lower pH than sample S_5 which was pasteurized may be due to higher microbial load fermenting sugars resulting in a medium of higher acidity as in sample S_6. Similarly, samples S_2 and S_3 which were treated with sodium metabisulphite, potassium sorbate and citric acid had different pH values with sample S_2 which was not pasteurized having a lower pH than sample S_3 which had undergone heat treatment. As shown in table 2, the pH of each sample decreased with time.

The initial pH values of the samples which ranged from 4.22 to 4.24 were similar to previously measured values ranging from 4.2 to 4.3 (Moraes et al., 2010). However, the final pH of the pulp after 21 days was higher than 3.0 to 3.2 obtained in other studies (Moraes et al., 2010). The differences may be attributed to differences in reagents used for treating the samples in this experiment and samples considered in previous studies. The pH of the unpasteurized sample, C_0 with no preservatives added changed from 3.92 to 2.66 within three days. The drop in pH may be a result of microbial degradation of nutrients producing acids and alcohols. Similarly, the pH of the pasteurized sample C_1 dropped from 3.92 to 2.75 after 4 days. The results are consistent with work done by other researchers where pH decreased with time (Ket al., 2012). Measurements of pH and other parameters for samples C_0 and C_1 were terminated after 3 and 4 days respectively because the samples had grown moulds that caused spoilage. The low pH favoured the growth of moulds.

An ANOVA test for time points variation for the mean pH was done at $\alpha = 0.05$ and gave a p-value of 0.057, showing that the different times had mean pH of mango pulp that were insignificantly different from each other. An analysis of variance (ANOVA) was conducted and showed a p-value of 0.009, indicating that the mean pH for the different samples were significantly different from each other. Least Significant Difference (LSD) was further used for pairwise comparisons of the mean pH for the

Table 2: Changes in pH of mango pulp of fruit from Chimanimani in Zimbabwe within three weeks of observation. C_0 was an unpasteurized sample without chemical preservatives. C_1 was a pasteurized sample but without chemical preservatives. Samples S_1, S_2, S_3, S_4, S_5 and S_6 had added chemical preservatives.

Time (Days)	pH							
	C_0	C_1	S_1	S_2	S_3	S_4	S_5	S_6
0	3.92	3.92	4.22	4.22	4.23	4.22	4.24	4.22
1	3.97	4.00	4.21	4.16	4.19	4.11	4.15	4.12
2	4.03	4.06	4.05	4.06	4.08	4.04	4.06	4.06
3	2.66	2.98	3.96	4.05	4.06	4.02	3.97	3.99
4	-	2.75	3.81	3.98	4.04	3.98	3.95	3.90
7	-	-	3.87	3.95	4.03	3.95	3.93	3.84
14	-	-	3.73	3.93	3.98	3.64	3.89	3.80
21	-	-	3.60	3.81	3.94	3.52	3.84	3.73
Mean	3.65±0.57	3.54±0.56	3.93±0.21	4.02±0.12	4.07±0.09	3.94±0.22	4.00±0.13	3.96±0.16

Means of pH values are significantly different ($p<0.05$). There are no significant differences ($p>0.05$) for means of pH values for samples S_1 to S_6.

Table 3: Changes in total soluble solids content of mango pulp of fruit from Chimanimani in Zimbabwe within three weeks of observation. C_0 was an unpasteurized sample without chemical preservatives. C_1 was a pasteurized sample but without chemical preservatives. Samples S_1, S_2, S_3, S_4, S_5 and S_6 had added chemical preservatives.

Time (Days)	Total soluble solids (°Brix)							
	C_0	C_1	S_1	S_2	S_3	S_4	S_5	S_6
0	13.9	17.5	9.0	9.0	9.0	9.0	9.0	9.0
1	14.2	15.0	9.0	9.0	9.0	9.0	9.0	9.0
2	14.0	15.5	9.0	9.0	9.0	9.0	9.0	9.0
3	14.0	15.9	9.0	9.0	9.0	9.0	9.0	9.0
4	-	14.7	9.0	9.0	9.0	9.0	9.0	9.0
7	-	-	8.8	9.0	9.0	8.8	8.9	9.0
14	-	-	8.6	9.0	9.0	8.2	8.0	8.9
21	-	-	7.8	9.0	9.0	8.0	8.0	8.6
Mean	14.0±0.1	15.7±1.0	8.8±0.4	9.0±0	9.0±0	8.8±0.4	8.7±0.4	8.9±0.1

Means of total soluble solids values are significantly different ($p<0.05$). There are no significant differences ($p>0.05$) in means of total soluble solids values for samples S_1 to S_6.

samples and it was observed that samples C_0 and C_1 were similar whilst sample S_1 up to sample S_6 were also insignificantly different from each other. The differences may be attributed to the effect of the chemical preservatives on the pulp sample. The similarities in the pH of the samples S_1 to S_6 may result from possible buffering action of the preservatives.

Total soluble solids

As shown in table 3, there was a marked decrease in total soluble solids of the unpasteurized pulp in sample C_0. The drop in soluble solids content may be caused by fermentation of sugars to alcohols and acids by microorganisms present such as yeasts. Sample S_5 which was treated with the same chemical preservatives as sample S_1 and pasteurized retained a higher content of soluble solids than sample S_1. The results show the additional effect of heat on stabilizing the sugar content in sample S_5. The heat of pasteurization possibly killed vegetative yeasts cells. There was no change in total soluble solids content of samples S_2 and S_3 during the period under review, possibly due to yeasts and moulds killed by potassium sorbate and bacteria destroyed by sulphur dioxide. Results for samples S_2 and S_3 show that by the time heat was applied to sample S_3, microorganisms causing fermentation of sugars had already died due to action of chemical preservatives. Sample S_4 had a higher drop in total soluble solids when compared with sample S_6. In sample S_6, the combined action of chemicals and pasteurization might have reduced microbial load resulting in minimal loss of soluble solids due to fermentation. Hence, sample S_4 which did not undergo heat treatment had a higher loss of soluble solids probably due to fermentation than sample S_6. Sample S_6 illustrates the Hurdle concept. Hurdle technology is a term applied when foods are preserved by a combination of processes (Jurrani et al., 2012; Moraes et al., 2010). Examples of hurdles are temperature, water activity, atmospheric conditions and preservatives. Hurdle technology advocates the deliberate combination of existing and novel preservation techniques in order to establish a series of preservative factors or hurdles that any micoorganisms present should not be able to overcome resulting in increased shelf life of the food product (Leistner and Gorris, 1995). The total soluble solids content of C_0 and C_1 were almost constant during the period of the study, but decreased towards the end in a way similar to previous findings (Hussain et al., 2003).

An analysis of variance (ANOVA) was carried out and gave a p-value of 0.371, showing that the different times had mean °Brix values of the mango pulp that were insignificantly different from each other. Further, an analysis of variance (ANOVA) was done to assess samples variation of the mean °Brix, a p-value of less than 0.001 was obtained, indicating that the mean °Brix values were significantly different for the different samples. Least Significant Difference (LSD) was then used for pairwise comparisons of the mean °Brix values of the samples and it was observed that samples C_0 and C_1 were significantly different from all other samples whilst sample S_1 up to S_6 were insignificantly different from each other.

Colour

In sample S_6 which was heat treated, sulphur dioxide was lost as sodium metabisulphite rapidly degraded leading to non-enzymatic browning reactions. Sulphur dioxide blocks enzymatic and non-enzymatic browning reactions (Fenemma, 1996; Ding et al., 2002). In sample S_4 which was not pasteurized, the pulp remained yellow. The available sulphur dioxide prevented enzymatic and non-enzymatic browning reactions resulting in retention of the yellow colour by

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Table 4: Changes in colour of mango pulp of fruit from Chimanimani in Zimbabwe within three weeks of observation. C_0 was an unpasteurized sample without chemical preservatives. C_1 was a pasteurized sample but without chemical preservatives. Samples S_1, S_2, S_3, S_4, S_5 and S_6 had added chemical preservatives

Time (Days)	Colour							
	C_0	C_1	S_1	S_2	S_3	S_4	S_5	S_6
0	Bright yellow	Bright yellow	Dark yellow	Dark yellow	Brownish yellow	Dark yellow	Brownish yellow	Brownish yellow
1	yellow	Pale yellow	Dark yellow	Dark yellow	Brownish yellow	Dark yellow	Brownish yellow	Brownish yellow
2	Dark yellow	Pale yellow	Dark yellow	Dark yellow	Brownish yellow	Dark yellow	Brownish yellow	Brownish yellow
3	Yellow /mould growth	Pale yellow	Dark yellow	Dark yellow	Brownish Yellow	Dark yellow	Brownish yellow	Brownish yellow
4	-	Yellow /mould growth	Dark yellow	Dark yellow	Brownish yellow	Dark yellow	Brownish yellow	Brownish yellow
7	-	-	Dark yellow	Pale yellow	Brownish yellow	Pale yellow	Brownish yellow	Brownish yellow
14	-	-	Pale yellow	Pale yellow	Brownish yellow	Pale yellow	Brownish yellow	Brownish yellow
21	-	-	Pale yellow	Pale yellow	Brownish yellow	Pale yellow	Brownish yellow	Brownish yellow

Table 5: Changes in odours of mango pulp of fruit from Chimanimani in Zimbabwe within three weeks of observation. C_0 was an unpasteurized sample without chemical preservatives. C_1 was a pasteurized sample but without chemical preservatives. Samples S_1, S_2, S_3, S_4, S_5 and S_6 had added chemical preservatives

Time (Days)	Odor							
	C_0	C_1	S_1	S_2	S_3	S_4	S_5	S_6
0	Fresh mango	Fresh mango	Pungent mango	Pungent mango	Cooked mango	Pungent mango	Cooked mango	Cooked mango
1	Strong mango	Fresh mango	Pungent mango	Pungent mango	Cooked mango	Pungent mango	Cooked mango	Cooked mango
2	stale mango	Fresh mango	Pungent mango	Pungent mango	Cooked mango	Pungent mango	Cooked mango	Cooked mango
3	stale mango	Fresh mango	Pungent mango	Pungent mango	Cooked mango	Pungent mango	Cooked mango	Cooked mango
4	-	Stale mango	Pungent mango	Pungent mango	Cooked mango	Pungent mango	Cooked mango	Cooked mango
7	-	-	Pungent mango	Pungent mango	Cooked mango	Pungent mango	Cooked mango	Cooked mango
14	-	-	Pungent mango	Pungent mango	Cooked mango	Pungent mango	Cooked mango	Cooked mango
21	-	-	Pungent mango	Pungent mango	Cooked mango	Pungent mango	Cooked mango	Cooked mango

the pulp. Observations made on colour of the pulp samples are recorded in table 4

Sample S_5 lost sulphur dioxide due to pasteurization resulting in brown colour of pulp, possibly due to caramelization reactions of sugars or Maillard browning reactions between sugars and proteins. Sample S_1 which was not heat treated was yellow, may be due to sulphur dioxide which blocked browning reactions. Similarly as for samples S_5 and S_6, sample S_3 which was also heat treated turned brown, while sample S_2 which was not

pasteurized remained yellow throughout the study period. There was no remarkable change in the colour of pulp for samples C_0 and C_1. However, the samples had grown moulds by the end of 4 days due to the absence of chemical preservatives. In contrast, samples C_0 and C_1, no mould growths were observed in samples S_1 to S_6 where chemical preservatives were added.

Odour

Samples S_3, S_5 and S_6 which were pasteurized had a cooked mango odour while samples S_1, S_2 and S_4 which were not heat treated had a pungent mango smell (Table 5). The pungent odour of unpasteurized pulp may be attributed to excess sulphur dioxide liberated in the samples. The heat-treated samples produced an odour that was less offensive than unpasteurized samples. The possible cause of the difference is loss of irritating sulphur dioxide and volatile products of fermentation from pasteurized pulp samples. The odours of samples C_0 and C_1 were those of fresh mango at the beginning, but turned stale at the end of 3 and 4 days respectively. Products of putrefaction and fermentation were smelled at the end of the 3 to 4 day period. The spoilage was caused by microbial growth resulting from lack of chemical preservatives which aid in prolonging the shelf life of the pulp by suppressing microbial proliferation.

CONCLUSION

From the results of the experiments performed, it was found that the use of sodium metabisulphite, citric acid, potassium sorbate and pasteurization increased the shelf life and stabilized the sensory properties of the pulp. Heat treatment inactivated enzymes that catalyze browning and fermentation reactions leading to pulp spoilage. The addition of sodium metabisulphite, citric acid and potassium sorbate was effective in preventing spoilage of the pulp. The Hurdle concept, which involves use of two or more preservation methods, provides an optimum procedure for preventing pulp spoilage and was illustrated in preservation of mango pulp in this study.

