PAK. J. FOOD SCI., 23(3), 2013: 124-132

ISSN: 2226-5899

Production and evaluation of gari produced from cassava (*Manihot esculenta*) substituted with cocoyam (*Colocasia esculenta)*

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ABSTRACT

Reliance on a few crops has been noted to have unhelpful consequences on ecosystems, food diversity and human health. Besides, neglected and underutilized species could play a prominent role in the struggle against hunger and serve as a key resource for agriculture and rural development. Cocoyam, an underexploited tuber in Nigeria was studied for its potential in gari making to reduce over reliance on cassava conventionally used for this product. Gari was produced from cassava mash substituted with varying proportions (0, 10, 20, 30, 50 and 100%) of cocoyam mash. The gari samples were evaluated to assess the chemical composition, physical and physicochemical properties. Additionally, the sensory attributes of the products were assessed. The moisture content of all the gari samples were generally below 8 % indicating safe level for prolonged storage. Substituting cassava with cocoyam at varying proportions resulted in 8–26 % reduction in fiber content, 8-49 % and 7-183 % enhancement in ash and protein contents, respectively. Cassava substitution with cocoyam significantly ($p<0.05$) increased pH level and reduced total titratable acidity of the gari samples. There were wide variations in the hydrogen cyanide (HCN) contents of the gari samples with gari produced from 100 % cocoyam mash having the least value of 0.24 mg/kg, and 100 % cassava gari had the highest value of 14.22 mg/kg. A progressive decrease in the swelling capacity was observed with increase in the substitution level of cocoyam in the mixture. The results of water absorption capacity, reconstitution index, syneresis and bulk density of the samples followed similar trend with that of swelling capacity. The gari samples had peak viscosity between 2054.75 and 2620.75 RVU, peak time ranged from 5.12 and 6.98 min and pasting temperature ranged between 72.94 and 86.78°C. Set back viscosity ranged from 1297.75 to 2425.75 RVU and breakdown viscosity from 149.50 to 566.25 RVU. The final viscosity of the samples ranged between 3429.75 and 4331.00 RVU. Gari produced from 100% cassava was the most preferred in terms of colour, taste and appearance while pure cocoyam gari was best preferred in terms of texture and aroma. No significant difference ($p<0.05$) was recorded for the overall acceptability attribute of 100% cocoyam gari and 100% cassava gari (control sample) which was best rated by the panelists. These findings indicate the potential of cocoyam in gari making.

Keywords: Gari, Cassava, Cocoyam, Chemical Compositions, Physico-chemical Properties, Sensory attributes

INTRODUCTION

Tropical root and tuber crops such as cassava (*Manihot esculenta* Cranz), Cocoyam (*Colocasia esculenta*) and yam (*Discorea* spp.) are the third important commodities of humans, after cereals and grains legumes which constitute either staple or subsidiary food for about a fifth of the world population (Ray *et al.*, 2009). In the tropics, cassava is an important food crop and is a major carbohydrate source. Cassava is grown in all the agro-ecological zones of Nigeria. It is a staple food for over a million people of the West African population (Oduro *et al.*, 2000; Afoakwa *et al.*, 2010). However, fresh cassava has limited storage life because of its high moisture content, its processing into relatively shelf stable intermediate and final products for various food applications is therefore necessary (Quaye *et al.*, 2009). Cassava can be transformed into various products such as ‘gari’, ‘fufu’, ‘lafun’ and many other West African traditional dishes (Obiele *et al.*, 2004; Afoakwa *et al.*, 2010).

Gari (a roasted fermented cassava meal) is the most popular cassava product consumed in West Africa and the most important food product in the diet of millions of Ghanaians and Nigerians (Afoakwa *et al.*, 2010). It is a staple food that used to be within the purchasing power of all categories of people in a society irrespective of their income (Sanni *et al.*, 2007). In the recent times, however, there is increasing use of cassava as source of ethanol for fuel, energy in animal feed, and starch for industry (Kolawole and Agbetoye, 2007). Additionally, with the Federal Government’s cassava export initiative in a bid to shore up its sources of foreign exchange earnings, the price of gari has skyrocketed suggesting the need to explore alternative sources for the product, to make it affordable to the common people in the country (Chukwuji et al, 2007). Continual dependence on cassava as the only raw material for gari production could lead to scarcity of the product and hike in price. Great success has been recorded in the use of sweet potato for gari (Sanni *et al.*, 2007; Sanni and Ikuomola, 2001). Exploration of other alternative sources such as cocoyam could be of great advantage considering the ever increasing market for gari both at home and abroad.