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Preservation of Ready-To-Prepare (RTP) Samosa Sheets

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ABSTRACT

Samosa (Indian stuffed pastry) primarily consists of a triangular (tetrahedral) golden-brown crisp coating with the inside cavity filled with a stuffing. Preparation of *samosa* sheet is tedious and time consuming since it requires dough preparation and sheeting and high skill of preparation. In India, many Indians consume *samosa* as snack food. In the present study, *samosa* sheet ingredients and process was optimized by varying ingredients and parameters such as salt (1.5, 2, 2.5%), oil (6, 8, 10, 12, 14, 16 %), water (44, 47, 48, 50, 52 %), dough mixing time (5, 7.5, 10, 15 min), frying time (0.75, 1, 1.25, 1.5, 2.0 min) and frying temperature (160, 170, 180, 190, 200°C). Optimization was done by changing one parameter at a time while others kept constant. Standardised *samosa* sheets were prepared into *samosa* and evaluated for quality parameters such as sensory analysis using nine-point hedonic scale. In the present study, *samosa* sheet with an oil content of 14%(v/w), water content of 47%(v/w), 2% salt, 10 min dough mixing time, 190°C frying temperature for 90 seconds resulted into best *samosa*. Further attempts were made to preserve *samosa* sheets using chemical preservatives such as calcium propionate (0.1 %, 0.2 %, 0.3% on flour weight basis), potassium sorbate (0.1 %, 0.2 %, 0.3% on flour weight basis) at different storage conditions viz. 35±2°C, 4±1°C &-18±1°C. From the present study it was observed that 0.3 % calcium propionate extended the shelf life of *samosa* sheet up to 15 days at 4±1°C and it extended more than 15 days at -18±1°C. Texture improvers such as guar gum (0.75% and 1%) and sodium stearoyl-2-lactylate (SSL) (0.5% and 0.75%) were also incorporated in *samosa* sheet to evaluate its effect on textural changes during storage.

Keywords: Samosa sheet, preservation of samosa sheet, texture improvers

INTRODUCTION

Samosa is a very common stuffed pastry consumed primarily in India and some other Asian countries such as Sri-lanka, Pakistan, Afghanistan, Bangladesh, Nepal etc. It consists of a triangular (tetrahedral) golden-brown crisp coating with the inside cavity filled with a stuffing. The coating is made from dough of refined flour or whole-wheat flour with some salt and mild spices. Crisp texture and formation of multi layers in the wheat flour dough coating after frying are some quality criteria of good *samosa*. This depends on the ratio of salt, oil, water content, mixing time. Many of the pastry products such as puff pastry, Danish pastry, pie pastry gives a good crispy and multi layer texture due to fat content, flour particle size and gluten formation (Berger, 2003). Once the coating is prepared, it is ready for the next process of stuffing. Stuffing most commonly consists of vegetables like cooked and mashed potatoes, onions, peas, green chilies, spices etc. Once the stuffing filled; coating is sealed and this is then deep-fried in oil until golden-brown color is developed. Traditionally, samosa fried in refined vegetable oil at temperatures ranging from 180°C to 220°C for five to ten minutes are of good quality and taste (Chapman, 2007; Sakhale et al., 2011). However, in the western countries samosas are baked in an oven instead of being fried. They are baked at around 180°C for 15 to 25 minutes depending on their size or until the crust appears golden-brown (Singhal et al., 2001).

Samosa is an easily perishable product with a shelf-life of only three to four days (Atta et al., 2008; Kakar 1998). After three-four days, the coating becomes soggy due to the moist stuffing, which makes it unacceptable to the consumer even though it is microbiologically safe. Hence, instead of preserving whole samosas, it is easier to preserve the samosa sheets as the process of samosa sheet making is tedious at the house-hold level and samosa sheets also allow the flexibility of the inside filling. Samosa sheets would thus supplement their home preparation in an easy way and cut down the preparation time drastically.

Samosa sheets are a simple and convenient way of making instant samosas. Samosa sheets also make the process of making samosas fast and mechanical, it is also a hygienic way of making samosas and maintenance of quality becomes simple with the standardization of the sheets and stuffing. The industrialization and urbanization of India is proceeding at a fast pace. The dietary and eating habits of Indians are rapidly changing. This has made outdoor eating popular and the need for fast foods intense (Sakhale et al., 2011). Snack foods have always found an important place in a common person's daily diet. In the total process of samosa preparation; samosa sheet preparation is the most challenging and tedious job which requires skill. If the ready to prepare samosa sheets are made available in the market, will defiantly

reduce the time of its preparation and will add to the convenience.

Presently, samosa sheets are not very well-liked in the general population. A reason for this may be due to the novelty of the product in the Indian market. A market survey shows that currently, ready-to-prepare samosa sheets are not available in the Indian (Mumbai) market. However, frozen ready-to-prepare samosas are available (Multi Food Industry, brand "prime-harvest mazedar"). Hence, in the present study efforts were made to standardize ingredients and process for preparation of samosa sheet. Further, samosa sheets were preserved by adding various additives in combinations with low temperature conditions.

Materials and Methods

Materials

Branded refined wheat flour (Aashirvad atta), refined oil (Saffola) and table salt (Tata salt) were procured from the local market, Mumbai, India. Citric acid (Monohydrate) was purchased from S D Fine Chemicals (Mumbai, India), Sodium Stearoyl-2-Lactate (SSL) and preservatives such as calcium propionate and potassium sorbate were gifted by Fine Organics, Mumbai, India. Guar gum was gifted by Tic Gums, USA. Butylated hydroxy anisole (BHA) was purchased from SRL Chemicals (Mumbai, India), and all other chemicals used in the present study were of analytical grade.

Materials and Methods

Proximate analysis of branded refined wheat flour
The moisture content was determined by AACC method, (AACC, 1976) dry gluten, protein, ash and fat content were determined by AOAC (1975) and carbohydrate by difference.

Preparation of samosa sheet and samosa
Wheat flour, salt and additives were weighed and water was added to form dough. Dough was then kneaded and thin sheets of 3 mm thickness were formed. These sheets were allowed to rest at 27^{o}C for 3 min so that the wet surface is dried. These sheets were then folded into triangular shapes. These triangular shapes were filled with vegetable stuffing and deep fried in vegetable oil. The flow diagram of samosa preparation is as follows

Weighing of ingredients
↓
All ingredients except water are mixed
↓
Water added slowly working
the ingredients into a dough
↓
Kneading of the dough
↓
Sheeting the dough into thin sheets (3mm thickness)
↓
Resting the sheets to dry the surface
(27°C ±3°C, 10 minutes)
↓
Folding the sheets into characteristic triangular shapes
↓
Deep frying in vegetable oil
↓
Fried samosa coatings ready to serve

Figure 1: Method for samosa sheet and samosa preparation

Optimization of samosa sheet ingredients by one factor at a time method

Optimisation of ingredients was carried out by choosing one factor at a time. For optimization of oil and water, 50g of flour was taken and oil was varied in the proportions viz., 6%, 8%, 10%, 12%, 14% and 16% on volume by flour weight basis. Water was added just enough till a soft sheetable dough was formed. Keeping all other parameters constant, the amount of salt in the dough was varied as 1.5%, 2% and 2.5% on flour weight basis.

Mixing time is important for the ultimate texture of the dough and hence of the final product. Optimum mixing is required for proper formation of the gluten network and making the dough soft and pliable. After the addition of all the ingredients the dough was mixed for 5, 7.5, 10 and 15 minutes. The sheets were fried at 160°C, 170°C, 180°C, 190°C and 200°C. The frying time was kept constant at 90 seconds after the frying temperature of 190°C was chosen, the frying time was varied from 45, 60, 75, 90 and 120 seconds. After the optimization of all parameters; process of *samosa* sheet making was optimized (Figure 1). Sensory evaluation was done by 9-Point hedonic scale with five semi-trained panelists. The average score of the panelists was determined for the samples under study and the samples with the highest score were chosen.

Effect of improvers on stickiness of samosa sheets
Effect of improvers on dough stickiness was evaluated using The Stable Micro Systems Chen–Hoseney Dough Stickiness Rig test, using accessories such as 25 mm perspex cylinder probe (P/25P), 50 kg load cell and SMS/Chen–Hoseney Dough Stickiness Cell (A/DSC) (Hoseney & Smewig, 1999).

Effect of improvers on spreadability of samosa sheets
Spredability of the dough was carried out using Stable Micro Systems Texture Analyzer. The probe used for carrying out the spreadability test was 100 mm diameter cylindrical probe. Following test setup was used: pre test speed: 1.0 m/s. test speed:1.7 m/s, distance: 10 mm, force value: 180 g. The dough of constant weight was prepared and rolled into fixed diameter and size. The prepared dough (native and reconstituted) was kept below the cylindrical probe. The force applied was constant, and the thickness and diameter of the dough sample after spreading was

measured using vernier caliper. From thickness (mm) and diameter (mm) values the Area = (Π/4) d^2 mm^2 and % relative spreadability was calculated.

Effect of improvers on texture of samosa prepared from preserved samosa sheets

Improver such as guar gum was varied from 0.75% and 1% on a flour weight basis and SSL was varied from 0.5% and 0.75% on a flour weight basis. *Samosa* sheets with added guar gum and SSL were packed in self sealable low density pouches of 60 gauge and kept at ambient temperature (27°C ± 3°C), refrigeration temperature (4°C ± 2°C) and frozen storage (-18°C ± 1°C) for 30 days. The samples were evaluated after interval of 2, 5, 10, 15, 30 days. The sheets were prepared into *samosa* and evaluated for sensory parameters as explained above.

Effect of preservatives and storage temperatures on shelf life of *samosa* sheets

Effect of three temperatures viz., ambient temperature (27°C ± 3°C), refrigerated conditions (4°C ± 2°C) and frozen (-18°C ± 1°C) on *samosa* sheet quality was evaluated by sensory analysis. Citric acid was chosen as the acidulant. The concentration of the acidulant was varied from 0.1%, 0.2%, 0.3% and 0.4% on flour weight basis. Potassium sorbate and calcium propionate was varied from 0.1%, 0.2% and 0.3% on a flour weight basis. *Samosa* sheets both control (without preservatives) and with added preservatives were prepared by adding above level preservatives and stored under three different temperature conditions. *Samosas* were prepared from the preserved *samosa* sheets after interval of 2, 5, 10, 15, 30 days. The prepared *samosas* were evaluated for sensory parameters using nine point hedonic scale. A score of 6 was selected as the threshold for acceptance and samples scoring a score of 6 or below were considered as 'unacceptable' thus excluded from further evaluation. Zero day sensory overall acceptability score for all *samosa* samples was 9. Panelists were advised to give absolute scores.

RESULTS AND DISCUSSION

Proximate composition of refined wheat flour:

The proximate composition of refined wheat flour used in the samosa preparation is as shown in Table 1. From Table 1 it is seen that moisture content of wheat flour was 12.94 %, protein content was 11.56 %, fat content was 1.06 %, total ash content was 0.49% and carbohydrate content was 73.95%.

Table 1: Proximate composition of the refined wheat flour used in the preparation of *samosa* sheets[a]

Proximate composition	% (w/w)
Moisture	12.94±0.35
Protein	11.56±0.2
Fat	1.06±0.1
Total Ash	0.49±0.07
Carbohydrates	73.95±0.02

[a]Note: Values are mean ±SD of three determinations

Optimization of *samosa* ingredients by one factor at a time method

Oil and water content optimization

It is seen from Table 2, sensory panelists preferred an oil content of 7ml as optimum for 50g of flour (14% v/w) and it got a maximum score of 7.4. Below 14%, the product did not develop the desired crispiness and flavor. Oil content above 14% made the end product very oily and thus received a lower score. 50g of flour with 7ml of oil required 23.5ml (47%v/w) of water to form soft, pliable dough. This was because the amount of water controls the quality, texture, taste, smell, volume, flavor, and mouthfeel of bakery products (Hui and Corke, 2006). The art of baking includes the use of optimum quantities of quality water (Cauvain and Young, 2000). Oil content improves the flavor and imparts desired texture (Baldwin *et al.*, 1972).

Salt content optimization

From Table 2, it is seen that a salt content of 2%, which scored 7.2 on the hedonic scale, was most preferred by the panelists. A salt content below 2% made the product bland and insipid which shows the important role as a major flavor enhancer. 2.5% salt content also has almost the same level of acceptability but 2% salt was chosen as it will reduce costs comparatively due to lesser usage of salt and the product will not become excessively salty in the event that other salts need to be added for their functional properties (preservatives etc.).

Mixing time optimization

From Table 2, it can be seen that the sensory panelists did not observe any noticeable change for products with dough mixing time of 10 and 15 minutes. Though there are minor differences in the scores of these samples, no consensus was reached placing one product above the other. So, a mixing time of 10 minutes with a hedonic scale score of 7.62 was chosen over 15 minutes as it would lead to lesser investment of energy for the mixing operation. This was due to water insoluble proteins in presence of water (gliadin and glutenin in equal amounts) formed a viscoelastic mass referred to as 'gluten' (Salunkhe et al., 1985)

Frying temperature optimization

At temperatures of 160°C, 170°C, the products were not fried properly and absorbed a lot of oil and so received low scores (Table 2). At 180°C, the product was fried properly but was not crispy enough. At 190°C, the product had the most acceptable color, texture and taste and hence received a maximum score of 7.75 from the panelists. At 200°C, slight case hardening was observed decreasing the overall acceptability of the product.

Frying time optimization

From Table 2, it can be seen that the panelists preferred a time of 90 seconds as it gave the most desired texture, color and taste to the product and thus received a maximum score of 7.62. At lower frying times, the product was not cooked completely. Also, the color development was not acceptable. At 120 seconds, the product absorbed more oil and the crispiness reduced, which resulted in a lower score of 6.3 for the sample.

Optimization of acidulant (citric acid)

From Table 2, it can be seen that the acidulant has a significant effect on the texture of the *samosa* sheets and hence on its overall acceptability. A citric acid content of 0.3% was chosen as the most acceptable concentration by the panelists receiving sensory overall acceptability score of 7.8. Citric acid contributed to the taste and the crisp texture of the product according to some panelists. A content of 0.4% improved the texture even more but contributed to an increased tartness which was not preferred by most of the panelists thus explaining the low score of 7.2 despite the increase in the texture. Citric acid was shown to have an effect on the inhibition of thermophilic bacteria (Fabian and Graham, 1953).

Effect of improvers on dough stickiness

Dough stickiness is an important quality factor that is related to the machineability and handling of the dough. The plot obtained is shown in Figure 2. The negative region of the plot was a result of 40g of force applied for 0.1s to compress the sample slightly. The maximum force reading (the highest positive peak), the positive area and the distance between the anchors set (travel), are all indicators of the stickiness of the dough. The highest positive

Table 2: Effect of oil, water, salt, dough mixing time, frying temperature, frying time and citric acid (CA) on the overall acceptability (OA) of *samosa* sheet

Oil (ml)	Water (ml)	OA	Salt (%)	OA	Dough mixing time (mins)	OA	Frying temperature (°C)	OA	Frying time (sec)	OA	CA (%)	OA
3	26	6.1±0.22	0	4.4±0.55	5	6.25±0.82	160	5.88±0.74	45	6.25±0.82	0	6.3±0.45
4	25	6.4±0.42	1.5	6.2±0.45	7.5	7.25±0.45	170	7±0.42	60	7.5±0.5	0.1	6.5±0.35
5	24.5	6.7±0.57	2	7.2±0.76	10	7.625±0.45	180	7.375±0.42	75	7.5±0.42	0.2	7.6±0.42
6	24	7.2±0.76	2.5	6.9±0.74	15	7.625±0.35	190	7.75±0.45	90	7.625±0.5	0.3	7.8±0.27
7	23.5	7.4±0.42					200	6.5±0.5	120		0.4	7.2±0.57
8	22	6.9±0.74										

[a] Values are mean±SD of three determinations

Table 3: Effect of improvers on the dough stickiness of the *samosa* sheet dough [a]

Additives	Dough Stickiness	Dough Adhesion	Cohesiveness/ Dough Strength
Control	30.32±1.46	3.46±1.04	2.14±0.84
0.75% SSL	27.28±0.76	1.66±0.24	0.57±1.44
1% Guar gum	37.94±3.22	8.44±0.66	7.22±0.3

[a] Values are mean±SD of six determinations

Table 4: Effect of improvers on the spreadibility of *samosa* sheet dough[a]

Additive	Weight of sample (g)	Thicknes(mm)	Diameter (mm)	Area mm^2	% Relative Spread ability
Control	10	9.01	30.9	750.02	100
0.75% SSL	10	8.07	33.85	900.22	120.02

1% Guar gum	10	10.74	30.58	735.28	98.03

[a]Values are mean±SD of six determinations

peak obtained has been used to represent the dough stickiness in this work. Table 3 shows the effect of additives on *samosa* sheet dough stickiness. It was seen that the addition of SSL caused a decrease in the stickiness of the dough sample. This is a positive effect as this would prevent the sticking of the *samosa* sheets in the package during its shelf-life. Guar gum increased the stickiness of the dough samples and hence is an undesirable change. Hydrocolloids are employed because of their ability to absorb large amounts of water (Rogers et al., 1988). The effect can be attributed to the hydroxyl groups in the hydrocolloid *structure*, which allows more water interactions through hydrogen bonding (Guarda et al., 2004).

Effect of improvers on dough spreadibility

Sheeting of dough is one of the most important steps in *samosa* sheet preparation followed by *samosa* making. To demonstrate the effect of addition of additives on sheeting ability of *samosa* sheet it was analyzed for its spreadability. From Table 4 it can be seen that when 50 g force was applied on 10 g dough prepared from maida (control), the round sheet of 9.01 mm thickness and 30.9 mm diameter was obtained. Whereas SSL (0.75%) incorporated dough showed increased sheeting ability (8.07 mm thickness and 33.85 mm diameter) resulted into 120.02% relative spreadability of the dough. This might be due to the incorporation of SSL. Addition of guar gum (1%) resulted into decrease in spreadability i.e. 98.03 %.

Effect of preservatives and temperature conditions on shelf life of *samosa* sheets

The *samosa* sheets were prepared using standard method (Figure 2), and till the 'resting' phase these sheets were packed in high density polyethelyne (HDPE) pouches. It was seen that *samosa* sheets are a highly perishable product. At room temperature (27°C ± 3°C), the *samosa* sheets developed a slimy texture due to the leaching out of oil. Also, some air pockets were observed which may indicate the initiation of fermentation. The sheets also got stuck to each other forming an mass. The same effect was seen in the refrigerated sample after two days.

The mechanism by which sorbic acid inhibits microbial growth may be due to its effects on enzymes. Sorbic acid inhibited dehydrogenases involved in fatty acid oxidation. Addition of sorbic acid resulted in the accumulation of β-unsaturated fatty acids that are intermediate products in the oxidation of fatty acids by fungi. This prevents the function of dehydrogenases and inhibits metabolism and growth (Brul et al., 1999). Calcium propionate is most effective against molds at pH values less than 5 or 6. The antimicrobial activity of the propionates results from the interference of the electrochemical gradients of the cell membrane, which disrupt transport processes (O'Connell et al., 1999). Table 5 shows the effect of preservatives on the shelf life of *samosa* sheets preserved at room temperature (27°C ± 3°C), refrigeration temperature (4°C ± 2°C) and frozen storage (-18°C ± 1°C) respectively.

It was seen that the type and concentration of the preservatives and the storage conditions had a significant effect of the shelf-life of the *samosa* sheets and hence it's overall acceptability. At 27°C±3°C, the shelf-life of the samples was the lowest as compared to the other storage conditions. The control sample got spoilt on the second day. Potassium sorbate and calcium propionate were both ineffective at a concentration of 0.1%. At 0.2%, sorbate sample had a shelf life of 5 days while that of the propionate was less than 10 days.

Table 5: Effect of different levels of preservatives on the overall acceptability of *samosa* sheets preserved at 27°C ± 3°C, 4°C ± 2°C and -18°C ± 1°C using Hedonic Scale Rating[a]

Storage temperature		Hedonic Scale Rating				
	Days	2	5	10	15	30
27°C ± 3°C	Control	5.6±0.55	XXX	XXX	XXX	XXX
	A1	5.8±0.44	XXX	XXX	XXX	XXX
	A2	7.2±0.44	6.2±0.44	XXX	XXX	XXX
	A3	7.4±0.55	6.6±0.55	5.8±0.44	XXX	XXX
	B1	6.2±0.44	XXX	XXX	XXX	XXX
	B2	7.4±0.55	6.4±0.55	5.6±0.55	XXX	XXX
	B3	7.6±0.55	7.2±0.44	6.8±0.44	6.2±0.44	XXX
4°C ± 2°C	Control	5.8±0.44	XXX	XXX	XXX	XXX
	A1	6.2±0.44	XXX	XXX	XXX	XXX
	A2	6.8±0.44	6.2±0.44	XXX	XXX	XXX
	A3	7.2±0.44	6.6±0.55	6.2±0.44	XXX	XXX
	B1	6.4±0.55	5.8±0.44	XXX	XXX	XXX
	B2	7.2±0.44	6.4±0.55	5.8±0.44	XXX	XXX
	B3	7.6±0.55	7.6±0.55	7.2±0.44	6.8±0.44	
-18°C ± 1°C	Control	7.8±0.44	7.8±0.44	7.6±0.55	7.2±0.44	
	A1	7.8±0.44	7.8±0.44	7.8±0.44	7.2±0.44	
	A2	7.8±0.44	7.8±0.44	7.8±0.44	7.6±0. 55	
	A3	7.8±0.44	7.8±0.44	7.8±0.44	7.6±0.55	
	B1	7.8±0.44	7.8±0.44	7.8±0.44	7.4±0.55	
	B2	7.8±0.44	7.8±0.44	7.8±0.44	7.6±0.55	
	B3	7.8±0.44	7.8±0.44	7.8±0.44	7.6±0.55	

[a] Values are mean±SD of five determinations
XXX – Samples were spoiled hence excluded from study

A1 = 0.1% w/w Potassium sorbate
A2 = 0.2% w/w Potassium sorbate
A3 = 0.3% w/w Potassium sorbate
B1 = 0.1% w/w Calcium propionate
B2 = 0.2% w/w Calcium propionate
B3 = 0.3% w/w Calcium propionate

Table 6: Effect of different levels of improvers on overall acceptability of *samosa* sheets preserved at 27°C ± 3°C, 4°C ± 2°C and -18°C ± 1°C using Hedonic Scale Rating.

Storage temperature		Hedonic Scale Rating			
	Days	2	5	10	15
27°C ± 3°C	Control	7.6±0.55	7.2±0.44	6.8±0.44	6.2±0.44
	A1	7.8±0.44	7.6±0.55	7.4±0.55	7.2±0.44
	A2	7.8±0.44	7.8±0.44	7.6±0.55	7.6±0.55
	B1	7.8±0.44	7.6±0.55	7.4±0.55	7.2±0.44
	B2	7.8±0.44	7.8±0.44	7.8±0.44	7.6±0.55
4°C ± 2°C	Control	7.6±0.55	7.6±0.55	7.2±0.44	6.8±0.44
	A1	7.8±0.44	7.8±0.44	7.6±0.55	7.2±0.44
	A2	7.8±0.44	7.8±0.44	7.6±0.55	7.6±0.55
	B1	7.8±0.44	7.8±0.44	7.6±0.55	7.2±0.44
	B2	7.8±0.44	7.8±0.44	7.8±0.44	7.6±0.55
-18°C ± 1°C	Control	7.8±0.44	7.8±0.44	7.8±0.44	7.6±0.55
	A1	7.8±0.44	7.8±0.44	7.8±0.44	7.8±0.44
	A2	7.8±0.44	7.8±0.44	7.8±0.44	7.8±0.44
	B1	7.8±0.44	7.8±0.44	7.8±0.44	7.8±0.44
	B2	7.8±0.44	7.8±0.44	7.8±0.44	7.8±0.44

[a] Values are mean±SD of five determinations

A1 = 0.75% w/w Guar gum
A2 = 1% w/w Guar gum
B1 = 0.5% w/w SSL
B2 = 0.75% w/w SSL