Cocoyam is grown in the tropical and sub-tropical regions of the world particularly in Africa for human nutrition, animal feed and cash income for both farmers and traders (Onwubuya and Ajani, 2012). Cocoyam is considerably high in crude and true proteins compared to other root crops, rich in ash, low in fibre and fat. Cocoyam is equally rich in certain micro elements such as P, Mg, Zn etc. It is moderately rich in carotene, ascorbic acid, thiamine, riboflavin and nicotinic acid (Eddy *et al.*, 2012). Though sweet potato has been utilized in gari production in Nigeria and Ghana through various unit operations such has not been demonstrated with cocoyam. Therefore considering the

ISSN: 2226-5899

nutritional quality of cocoyam, the high starch quality (i.e fine starch grains), the utilization of cocoyam in gari production is worth exploration. The aim of this research work, therefore, was to evaluate the chemical and physico-chemical parameters of gari produced from cassava substituted with cocoyam with a view to assessing the suitability of cocoyam in gari production. This could bring down the hike in the price of gari and increase the utilization of cocoyam which is hitherto underutilized in Nigeria.

MATERIALS AND METHODS

Materials

Cassava and cocoyam were purchased from a local market in Ogbomoso, south western Nigeria. The tubers were manually cleaned to remove adhering soil and other extraneous materials before processing. All reagents that were used in this study were of analytical grade.

METHODS

Sample Preparation

The methods of Sanni (2001) and Onuorah *et al.* (2004) were adopted with slight modifications for the processing of the gari products. Peeled diced cocoyam pieces were soaked in 0.1% potassium metabiosulphite for 20 min to prevent browning and were pressed for 24 h before adding to cassava mash already pressed for 48 h. The mixture of cassava-cocoyam mashes were fermented further for another 24 h before subsequent processing into gari. The dried granules of cocoyam-gari were packed into polyethylene bag and heat sealed (Plate 1) before keeping in plastic container with cover until needed for analyses.

Chemical Analysis

The chemical analysis of the gari products were carried out to determine the following: moisture content, ash content, crude fibre, crude protein, crude fat, carbohydrate (by difference), pH, total titrable acidity (TTA) and total hydrogen cyanide according to the methods of AOAC (2005).

Physical and Physico-Chemical Properties

The bulk density was determined using the method of Nwanekezi *et al.* (2001). Swelling capacity for the samples was determined according to the method adopted by Sanni *et al.* (2001). Reconstitution index and water absorption capacity were determined according to the procedure described by Egounlety (1994) and Iwuoha (2004) respectively. Syneresis was determined using Singh and Walekhwa (2004). The pasting characteristics were determined with a Rapid Visco Analyser (RVA), (model RVA 3D+, Network Scientific, Australia) according to the method of Pérez *et al*. (1998).

Sensory Evaluation

Sensory evaluation was conducted to determine consumer preferences and acceptability of the samples, using a 7-point hedonic scale for the degree of likeness. In scaling, 7 represents “like extremely”, midpoint 4 represents “neither like nor dislike” and runs down to one which represents “dislike extremely”. The quality parameters assessed include; colour, texture, taste, aroma, appearance and overall acceptability. Fifty (50) untrained panelists who are regular gari consumers were selected randomly and used for the sensory evaluation. Samples were coded and randomly presented in clean ceramic plates all at the same time and were assessed in both dried and reconstituted forms, cold and hot water were supplied for personal reconstitution of samples.

Statistical analysis

All treatments were replicated twice for reproducibility and analysis done in duplicate. The statistical analysis of the data was done with Statistical Analysis Systems (SAS, 1999) package (Version 8.2 SAS Institute Inc.). Statistically significant differences ($p < 0.05$) in all data were determined by analysis of variance procedure while least significant difference was used to separate the means.

RESULTS AND DISCUSSION

Proximate Composition of the Gari Samples

The results of the proximate composition of the gari samples are presented in Table 1. The values for the moisture content ranged from 6.32-7.29% with the 100% cassava gari having the highest value (7.29%). The moisture content decreased generally as the level of substitution with cocoyam increased. All the gari samples generally had low moisture contents which are within the range of values reported for sweet-potato gari by Sanni *et al.* (2007). Irtwanga and Achimba (2009) stated that good quality gari should be well dried and thus of low moisture content for good storability.