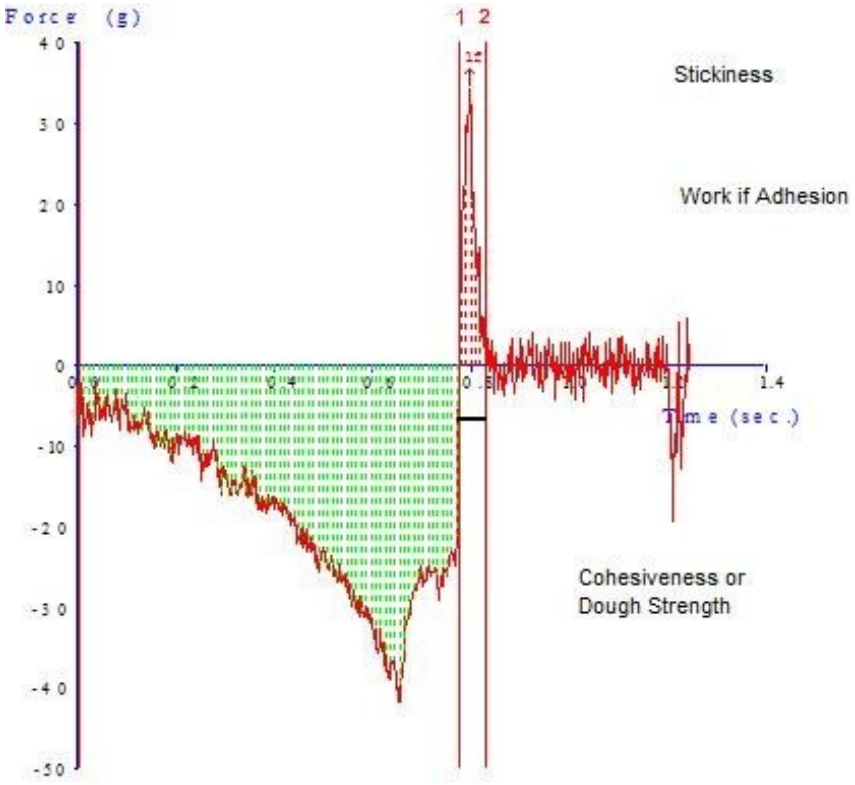

Figure 2: Typical dough stickiness graph obtained using Chen-Hoseney Dough Stickiness Rig test for *samosa* sheet dough

A 0.3% sorbate content extended the shelf-life slightly but not for more than 10 days. Samples with 0.3% calcium propionate had a shelf life of 15 days and so calcium propionate at a concentration of 0.3% w/w was chosen as the best preservative for *samosa* sheets stored at 27°C±3°C or 4°C±2°C. For refrigerated storage (4°C±2°C), it was seen that a longer shelf-life was obtained. Samples with 0.1% propionate had a shelf-life of 5 days. This was further increased to 10 days by 0.2% propionate. A 0.3% propionate sample gave a shelf-life of more than 15 days. For frozen storage (-18°C±1°C), the samples showed hardly any signs of spoilage even after 10 days of preservation. It was seen that preservatives, at concentrations as low as 0.1%, also proved to be effective in extending the shelf-life of the product. After 15 days, the quality of the samples deteriorated marginally but still the samples were acceptable.

Effect of improvers on the samosa sheet and samosa prepared from it

The (table 60 shows the effect of improvers on the oil binding capacity of *samosa* sheets preserved at room temperature (27°C ± 3°C), refrigeration temperature (4°C ± 2°C) and frozen storage (-18°C ± 1°C) respectively. The effect of addition of improvers such as SSL, guar gum showed a very good effect compared to control sample. It was seen that the addition of emulsifiers had a positive effect on the overall acceptability of the product. 0.75% SSL and 1% guar gum scored the highest and also improved the texture of the dough along with the retention of oil. 0.75% SSL and 1% guar gum scored the highest even under refrigerated (4°C ± 2°C) and frozen (-18°C ± 1°C) storage. Thus both these concentrations were chosen and these samples were further analyzed to select an emulsifier.

CONCLUSIONS

The Present study the process of *samosa* sheets was optimized and efforts were done to increase the shelf-life of the *samosa* sheets. Preservatives like calcium propionate and potassium sorbate were tried and it is observed that 0.3% calcium propionate at refrigerated storage (4°C±2°C) gave shelf-life of 15 days. Effect of texture emulsifiers like SSL and guar gum were studied and it is found that SSL causes decrease in the stickiness of the dough sample where as guar gum increased the stickiness of the dough sample which is undesirable. The optimized values gave successful results and increased the shelf-life of *samosa* sheets drastically.

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Value addition in strawberry: a tool for long term storage- a review

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ABSTRACT

The perishable nature of many agricultural produce limits the access to profitable markets and causes dwindling income of small farmers. Post-harvest losses of fruits and vegetables are a serious issue in developing countries as compared to developed countries. The total losses from harvest to the point of consumption are as high as 30-40%, which is worth in million of rupees. About 10-15% of fresh fruits shrivel and stale, lowering their market value and consumer acceptability. Enhancement in post-harvest processing and preservation technologies are the critical strategies to add value to the food crops. Fruits play an important role in satisfying the demands for nutritious, full of flavours and attractive natural colours with high therapeutic value. They are rich in antioxidants, vitamins, minerals and dietary fiber. Some fruits have excellent flavour and very attractive in color, apart from nutritional, therapeutic and medicinal value. Strawberry is one of such fruit, which has short shelf life and is being highly perishable in nature. The best way of utilizing the delicate commodities in seasonal glut is through processing, value addition and to reduce the fruit wastage. Strawberries are available in Pakistani fruit markets during spring season. The demand and consumption of this delicate fruit is increasing in Pakistan as well as in other parts of the world. The nutrients, natural flavours and attractive color can be preserved through processing of fruit into various products i.e sweetened preserves, jams, jellies, whole frozen berries, dehydrated slices, freeze dried, spray dried and canned products. The present paper represents an overview on strawberry fruit processing and its possible value added products with special emphasis to Pakistani market.

Key words: Strawberry fruit, Nutritional aspects, Export value, Value added products

Introduction

Strawberry (Fragaria ananassa) belongs to family Rosaceae (rose family), is low herbaceous perennials with edible red fruits, native to temperate and mountainous tropical regions (Childer, 1980). The name is derived from old english strēawberiġe, which is a compound of streaw meaning "straw" and berige meaning "berry".

Nutritional facts of strawberries

Nutritionally, strawberry contains low calorie carbohydrate and a potential source of vitamin C, fiber and provides more vitamin C than oranges. The main constituents are; Vitamin C is (64.0 mg), water (91.75 g), protein (0.61 g), fat (0.37g), carbohydrate (7.02g), fiber (2.3g), calcium (14.0mg), potassium (166.0mg/160g) respectively, vitamin-A 27 IU, pH ranges from 3.27 – 3.86, which helps in stabilizing color. Acidity ranges between 0.58 - 1.35%, citric and malic acids are primary organic acids contributing excellent flavour. Soluble solids are in the range of 8.0-11.5% and are ideal for the development of juice concentrate. Soluble solids/acid ratio 8.52 - 13.79 is good balance of sweet-tart flavour (Ayub *et al.,* 2010). It is grown on an area of around 78 hectares with annual production of about 274 tonnes in Pakistan during the crop year 2009-10. The average per acre yield of this fruit crop is very low as compared to other strawberry growing countries of the world due to defective agronomic practices, lack of appropriate research work and lack of economic and market value of strawberry cultivation among the strawberry cultivators (Aslam and Rasool, 2012).

Present status and export value in Pakistan

Strawberry is cultivated at lower altitudes in KPK during the month of November while it gives fruits in April till late May, adding that fruit maturity period is short and ranges from 30-40 days. The grass income per acre for strawberry, wheat and sugarcane is estimated at Rs. 154,751, Rs. 16,094 and Rs. 39,059 respectively. The cost for various operations is summed to Rs. 42,890 resulting in net income at Rs. 111,861 for strawberry on per acre basis approximately. The income for all the important Rabi crops showed comparative edge to the strawberry, as its net revenue was about four times higher than sugarcane and about nine times higher than wheat crop. On the other hand, the wholesale price of the fruit comes down to Rs. 50 per kg during the second fortnight of March when the crop production touches its peak (Afridi *et al.,* 2009). The income from per acre of

strawberry crop is estimated to Rs. 100,000 per season. The main promising varieties grown in Pakistan are Corona, Mission, Superfaction, Tuft, Sweet Charlie and Festival. Pakistan has very limited exports of strawberry fruit. The greater size of the European market depicts that it may be the leading potential market for Pakistani strawberry and it has a sufficient seasonal advantage over the other producing countries. Germany is the prime single potential market which can absorb roughly 6,000 metric tons of fresh strawberries per week and has another export destiny and the remaining potential markets are France, United Kingdom and Japan. The main reasons for restricted production in Pakistan are adverse nature of climate and quality variation like size and taste. To capture the potential exportable markets and compete with the other exporting countries the problems has to be removed in terms of quality, quantity and perish ability of fruit (Aslam and Rasool, 2012).

Value addition or value added products

The value addition is a process to adding value to a food commodity which may include any unit operation during product development from simple washing to complex manufacturing process. Adding valued features to products is a common method used to market a product or food material, since it can help increase sales and profit. A number of value added products can be developed from Strawberry fruit for long term usage. The processing steps for strawberry products are similar to that of other fruits. However specific value added products have been developed through processing techniques like freezing, drying, canning (Linda and Mitcham, 2007).

Frozen strawberries

Freeze-drying is used for the preservation of sensitive materials and the facilitation of transport and is carried out in two stages; the product is first frozen and then the ice is removed by sublimation directly from the solid to the vapor phase. During freeze-drying, ice sublimation create pores or gaps with different characteristics and causes significant changes in shape and volume of the food products (Krokida *et al.,* 1998).

Frozen strawberries can be used as such or as raw material for the development of different value added products. For example, individual quick frozen strawberries are used for freeze drying. The frozen strawberries can also be sliced or diced for use in ice cream, jelly and jam. It is important to select uniform sized, red coloured strawberries for freezing purpose. The process consists of following steps:

Fruit reception → precooling → removal of stem and caps → air circulation for removal of extraneous material → inspection and grading → washing with chlorinated water →quick freezing of fruit (at -40^{0}C) in a blast air freeze tunnel → packing and storage under freezing temperature (at-18).

Quick freezing reduces formation of large ice crystals, which causes drip loss upon thawing. Packing in heavy weight, moisture resistant and air tight packing material is helpful in minimizing the problems during storage (Linda and Mitcham, 2007).

Strawberry puree

Fruits are processed to make puree or pulp and utilized in various products. It is critical that mouldy and rotten fruits are not used for processing into puree. The fruit before processing are sorted, washed and separated the bruised one, however it is not necessary to remove the berry cap. The pulper meshes (mesh size 0.5-10 mm) the fruit into a smooth pulp or puree and also removes skin and seeds. Often the puree is pasteurized through a vacuum system to maintain colour and flavour (88^{0}C for 2 minutes) and cooled to about 15^{0}C. The final product is filled and stored at refrigeration temperature (Hui *et al.,* 2006).

Drying strawberry fruit

Air drying is an ancient process used to preserve foods in which the food material is exposed to a continuously flowing hot stream of air where moisture evaporates (Ratti, 2001). Osmo air-dried fruits are based on a novel approach towards dehydration. Slices of fruits are processed in two stages. In the first phase most of the water is removed using sugar syrup as an osmotic agent. In the second phase air drying is done where the moisture content is further reduced to about 15%. The process is simple and involves operations like selection of fruits, cleaning, washing, peeling, curing and slicing and dicing. To remove water by osmosis the prepared fruit slices are steeped in sugar solution. The slices are then drained, dried in a hot air drier and dehydrated slices are packed in plastic polyethylene pouches. The osmo-air dehydrated product is near to the fresh fruit in terms of colour and flavour. The osmo-air dehydrated product can be used in ready-to-eat foods, ice cream, fruit salad, kheer, cakes and bakery products.

Strawberry powder by spray drying

Drying techniques such as spray drying, freeze drying, tunnel drying have been invented to produce better quality products with more control on processing conditions. Spray drying technique is used for microencapsulation of food ingredients (Desobry *et al.,* 1997). It is a simple inexpensive method in which either proteins or polysaccharides or a combination of both can be used to create the shell. It takes a liquid stream of strawberry juice and separates the solute or suspension as a solid and the solvent into vapours. The solid fruit material is usually collected in a drum or cyclone. The liquid input stream of fruit is sprayed through a nozzle into a hot vapour stream and vaporised. Solids form as

moisture quickly leaves droplets. Droplet sizes can range from 20µm to 180µm depending on the nozzle. Spray dryers dry a product very quickly they turn a fruit solution or slurry into a dried strawberry powder in a single step.

Canning of strawberry fruits

Canning is a method of food preservation in which the food material is subjected to elevated temperature and pressure, packed in hermetically sealed container which provides unfavourable conditions for microbial growth. Strawberries produce and ripen quickly over a period of just a couple of weeks, so canning is one of the best methods to preserve them. Strawberries are canned using the boiling water-bath method. Canned strawberry is suitable for cooking, used for preparation of pudding, cakes, can be mixed with ice cream and used with a lot of more products (Linda and Mitcham, 2007). The basic steps involved in canning process are sorting, grading, de-capping, syruping, filling in jars or cans, exhausting, sealing and storage (Anon, 2006).

Conclusion

In the recent scenario, future of strawberry's cultivation, processing and export is very bright in Pakistan. Like the beekeeping venture, the cultivation of strawberry as well as value addition has a great potential to increase the income of small farmers. Unfortunately the annual production of strawberry fruit in Pakistan is not sufficient to meet the export potential due to insufficient cultivated area. There is need to increase the area of cultivation for maximum high yield production in Pakistan. This turn will lead to boost the export potential as well as the availability of value added products on long term basis, which will ensure welfare and increasing economic development in the country.

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Extraction of starch from Water Chestnut (Trapa bispinosa Roxb) and its application in yogurt as a stabilizer

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Abstract

Stabilizer is an important ingredient in yoghurt. For the stabilizing stabilizer is usually used in yoghurt to impart viscosity, improve mouth feel, extend milk solids and prevent wheying off. Starch is the preferred thickening agent in yoghurt due to its creamy texture, processing ease and low cost when compared with other hydrocolloids. Water chestnut (Trapa bispinosa) is the major source of starch which contains approximately 72 % starch. Starch is extracted from Water chestnut (Trapa bispinosa) and it is used in yoghurt as stabilizing agent. Enriched of yoghurt with Trapa bispinosa starch at different levels was studied with physicochemical and sensory analysis. Yoghurt prepared by incorporation of Trapa bispinosa starch @ 0.5%, 0.75%, 1% and 1.25% was compared for these characteristics to the yoghurt containing stabilizer gelatin @ 0.5% w/w. Physiochemical parameters (fat, pH, acidity, synersis, water holding capacity, viscosity, protein etc), sensory evaluation and microbial analysis (total viable count and coliform test) were studied. Use of Trapa bispinosa starch produced better results in terms of lowering synersis and increasing water holding capacity, viscosity and overall acceptability for all sensory attributes. Addition of Trapa bispinosa starch did not influence the taste and overall acceptability. Trapa bispinosa starch @ 1.25% gave most excellent results for water holding capacity, synersis and viscosity and Trapa bispinosa starch @ 0.75% gave most excellent results for all sensory attributes. Yoghurt shelf life was increased upto 25 days.

Key words: Starch, Water chestnut, Stabilizer, Yogurt, whey

Introduction

Singhara is the common name of water chestnut (Trapa bispinosa Roxb.). It was thought that family of Trapa natans is Trapaceae a mongeneric family, widely dispersed in the Eastern Hemisphere (Cook et al., 1974). However, current molecular research places Trapa species in the Lythraceae in the order Myrtales (The Angiosperm Phylogeny Group, 1998). Due to morphological variation in species of Trapa, there had been little agreement about the number of species in the genus. Trapa bispinosa Roxburgh and Trapa bicornis Osbeck having two horns are the two common cultivated species of Asia. According to many botanists now only one species Trapa natans consisting two varieties Trapa natans var. bispinosa Roxb and Trapa natans var. natans L. Water caltrop or water-nut are included in Trapa natans var. natans now broadly distributed in Africa, Eurasia and the northeastern United States, bears a four horned fruit (Crow and Hellquist 2000) and singhara nut, bull nut or water chestnut are included in Trapa natans var. bispinosa Roxb. distributed in India, Japan, southeast Asia and China produces a fruit with two stout curved horns (Herklots 1972).

Water chestnut plant is free floating which is grown in ponds, marshy lands or shallow water fields in tropical and sub-tropical countries (Takano and Kadono, 2005). The color and shape of its outer cover are the interesting features of water chestnut in which the kernel is enclosed. Thick jet-black is the outer pericarp of water chestnut whose shape likes a horn protruding from the buffalo head. The outer covering of water chestnut is tough and due to which it is very difficult to peel off to obtain the edible part (Tulyathan et al., 2005). During fasting days in Indian subcontinent fruit is used as substitute for cereal. Thin brown spermoderm is the outer layer of edible white meat. The edible white meat has 5g a normal weight. Approximately 70% carbohydrate on dry weight basis presents in white meat. Approximately 4.7% protein, with the higher biological value presents in seeds than wheat protein (Suhrahmanyam et al., 1954). Calcium, iron, phosphorus, thiamine and ascorbic acid are present in significant amount which make the water chestnut an important food source (Bargale et al., 1987). The common method which is used to soft the outer covering of water chestnut called steaming and at the same time cooked the internal meat. Then it may easy to remove outer cover and obtaining starchy white meat. The edible part is generally eaten as a snack or used as an element in many foods (Rodrigues et al., 1964). Cereal grains e.g. wheat, rice and corn and tubers e.g. cassava and potato are the major source of starch. Starches are very functional in food and industrial applications due to its thickening power (Moorthy, 2002). Water chestnut is an alternative source of carbohydrate. Flour obtained from fruit is valuable for textile sizing and is used in ice cream manufacture as a substitute of corn starch.

The physicochemical characteristics, such as growth

period, mineral content, cultivars, environmental factors, granule size, and amylose/amylopectin ratio are depended on starch properties (Noda et al., 2004). Crop of water chestnut yield per acre with a growth cycle of 6-8 months is approximately 93 Mounds. The annual worldwide production is 66.5 million tons of starch (FAOSTAT, 2002). To the authors' knowledge that Trapa bispinosa starch is used as part of recipe in many foods to improve the textural properties, such as stabilizing of sauces and soups locally but not use as a stabilizing agent in yoghurt. In Asians countries potential source of commercial starch is water chestnut due to its simple implementation and management. Due to its water-soluble hydrocolloid property it provides function to control of water by gelling and thickening (Tsuchiya et al., 1987).

The role of milk and milk products is vital in the human diet. Those nations are healthier whose consumption is high for these products. The new verities of fermented milk products are regularly entering in the consumer market. Yoghurt is probably the most popular product between all fermented milk products like sour cream, butter milk, ropy milk, acidophilus milk, cheese because it has pleasant aromatic flavour and thick creamy consistency (Humet al., 2003). It is made in variety of composition such as fruits, sugar and gelling agents. Streptococcus thermophilus and Lactobacillus delbrueckii spp. Bulgaricus are essential flora of yoghurt. Approximately equal numbers of both species should be present to develop a satisfactory flavour. Acetic acid, diacetyl and acetaldehyde are volatile compounds produced by the yoghurt bacteria.

The word yoghurt originated from Turkey, dating back to 2000 B.C, when Middle Eastern civilizations used fermentation for preserving milk. Yoghurt has been considered useful in Turkey, India, America, Egypt, Rumania, Yugoslavia, Central Europe and Russia since ancient times. An important role has been played by the yoghurt in the diet of these nations. Now a days, the importance of dairy products has increased manifold due to the awareness among the people about the nutritive value of yoghurt. It has been established that fermented milk products, of which yoghurt is one increase nutritive value of food as compared to original milk. Different ingredients like cereals, gums, fibers and starches, legumes mixes, fruit pulp and juices of many medicinal plants added in yoghurt to enhance the therapeutic and quality value of it.

Yoghurt is an important food ingredient in most of societies. It is believed that consumption of yoghurt and other dairy products is very beneficial for health. The nutrient value of curd or yoghurt depends on the milk composition and substances added to it during manufacturing. Yoghurt can be manufactured from skimmed or whole milk and it can be sweetened, plain or flavoured with fruit juices, cane sugar etc (Srividya and Rao 2003). Yoghurt is popular milk product with significant health beneficial effects and higher nutritional value. In recent years a lot of interest had raised by immunological potentials of Lactobacillus acidophilus due to their properties of immune stimulating. In vitro, on cells of the immune system stimulatory properties had displayed by several strains of lactic acid bacteria (LAB), including macrophages. Probiotics encompass of about 65 % of the world functional market of food (Agarwal, 2005).

MATERIAL AND METHODS

Procurement of raw material

The water chestnut (Trapa bispinosa Roxb.) was obtained from local market of Narowal for extraction of starch. Buffalo milk for making yoghurt was procured from Dairy farm, University of Agriculture, Faisalabad. Food grade stabilizer, gelatin was obtained from local market. Commercial freeze dried starter cultures of Streptococcus thermophilus and Lactobacillus bulgaricus was obtained from the local distributor of Christ Hansen.

Extraction of starch from water chestnut

The selected dehydrated water chestnuts were ground or crushed and 1 kg of sample was mixed with 2L of water, while sustaining pH 9.0 by an addition of 0.2% sodium hydroxide solution. 100 and 170 mesh sieves were used for the filtration of the slurry and centrifuged at 3000 rpm using a BECKMAN COULTER Allegra™ X-22 centrifuge. The residue was washed with water in order to discolor it and then air dried in an oven at 45 ± 1 ºC for 24 hours (Tulyathan et al., 2005).

Analysis of Water Chestnut Starch

Protein content of water chestnut

The Protein content of water chestnut was estimated by Kjeldal method described in AOAC (2000). The sample (4g) was first digested with 30 mL concentrated sulphuric acid in the presence of digestion mixture for 5-6 hours or till the digestion mixture acquired light green or transparent colour. This material was diluted to 250 mL and distillation was done by taking 10 mL of diluted material and 10 mL of 40% NaOH solution in dilution apparatus. The ammonia thus obtained was collected in 2% boric acid solution containing methyl red as an indicator, finally the distillate was titrated against 0.1N H_2SO_4 solution till light pink end point. The crude protein percentage was calculated by multiplying nitrogen with a factor 6.25 as given below

$$\text{Crude protein} = \text{Nitrogen (\%)} \times 6.25$$

Energy content of water chestnut

The Energy was calculated by oxygen bomb calorimeter (C2000 basic KIKA WERKE).

Total carbohydrates

The available or soluble carbohydrates were measured by the method of (Dubois *et al*., 1956). Eight consecutive volumes of 0.1 percent glucose solution were transferred to eight test tubes while the ninth was kept blank. All the test tubes were treated with 1 mL of 0.25 N of H_2SO_4 plus 1 ml of 0.85 percent aqueous phenol. Then distilled water was added to each test tube to make the volume constant. Finally 5 mL concentrated H_2SO_4 was added to each test tube and mixed till orange yellow colour developed the intensity which was measured at 490 nm using spectrophotometer. A standard curve was also drawn by plotting the absorbance against concentration of glucose. The weighed amount of samples were blended with distilled water till homogeneous mass was obtained, then volumes were measured in measuring cylinders. Out of whole volume, 5 mL of blend mass was centrifuge to separate soluble and insoluble fractions from soluble portion.0.1 mL sample solution was drawn and treated with reagents as used in the case of standard curve, and then from the standard curve, the concentration of carbohydrates against absorbance was read directly in 0.1 ml sample solution. Then amount of carbohydrate in weighed sample was calculated. Percentage of carbohydrate was calculated as:

$$\text{Carbohydrate (\%)} = \frac{\text{Amount of carbohydrate}}{\text{Weight of sample}} \times 100$$

Ash contents

The ash content of sample was determined by incinerating weighed amount of the samples in a muffle furnace at 550°C for 4-5 hours till white ash is obtained (AOAC, 2000). Before putting in muffle furnace, charring of the sample was done so that the sample becomes smoke free. The ash was calculated according to the following formula:

$$\text{Ash (\%)} = \frac{\text{Wt. of ash}}{\text{Wt. of sample}} \times 100$$

Moisture contents

The moisture content of Trapa bispinosa starch was determined as described in AOAC (2000). 5 grams of sample was taken in dried and weighed china dish and put in oven for 24 hours. After that moisture content was calculated

Yoghurt preparation

The Fresh milk was used for yoghurt preparation. Milk was first standardized at 3% fat. After standardization, milk was divided into five equal portions and stabilizer was added as given in Table (1).

Analysis of yoghurt

pH of yogurt

The pH was instantly determined by using the pH meter (WTW series pH-720). An ample quantity of yoghurt sample was taken in a beaker and adjusted according to room temperature in which electrodes of pH meter were dipped and reading was recorded after standardizing the instrument with buffer solution of pH 4 and 7 and reading was directly recorded (Ong *et al*., 2007).

Table 1: Treatment plan of Stabilizers used for yogurt preparation

Yoghurt samples	Trapa bispinosa starch (% w/v)	Gelatin (% w/v)
S_0	,	0.5
S_1	0.5	,
S_2	0.75	,
S_3	1.0	,
S_4	1.25	,

Acidity of yogurt

The direct titration method was used to determine the acidity described in AOAC (2000). A suitable amount of well mixed and homogenous sample was taken in 100 mL Erlenmeyer flask, diluted it with double quantity of distilled water and titrated against 0.1N NaOH using phenolphthalein as an indicator till light pink color was achieved. The volume of NaOH used was measured. The acidity was calculated.

Fat content of yogurt

The fat was examined according to method described by (British Standard Institution, 1955). 10 mL of sulphuric acid (specific gravity 1.082) was taken in butyrometers followed by 11.3 mL of yoghurt sample. Then 1 mL of iso-amyl alcohol was added. After mixing properly the butyrometers were placed in centrifuge machine at 1100 rpm, at 65°C temperature for five minutes. The reading of separated fat was promptly noted on the scale of butyrometer.

Synersis of yogurt

For synersis of yoghurt samples, 5mL of sample was centrifuged at 5000 rpm for 20 minutes at 0°C and separated whey was measured after 1 minute. Whey separation amount was expressed as volume of separated whey per 100 ml of yoghurt (Rodaret *et al*., 2004). Synersis was measured during 1st day, 5th day, 8th day, 11th day, 14th day, 17th day and 24th day and the measurements were made in triplicate for each yogurt sample to avoid errors.

Microbiological analysis of yoghurt

Total viable count

The total viable count of the yogurt was measured according to the method of (Yousaf and Calstrom, 2003). Plate count agar (oxide) was dissolved 15 g of plate count agar in 1 litre of distilled water and sterilized at 121°C for 15 minutes. Prepared the dilutions blank by putting 9 mL amounts of normal saline into sterile test tubes. With a micropipette dipped in a half inch, delivered 1 mL of well mixed (with help of blender) yoghurt sample into the first dilution blank about half an inch above the level of the liquid. Blew out and discarded the tip. With a fresh tip, dip half an inch into the liquid, sucked up and down ten times to mix. Took 1 mL and transferred to the next dilution blank, discarded the tip and continued for the required number of dilutions. Shifted 1 mL contents from each dilution on to the surface of separate nutrient agar plates and spread well. Then it was incubated for 24 hours at 37°C and after that average number of colonies from those dilutions that showed the colonies size ranging from 30 to 300 with the help of colony counter.

Viable bacterial count = Average number of colonies × dilution factor/volume factor

Sensory evaluation

The overall acceptability of Trapa bispinosa starch addition yoghurt was assessed by the panel of post graduate students from National Institute of Food Science and Technology, University of Agriculture, Faisalabad according to the method proposed by (Munir and Hunter, 1992). Flavour, acidity, appearance body and texture and overall acceptability were the attributes for sensory evaluation of yoghurt. As recommended by IDF (1987), the attributes of body and texture and flavor were given priority over others. Overall acceptability was obtained by adding the scores of all attributes. Yoghurt samples were coded with numbers and presented together to panel members in day light. Water was provided for rinsing mouth after each sample. Yoghurt samples were evaluated sensory at 1st day, 5th day, 8th day, 11th day, 14th day, 17th day and 24th day of storage.

Statistical analysis

The parameters of this study were designed according to two factor completely randomized design. Effect of Trapa bispinosa integration in experimental treatments, with or without its addition was checked by ANOVA. Duncan's Multiple Range Test was used to conclude statistically different groups (Steel et al., 1997).

RESULTS AND DISCUSSION

Results of Trapa bispinosa starch

The research project was planned to study the effect of different concentration of stabilizer i.e. starch extracted from Trapa bispinosa on the stability and quality of yoghurt, through physical, chemical, microbiological and sensory evaluation. Gelatin was used as controlled @ 0.5% concentration to compare its effects with different concentrations (0.5%, 0.75%, 1% and 1.25%) of Trapa bispinosa starch. The starch extracted from water chestnut (Trapa bispinosa Roxb.) revealed that its protein content 0.15%, ash 0.06%, Moisture content 7.6%, total carbohydrates 97% and overall energy of 493 and results are given in Table (2).

Table 2: Chemical analysis of Trapa bispinosa starch

Protein %age	0.15
Ash %age	0.06
Moisture % age	7.6
Total carbohydrates % age	97
Energy calories/100g	493

pH of yogurt

Negative logarithm of the hydrogen ion concentration is called pH. It is more authentic means of measurement than titratable acidity. The titratable acidity provides a measurement of the quantity of acid present whereas pH gives the measurement of the power of that acid. Yoghurt is a perishable food and due to acidity production pH of the perishable foods decreased by increasing storage time. The results regarding pH of yoghurt samples, depicted in the Table (3) shows that pH of the yoghurt samples significantly affected due to the different concentration of starch (extracted from Trapa bispinosa) and due to storage. The pH values of yoghurt samples were decreased as storage time increased, which would be due to conversion of lactose into lactic acid. The decrease in pH from 1st to 24th days of storage was 4.90 to 4.27, 4.85 to 4.4, 4.84 to 4.36, 4.89 to 4.44 and 4.89 to 4.43 for samples SO, S1, S2, S3, and S4 respectively. Results revealed that the yoghurt having 0.50% Trapa bispinosa starch had the least decreased in pH and less production to acidity. These results are according to the findings of (Kamaruzzaman and Rehman, 2000) who reported pH decreased during the storage of yoghurt due to production of lactic acid.

Acidity of yogurt

Acidity and pH exhibit a negative relationship because when acid started to produce, acidity level increases, on the other side pH decreases with same ratio. Acidity is particularly means as a determination of sum of lactic acid production in dairy products. The results (Table 4) showed that acidity of the yoghurt samples are affected significantly due to the different concentration of starch (extracted from Trapa bispinosa) and due to storage. The mean values of acidity showed that acidity of all samples increased during storage irrespective to different concentrations of stabilizers. Increase in acidity from 1st to 24th day was 0.82 to 1.47, 0.88 to

1.42, 0.85 to 1.63, 0.83 to 1.54 and 0.87 to 1.56% for S0, Sl, S2, S3 and S4 respectively. The difference between initial and final values was 0.59, 0.34, 0.78, 0.71 and 0.69 of S0, Sl, S2, S3 and S4 respectively. The increase in acidity is due to conversion of lactose into lactic acid by the activity of lactic acid bacteria. Maximum increase in acidity was 1.40 for S2 and minimum increase is 1.52 for S1, it shows that S1 has more capability to resist against changes, resultantly microbial activity remained in control and there was less whey separation. Reason behind this is that stabilizer has ability to bind the water. These results were according to the findings of Bilal (1995), Shin et al. (1991), Cheah-ying (1990), Mehna and Mehna (1989), Peterson (1989) and Rehman 1987) who examined that during storage acidity increased due to microbial activity and lactose converted into lactic acid.

Fat content of yogurt

Fat enhances the flavour of any food product and it also increased the value of the product in which overall appearance, structure and taste of the product is improved. There is variation for fat contents among different breed of milking animals, further more there is extremely wide variation of fatty acids. Most of these are present in various forms of glycerides.

The results concerning the fat of yoghurt samples represented in table (5) shows that variable

Table 3: Means values of yoghurt€s pH

Days	S_0	S_1	S_2	S_3	S_4	Means
1	4.9^{a}	4.85^{b}	4.84^{b}	4.89^{a}	4.89^{a}	4.87^{A}
5	4.82^{c}	4.60^{f}	4.64^{d}	4.6^{f}	4.62^{e}	4.65^{B}
8	4.61^{ef}	4.57^{g}	4.54^{h}	4.58^{g}	4.57^{g}	4.57^{C}
11	4.57^{g}	4.53^{hi}	4.5^{k}	4.53^{hi}	4.52^{ij}	4.53^{D}
14	4.41^{o}	4.47^{l}	4.52^{ij}	4.52^{ij}	4.51^{ijk}	4.48^{E}
17	4.35^{p}	4.45^{m}	4.45^{m}	4.51^{ijk}	4.5^{k}	4.45^{F}
24	4.27^{q}	4.40^{o}	4.36^{p}	4.44^{mn}	4.43^{n}	4.38^{G}
Means	4.56^{B}	4.55^{C}	4.55^{C}	4.58^{A}	4.57^{A}	

Table 4: Mean values of yoghurt€s acidity (%)

Days	S_0	S_1	S_2	S_3	S_4	Means
1	0.82^{y}	0.88^{t}	0.85^{u}	0.83^{y}	0.87^{t}	0.85^{G}
5	1.12^{s}	1.20^{q}	1.31^{no}	1.30^{o}	1.26^{p}	1.23^{F}
8	1.17^{r}	1.30^{o}	1.38^{k}	1.32^{n}	1.27^{p}	1.28^{E}
11	1.38^{k}	1.31^{no}	1.51^{e}	1.39^{ik}	1.39^{ik}	1.39^{D}
14	1.40^{j}	1.34^{m}	1.53^{d}	1.44^{h}	1.46^{g}	1.43^{C}
17	1.44^{h}	1.36^{l}	1.59^{b}	1.53^{d}	1.49^{f}	1.48^{B}
24	1.47^{g}	1.42^{i}	1.63^{a}	1.54^{d}	1.56^{c}	1.52^{A}
Means	1.25^{D}	1.25^{D}	1.40^{A}	1.33^{B}	1.32^{C}	

Table 5: Mean values of yoghurt€s fat contents (%)

Days	S_0	S_1	S_2	S_3	S_4	Means
1	5.10^{a}	5.05^{B}	5.00^{d}	5.03^{c}	5.05^{b}	5.05^{A}
8	5.09^{a}	5.04^{bc}	5.00^{d}	5.03^{c}	5.05^{b}	5.04^{A}
16	4.95^{e}	4.99^{d}	4.93^{f}	4.91^{g}	4.91^{g}	4.94^{B}
Means	5.04^{A}	5.03^{B}	5.00^{C}	4.99^{D}	4.98^{E}	

Table 6: Mean values of yoghurt€s synersis (ml/100ml)

Days	S_0	S_1	S_2	S_3	S_4	Means
1	1.89^{f}	2.01^{b}	1.95^{d}	1.90^{f}	1.93^{e}	1.94^{B}
5	1.92^{e}	2.07^{a}	1.97^{c}	1.97^{c}	1.97^{c}	1.98^{A}
8	1.40^{s}	1.63^{g}	1.53^{h}	1.43^{i}	1.38^{k}	1.39^{C}
11	0.55^{t}	1.33^{mn}	1.41^{j}	1.41^{j}	1.28^{p}	1.19^{D}
14	0.51^{u}	1.32^{no}	1.37^{kl}	1.39^{j}	1.27^{pq}	1.17^{E}
17	0.48^{v}	1.31^{op}	1.36^{l}	1.26^{qr}	1.26^{qr}	1.13^{F}
24	0.46^{w}	1.28^{p}	1.34^{m}	1.33^{mn}	1.25^{r}	1.13^{F}
Means	0.97^{D}	1.56^{A}	1.56^{A}	1.53^{B}	1.48^{C}	

Table 7: Mean values of yoghurt€s total viable count

Days	S_0	S_1	S_2	S_3	S_4	Means
5	$1.70x10^{7g}$	$1.72\ x10^{7f}$	$1.71x10^{7fg}$	$1.76\ x10^{7e}$	$1.77\ x10^{7e}$	$1.73\ x10^{7B}$
8	$2.21\ x10^{7d}$	$2.30\ x10^{7c}$	$2.31\ x10^{7bc}$	$2.32\ x10^{7ab}$	$2.33\ x10^{7a}$	$2.29\ x10^{7A}$
Means	$1.95\ x10^{7C}$	$2.01\ x10^{7B}$	$2.01\ x10^{7B}$	$2.04\ x10^{7A}$	$2.05\ x10^{7A}$	

centrations of starch (extracted from Trapa bispinosa) and storage conditions have significant affect on fat. The results related to changes in fat concentration are presented in Table 4.10. Difference between initial and final values of Fat contents from 1st day to 24th day were 0.15, 0.32, 0.07, 0.12 and 0.14 percent for SO to S4 respectively. These results were in accordance to Mehana and Mehana (1989) who said that N stabilizers had no significant effect on fat contents of yoghurt while according to Ahmad (1999) acidic storage for longer periods of time or lipolytic activity of microflora caused the reduction of fat contents. However no rancidity was observed due to low storage temperature of yoghurt.

Synersis of yogurt

Synersis (whey separation due to shrinkage of gel) is the major problem for yoghurt during storage. The results in (Table 6) shows that synersis of the yoghurt samples are affected significantly due to the different concentration of starch (extracted from Trapa bispinosa) and due to storage. The decreased in values synersis (Table 4.16) were 1.89 to 0.46, 2.01 to 1.28, 1.95 to 1.34, 1.90 to 1.33 and 1.93 to 1.25 for samples S0 to S4 respectively. Differences between initial and final values were 1.43, 0.73, 0.61, 0.57 and 0.68 for S0 to S4 respectively. Maximum decrease in whey separation was for S0 and Sl. Against reduction in synersis both S0 and Sl showed excellent results, while other samples also showed good results against synersis.

Total viable count of yogurt

The results regarding the total viable count of yoghurt samples described in (Table 7) showed that total viable count of the yoghurt samples significantly affected due to different concentrations of starch (extracted from Trapa bispinosa) and due to storage. The data regarding total viable count of all samples during storage time is presented in Table 4.18. Total viable count of all yoghurt samples increased during storage interval. Increase in total viable count from 1st to 24th days of storage was $1.70x10^7$ to $2.21x10^7$, $1.72x10^7$ to $2.30x10^7$, $1.71x10^7$ to $2.31x10^7$, $1.76x10^7$ to $2.32x10^7$ and $1.77x10^7$ to $2.33x10^7$ for S_0, S_1, S_2, S_3 and S_4 respectively. The results were in the agreement with that of Younis et al. (2000) who showed that there was a significant effect during storage interval

Appearance of yogurt

Appearance is an important factor to judge liking or disliking of consumers for product. The results regarding sensory appearance of yoghurt samples manifested in the (Table 7) showed that sensory appearance of the yoghurt samples significantly affected due to the different concentration of starch (extracted from Trapa bispinosa) and due to storage while the interaction of storage and samples non significant. The mean score for appearance (Table 4.21) from 1st to 24th day of storage ranged from 8.00 to 6.00, 8.00 to 6.00, 8.67 to 7, 8.67 to 7.00 and 8.00 to 6.00 for S0 to S4 respectively. Difference between initial and final values was 2.00, 2.00, 1.67, 1.67 and 2.00 for all samples from S0 to S4 respectively. The results of my study are in line with (Marcotte et al., 2001) who found that yoghurt was remained satisfactory for about 1 month at refrigeration temperature; this might be due to slight growth of psychrotrophs.

Texture of yogurt

Among sensory evaluation, the 2nd most important character of yoghurt is apparent body and texture for consumer liking or disliking. It's affected by many factors like total viable count and acidity, as increased in yeast, acidity and mould count, body and texture decreased. It might be due to activity of yeast and mould that affect the action of stabilizers. The mean score (Table 9) for body and texture from 1st to 24th day of storage ranged from 8.00 to 3.33, 8.00 to 7.00,

Table 8: Mean values of yoghurt€s appearance

Days	S_0	S_1	S_2	S_3	S_4	Means
1	8.00^{ab}	8.00^{ab}	8.67^{a}	8.67^{a}	8.00^{ab}	8.27^{A}
5	8.00^{ab}	8.00^{ab}	8.00^{ab}	8.00^{ab}	8.00^{ab}	8.00^{A}
8	8.00^{ab}	8.00^{ab}	8.00^{ab}	8.00^{ab}	8.00^{ab}	8.00^{A}
11	7.00^{bc}	8.00^{ab}	8.33^{ab}	8.00^{ab}	7.00^{bc}	7.67^{AB}
14	7.00^{bc}	8.00^{ab}	7.00^{bc}	7.00^{bc}	6.00^{c}	7.00^{BC}
17	6.00^{c}	7.00^{bc}	7.00^{bc}	7.00^{bc}	6.00^{c}	6.60^{C}
24	6.00^{c}	6.00^{c}	7.00^{bc}	7.00^{bc}	6.00^{c}	6.40^{C}
Means	7.14^{AB}	7.57^{AB}	7.71^{A}	7.67^{A}	7.00^{B}	

Table 9: Mean values of yoghurt€s body and texture

Days	S_0	S_1	S_2	S_3	S_4	Means
1	8.00^{ab}	8.00^{ab}	8.67^{a}	8.67^{a}	8.67^{a}	8.40^{A}
5	7.00^{bc}	8.00^{ab}	8.00^{ab}	7.00^{bc}	7.00^{bc}	7.40^{B}
8	7.00^{bc}	7.00^{bc}	8.00^{ab}	8.00^{ab}	8.00^{ab}	7.60^{B}
11	7.00^{bc}	7.00^{bc}	8.33^{ab}	7.33^{abc}	7.00^{bc}	7.33^{B}
14	7.00^{bc}	7.00^{bc}	7.00^{bc}	7.00^{bc}	7.00^{bc}	7.00^{BC}
17	6.00^{c}	7.00^{bc}	7.00^{bc}	7.00^{bc}	6.00^{c}	6.60^{CD}
24	3.33^{d}	7.00^{bc}	7.00^{bc}	7.00^{bc}	6.00^{c}	6.07^{D}
Means	6.48^{C}	7.29^{AB}	7.71^{A}	7.43^{AB}	7.09^{B}	

Table 10: Mean values of yoghurt€s flavor

Days	S_0	S_1	S_2	S_3	S_4	Means
1	8.00^{a}	8.00^{a}	8.00^{a}	8.00^{a}	8.00^{a}	8.00^{A}
5	8.00^{a}	8.00^{a}	8.00^{a}	8.00^{a}	8.00^{a}	8.00^{A}
8	7.00^{ab}	7.00^{ab}	8.00^{a}	7.00^{ab}	8.00^{a}	7.40^{A}
11	6.00^{bc}	6.00^{bc}	7.00^{ab}	7.33^{ab}	7.00^{ab}	6.67^{B}
14	6.00^{bc}	6.00^{bc}	7.00^{ab}	7.00^{ab}	7.00^{ab}	6.60^{B}
17	5.00^{cd}	7.00^{ab}	7.00^{ab}	7.00^{ab}	6.00^{bc}	6.40^{B}
24	5.00^{cd}	4.00^{d}	6.00^{bc}	6.00^{bc}	6.00^{bc}	5.40^{C}
Means	6.43^{C}	6.57^{BC}	7.29^{A}	7.19^{A}	7.14^{AB}	

Table 11: Mean values of yoghurt€s overall acceptability

Days	S_0	S_1	S_2	S_3	S_4	Means
1	8.00^{a}	8.00^{a}	8.00^{a}	8.00^{a}	8.00^{a}	8.00^{A}
5	7.00^{ab}	8.00^{a}	8.00^{a}	7.00^{ab}	8.00^{a}	7.60^{A}
8	7.00^{ab}	7.00^{ab}	8.00^{a}	7.00^{ab}	8.00^{a}	7.40^{B}
11	6.00^{bc}	7.00^{ab}	7.00^{ab}	7.33^{ab}	7.00^{ab}	6.67^{BC}
14	6.00^{bc}	7.00^{ab}	7.00^{ab}	7.00^{ab}	7.00^{ab}	6.80^{BC}
17	5.00^{c}	7.00^{ab}	7.00^{ab}	7.00^{ab}	6.00^{bc}	6.40^{CD}
24	5.00^{c}	6.00^{bc}	6.00^{bc}	6.00^{bc}	6.00^{bc}	5.80^{D}
Means	6.29^{B}	7.14^{A}	7.29^{A}	7.05^{A}	7.14^{A}	

8.67 to 7.00, 8.67 to 7.00 and 8.67 to 6.00 for S0, S1, S2, S3 and S4 respectively. Among all the samples of yoghurts the highest scores were awarded to S1 followed by S2, S3, S4 and S0.

Flavor of yogurt

Thermal degradation of some constituents of milk forms a volatile compound which produced the flavor in yoghurt. Acetaldehyde is one of the important aroma compounds. Flavor is one of the most important factors among sensory evaluation of the product to check the consumer•s response towards product. The data represented in Table (10) indicated that there was decreased in flavor from 1st day to 24th day. Decrease in flavor score was 8.00 to 5.00, 8.00 to 4.00, 8.00 to 6.00, 8.00 to 6.00 and 8.00 to 6.00 for S_0 to S_4 respectively. Difference between initial and final values was 3.00, 4.00, 2.00, 2.00 and 2.00 for all samples from S_0 to S_4 respectively.

The results revealed that S2, S3 and S4 got highest points as compared to other samples (S0 and S1), in these samples only 2 points decreased during storage periods of 24 days. While all samples remained in acceptable range due to addition of stabilizer and also stored at low temperature, but overall Trapa bispinosa starch addition samples gave best flavour results. These results were according to the findings of Kamaruzzaman at al., (2002), who reported a decrease in yoghurt flavour when stored at low temperature.

Overall acceptability of yogurt

Processing conditions, culture, stabilizers and storage conditions are the main factors which affect the quality of the yoghurt. The results regarding the overall acceptability of yoghurt samples depicted in (Table 11) showed that overall acceptability of the yoghurt samples significantly affected due to different concentrations of starch (extracted from Trapa bispinosa) and due to storage while the interaction of storage and samples non significant. The data presented in Table 4.29 indicated that there was decreased in overall acceptability from 1st day to 24th day. Decrease in overall acceptability score was 8.00 to 5.00, 8.00 to 6.00, 8.00 to 6.00, 8.00 to 6.00 and 8.00 to 6.00 for S_0 to S_4 respectively. Difference between initial and final values was 3.00, 2.00, 2.00, 2.00 and 2.00 for all samples from S_0 to S_4 respectively. It is revealed from results that S_1, S_2, S_3 and S_4 got highest points as compared to other sample i.e. S_0 in these samples only 2 points decrease during storage periods of 24 days. Overall Trapa bispinosa starch addition yoghurt samples gave best but the S_2 gave the most excellent overall acceptability results. Overall acceptability of Trapa bispinosa starch enriched yoghurt decreased due to decrease in viscosity, yoghurt flavor and increase in sourness or acidity (Imeson, 1997).

CONCLUSION

It is concluded at the end from all results that Trapa bispinosa starch gave better results for synersis, water holding capacity, viscosity and for all sensory attributes. Among different concentrations of stabilizer further Trapa bispinosa starch with the concentration of 0.75% gives most excellent result for water holding capacity, synersis and overall sensory attributes. It is also concluded that starch (extracted from Trapa bispinosa) addition @ 1.00% gives most excellent results for, viscosity, protein and total viable count. It also observed that yoghurt can be stored up to 25 days when kept at 4°C by giving proper storage and especially packaging condition.

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The content of zinc, copper, lead and cadmium in some vegetables of Kyrgyzstan

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ABSTRACT

Due to anthropogenic pollution of the environment in recent years, the constant monitoring of toxic metal contents in foods of plant origin is becoming essentially necessary. This work evaluated some toxic metals (Cu, Zn, Cd, Pb) in thirteen (13) vegetables grown, sold and constantly in the diet of residents of the Chui region of the Kyrgyzstan for the purpose of ensuring healthy diet among the populace. The toxic metals were determined using inverse voltammetric technique. Green vegetables such as garlic chives, parsley and dill had the highest average concentrations of Cu with 4.246, 4.030 and 3.909 mg/kg, respectively. Also found the highest concentrations of zinc in garlic chives, green radish and potatoes with average contents of 3.045, 2.988 and 2.469 mg/kg, respectively. The concentration of cadmium in red beets, green radish and cabbage with an average content of 0.025, 0.024 and 0.023 mg/kg, was found respectively. However, in certain vegetables samples such as dill, sweet pepper and parsley we observed relatively high values of 0.291, 0,257 and 0.257 mg/kg respectively, but were not considered harmful. The results showed that the concentrations of copper, zinc and lead in the analyzed vegetables did not exceed the maximum allowable concentration (MAC) recommended by Sanitary rules and norms (SanPIN) guidelines, but the cadmium content was approaching the maximum permissible concentration, indicating the need for more caution to prevent the cadmium content of the vegetables from exceeding the safe limit.

Key words: Heavy metals, vegetables, concentration, voltammetry, Kyrgyzstan.

INTRODUCTION

Plants play an important role not only for geochemical cycling of microelements but also as an intermediate reservoir from which the microelements partially transfer into humans and livestock through polluted soil, water and air (Kabata-Pendias and Pendias, 1989). The uptake of these heavy metals by plants especially leafy vegetables is an avenue of their entry into the human food chain with harmful effects on health (Ihekoronye and Ngoddy, 1985). The uptake of heavy metals by plants grown in polluted soils has been studied to a considerable extent (Yussuf and Oluwole, 2009; Sukreeyapongse *et al.*, 2002). Heavy metal contamination in plant food materials cannot be underestimated as these commodities are important components of human diet. Heavy metal contamination of the food items is one of the most important aspects of food quality assurance (Radwan and Salama, 2006; Khan et al., 2008). International and national regulations on food quality have lowered the maximum permissible levels of toxic metals in food items due to an increased awareness of the risk these metals pose to food chain contamination (Radwan and Salama, 2006).

With increased anthropogenic pressure and worsened environmental and economic conditions, safe food consumption is an important health factor. Several cases of human disease, disorders, malfunction and malformation of organs due to metal toxicity have been reported (Türkdogan *et al*., 2003; Alam and Tanaka, 2003; Damek-Poprawa and Sawicka-Kapusta, 2003; Arora *et al.*, 2008). Studies on the impact of toxic metals on the human body include the effects of high concentrations of zinc and cadmium, which alter the composition of blood and may result in cancer (Maret and Sandstead, 2006; Nriagu, 2007; Nawrot *et al*., 2008).

Toxic metals may be absorbed by vegetables through several processes and finally enter the food chain at high concentrations capable of causing a serious health risk to consumers. Their toxicity can damage or reduce mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver and other vital organs. Long term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that cause muscular dystrophy, and multiple sclerosis (Kihampa et al. 2010). Consequently, many trace elements are not only essential

for humans and animals but some are also considered harmful and a source of various illnesses.

The mineral composition of food raw materials is determined by regional features and is constantly changing depending on the environmental, including soil – climatic conditions, industrial activity, agricultural practices, technology, food production, etc. therefore, planned, systematic and differentiated on a regional basis studies of trace element composition of the food, systematization of the available information is one of the main objectives of nutrition and food chemistry.

There is paucity of information on the heavy metal contents in commonly consumed vegetables in Kyrgyzstan. Therefore, this paper provides a comprehensive evaluation of toxic metals in important vegetables of Kyrgyzstan. The goal of this study was to establish levels of toxic metals in plant food materials, particularly in vegetables. In addition, the result of this study can be used for the development of a database for toxic metals in food products in the Kyrgyzstan.

MATERIALS AND METHODS

Materials

Fresh vegetable samples including garlic chives, cabbage, potatoes, onion, carrots, cucumbers, sweet pepper, parsley, radish, green radish, red beet, tomatoes and dill were obtained from a private farmer in the Chui region during different growing seasons of the year. The samples were transferred quickly to the laboratory for analysis.

Methods

Sample Preparation

Sampling was carried out in accordance with KMS 40.205-99. Samples were washed with deionized water. All samples were oven dried at 80-100 ^{0}C before charring at 150±50^{0}C on a hot plate in a fume cupboard. Dry ashing of the charred samples was carried out in a muffle furnace at a temperature of 450±50^{0}C. The resulting ash was dissolved in 1-2 ml concentrated hydrochloric acid at low heat on a hot plate and the solution was evaporated to near dryness. The precipitate was later dissolved in 10 ml deionized water.

Reagents and options

The concentration is determined by standard addition of Zn, Cd, Pb, Cu by using mercury-film electrode. All reagents which used in this method must be in pure quality (analytical grade). Only ultrapure water should be used. Standard solutions:

Stock solutions were prepared from the state standard samples with certified concentrations of metals 10.00 mg/cm3. (Zn2 +, Cd2 +, Pb2 +, Cu2 +) = 10.00; 1.00; 0.10 mg / L were prepared by diluting the original solution into a volumetric flask with a capacity of 50.0 cm3 with deionized water (GOST 51301-99, 1999).
Peak potential (Zn) - (-0.90± 0.05) V
Peak potential (Cd) - (-0.60± 0.05) V
Peak potential (Pb) - (-0.40± 0.05) V
Peak potential (Cu) - (-0.10± 0.05) V

Voltammetric determination of zinc, cadmium, lead and copper

Voltammetric analyzer TA-1(Polarographic) was used with formic acid and UV-irradiation with software working in MS-DOS. Electrodes used were mercury-film electrode and a teflon rod with extruded silver wire, anode reference electrode filled with a 1 M solution KCI, silver electrode. All the reagents used were analytical grade. The electrochemical cell (cups) and electrode were properly cleaned with 10 ml deionized water and 0.1-0.2 ml concentrated formic acid to ensure purity. Sensitivity of device was 4.109 A/mm and the time for electric charge accumulation was 120 s. The solution was stirred with a magnetic stirrer within 300 s. During the deposition stage, a potential of -1.4 V was adjusted and the solution was stirred with a magnetic stirrer for 120-300s depending on the concentration in the analyze. After 5 sec the voltammogram in the range of potentials from -1.2 to +0.05 V were recorded. Potential was stopped at 0.05 V and held until dissolution of impurities from the surface of electrode while stirring the solution for 20 s. The aliquots of the sample (1.0-2.0ml) were added and placed in the electrochemical cell followed by UV-irradiation.The concentration of the heavy metals, Zn, Cd, Pb, Cu, were determined by comparison with standard solutions (GOST 51301-99, 1999).

RESULTS AND DISCUSSION

Heavy metal average and range concentrations in the thirteen vegetable samples of Kyrgyzstan are presented in (Table 1 and 2).
Copper (toxic trace element). Green vegetables such as garlic chives, parsley and dill had the highest average concentrations of Cu with 4.246, 4.030 and 3.909 mg/kg, respectively. These levels are very close to but below the maximum allowable concentration (MAC) stated for vegetables, i.e. 5.0 mg/kg by SanPIN guidelines (SanPIN 2.3.2. 560-96, 1996). All other vegetables had copper in the range of 0.519 to 1.641 mg/kg which are far below MAC for vegetables according to SanPIN guidelines. Copper is a trace element that is essential for human health. The observation for other vegetables apart from the green vegetables agrees with the report of Chove et al. (2006) who estimated copper levels in two common vegetables in Tanzania to be in the range of 0.885 to 1.39 mg/100g (dry weight). Akan et al. (2013) reported

Table 1: Toxic metals concentrations of some vegetables (mg/kg, wet weight matter)

Vegetable	Cu		Zn	
	Mean±SD[b]	Range	Mean±SD	Range
Garlic chives (n = 4)[a]	4.246±0.195	3.958-4.376	3.045±0,367	2.764-3.558
Cabbage (n = 4)	0.595±0.113	0.460-0.736	0.506±0.269	0.175-0.832
Potatoes (n = 4)	0.738±0.118	0.572-0.849	2.469±0.179	2.249-2.639
Onion (n = 7)	0.703±0.616	0.244-2.078	0.976±0.372	0.465-1.654
Carrots (n = 4)	1.459±0.084	1.357-1.553	0.888±0.048	0.849-0.954
Cucumbers (n = 5)	0.937±0.205	0.646-1.209	1.426±0.659	0.710-2.496
Sweet pepper (n = 4)	1.547±0.358	1.104-1.949	0.704±0.163	0.540-0.892
Parsley (n = 4)	4.030±0.522	3.250-4.329	1.777±0.290	1.432-2.10
Radish (n = 4)	0.910±0.260	0.562-1.133	0.650±0.080	0.551-0.742
Green radish (n = 4)	0.519±0.336	0.185-0.852	2.988±1.333	1.683-4.795
Red beet (n = 3)	1.482±0.106	1.385-1.595	1.957±0.089	1.860-2.035
Tomatoes (n = 3)	1.641±0.306	1.410-1.988	0.711±0.232	0.478-0.943
Dill (n = 4)	3.909±0.489	3.258-4.418	1.316±0.172	1.07-1.44

Table 2: Toxic metals concentrations of some vegetables (mg/kg, wet weight matter)

Vegetable	Cd		Pb	
	Mean±SD	Range	Mean±SD	Range
Garlic chives (n = 4)[a]	0.017±0.00028	0.017-0.018	0.177±0.063	0.113-0.249
Cabbage (n = 4)	0.023±0.0081	0.012-0.032	0.066±0.077	0.049-0.080
Potatoes (n = 4)	0.015±0.0014	0.014-0.017	0.102±0.060	0.080-0.154
Onion (n = 7)	0.012±0.0069	0.0023-0.019	0.022±0.0076	0.015-0.034
Carrots (n = 4)	0.019±0.0009	0.019-0.021	0.027±0.0019	0.025-0.029
Cucumbers (n = 5)	0.011±0.0064	0.004-0.022	0.071±0.062	0.034-0.180
Sweet pepper (n = 4)	0.017±0.0062	0.009-0.024	0.257±0.168	0.077-0.469
Parsley (n = 4)	0.020±0.0054	0.014-0.026	0.257±0.023	0.230-0.277
Radish (n = 4)	0.018±0.0071	0.008-0.024	0.033±0.0056	0.028-0.040
Green radish (n = 4)	0.024±0.0018	0.021-0.025	0.112±0.065	0.028-0.171
Red beet (n = 3)	0.025±0.0007	0.024-0.026	0.017±0.0009	0.017-0.019
Tomatoes (n = 3)	0.006±0.0042	0.001-0.009	0.062±0.012	0.053-0.075
Dill (n = 4)	0.007±0.0011	0.006-0.008	0.291±0.058	0.228-0.344

Notes:
[a]Number of samples (monthly).
[b]Data are presented as means±SD (Standard deviation).

21 to 3.22 mg/kg Cu levels for some vegetables in Nigeria which is also similar to the copper level in many of the vegetables studied in this work. Also the copper levels in the thirteen vegetables were below the limit recommended by the Food and Agricultural Organization (FAO) and the WHO/EU joint guidelines.

Zinc (toxic trace element).We also found the highest concentrations of zinc in garlic chives, green radish and potatoes with average contents of 3.045, 2.988 and 2.469 mg/kg, respectively, and the lowest level was recorded in cabbage with 0.506 mg/kg. In general, observed zinc levels were not considered problematic since concentrations were 3.3 to 20 times lower than MAC (10.0 mg/kg) recommended by SanPIN guidelines. Kabata-Pendias and Pendias (1989) noted that the root systems of plants contain a greater proportion of zinc than their aerial part. Haynes (1980) observed that zinc is accumulated in root plants. Udris and Neiland (1981) confirmed that root plants are characterized by high concentrations of zinc of up to 100-200 mg/kg. Kihampa et al. (2010) reported 18.61 and 122.88 mg/kg dry weight for zinc in vegetables grown in the vicinity of the closed dumpsite.

Cadmium (toxic trace element). Our studies have found large concentrations of cadmium in red beets, green radish and cabbage with an average content of 0.025, 0.024 and 0.023 mg/kg, respectively. Dill and tomatoes had the least values and were almost 3.6 and 4.1 times less in cadmium than red beets. Okwulehie and Ogoke (2013) reported cadmium in the range of 0.7-0.94 ppm for certain mushrooms in Nigeria. Banerjee et al. (2010) recorded cadmium levels in some fruits and vegetables in India to be in the range of 0.03 to 7.32 µg/g dry weight. Cadmium content of all the analysed vegetables in Kyrgyzstan was below those reported in the literature. It is also below the MAC limit stipulated in the SanPIN guidelines.

Lead (toxic trace element). In our study the average *lead* content in vegetables did not reach the upper concentration limit of 0.5 mg/kg as recommended by SanPIN guidelines. However, in certain vegetables samples such as dill, sweet pepper and parsley we observed relatively high values of 0.291, 0,257 and 0.257 mg/kg respectively, but were not considered harmful. Other vegetables had their lead values in the range of 0.017 to 0.177 mg/kg. Agbenin et al. (2010) found higher concentrations of lead in lettuce (3.78 to 15.6 mg/kg on a dry weight basis) compared with the concentration found in amaranthus (1.30 to 3.54 mg/kg on a dry weight basis) planted in the same field. In another study, reported lead concentrations in parsley and pumpkins were lower than concentrations reported for other vegetables including cabbages, carrots, cauliflower, cucumbers, eggplants, green peppers, spinach, sweet potatoes and tomatoes (Mbabazi et al., 2010). Sangster et al. (2012) also reported around 16.25 mg/kg lead for certain vegetables in Nebraska. The lead contents in the vegetables from Kyrgyzstan are very low compared to many of the reports in the literature and most importantly fall below the limits recommended by the SanPIN guidelines signifying that the consumption of the vegetables has no health hazard implication.

CONCLUSIONS

The present study provides data on heavy metal constituents in vegetables grown, sold and eaten in Chui region in Kyrgyzstan. In the whole plant materials studied from the region green vegetables such as garlic chives, dill and parsley had highest concentrations of copper, highest zinc values were recorded in garlic chives, radish and potatoes. Red beet, radish and cabbage had higher concentrations of cadmium while highest lead concentrations were observed in dill, sweet pepper and parsley. However, in general the levels of all the metals studied were lower than those recommended by SanPIN maximum allowable concentration of Cu-5.0 mg/kg; Zn-10.0 mg/kg; Cd-0.03 mg/kg; Pb-0.5 mg/kg and far below the Food and Agricultural Organization (FAO) and the WHO/EU joint limits of 0.1 µg/g Pb; 0.1 µg/g Cu; 0.1 µg/g Zn and 0.02 µg/g Cd (Akan et al., 2013). The results indicate that all the vegetable samples analyzed in this study had low levels of heavy metals and would not pose any health risk to the consumers in the area of production. Further studies should be conducted to cover a wide range of production in Kyrgyzstan to corroborate this finding.

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