The maximum value for crude fibre was 3.13% (100% cassava gari) while the minimum value was 2.32% (90% cassava: 10% cocoyam) as presented in Table 1. There was significant difference ($p<0.05$) between pure cassava gari and those substituted with cocoyam mash which could be due to differences in the initial fibre content of the fresh samples. There was significant reduction in the values with substitution of cassava with cocoyam in the gari products. Higher fibre content was reported for cassava than cocoyam by Odebunmi et al. (2007). This range falls below the value (6.13%) reported for gari from pure cassava by Oluwamukomi and Adeyemi (2013) which could be associated with varietal differences in the materials used. However the range of values observed in this study agrees with those reported for gari produced from pure cassava by Irtwanga and Achimba (2009). A significant enhancement in the ash content (8-44%) in the

ISSN: 2226-5899

cocoyam containing gari samples was observed compared to gari from 100% cassava (control). This observation deviates from the findings of Oluwamukomi and Adeyemi (2013) who reported 1.18% ash content for cassava gari. The increase noted in the ash content of cocoyam containing gari may be attributed to the high mineral content of cocoyam (Eddy *et al*., 2012).

The highest value of crude protein obtained for the samples were 5.21% (100% cocoyam gari) while the least was 1.84% (control). This is expected as cocoyam is fairly rich in protein (Odebunmi *et al*., 1999; Eddy *et al*., 2012). Irtwange and Achimba (2009) gave the crude protein content of their gari in the range of 2.33 to 2.55%. Komolafe and Arawande (2010) gave the protein contents of gari samples in the range of 1.04 to 1.40%. According to Obatolu and Osho (1992) gari should contain 0.7 to 1.2% protein. The protein contents in this study are higher than the ones reported by previous researchers. This observation indicates nutritional advantage gari produced from cassava substituted with cocoyam will have over to the conventional gari been produced from cassava if adopted as a complement or alternative raw material in gari processing.

The range of values for crude fat falls between 1.52% and 2.45% with 100% cocoyam having the highest value and those substituted with 10% cocoyam mash having the least value. These values were generally lower than those reported for pure cassava gari by some authors which could be as a result of varietal differences (Oluwamukomi and Adeyemi, 2013; Akindahunsi et al., 1999). It was however noted that there was significant increase in the values of those substituted with cocoyam except gari containing 10% cocoyam mash. Gari is not a rich source of fat except when fried with palm oil for colour enhancement. The significant increase might not be unconnected with the initial fat content which has been reported to be higher in cocoyam than cassava (Odebunmi *et al*., 2007).

Significant differences were not observed among the carbohydrate values for the control and those substituted with cocoyam mashes except for the ones containing 50 and 100% cocoyam which might be associated with their relatively high protein content since carbohydrate was determined by difference. Both cocoyam and cassava are mainly carbohydrate food and previous report has shown that the starch of cocoyam is easily digested because of its fine granules (Okpala *et al*., 2012) which will be an added advantage if incorporated into gari making.

Cyanide content, pH and total titratable acidity of the samples

There was significant reduction in the cyanide level of gari produced with cocoyam in the mashes (Table 2). Gari from pure cassava had 14.22 mg/kg while those produced from 100% cocoyam had 0.24 mg/kg indicating about 98 to 14% reduction in the cyanide level. These relatively low cyanide levels could be attributed to cassava processing which involves grating, fermentation and roasting that have been reported to lowering total cyanide in fresh peeled roots (Akindahunsi *et al*., 1999). Furthermore cocoyam has low level of hydrocyanic acid contributing significantly to reducing the residual cyanide content in gari produced from cassava-cocoyam mashes (Eddy *et al*., 2012). The values obtained are lower than the recommended safe level of 20mg/kg (NIS, 2004; Oluwamukomi and Adeyemi, 2013). Since the values obtained for all the gari samples are below the safe level, the products can therefore be considered adequate and safe for human consumption as regards cyanide poisoning.

The pH value progressively and significantly increased from 3.47 (control) to 6.03 (100% cocoyam gari) and the acidity (TTA) correspondingly decreased from 0.97% (control) to 0.60% (100% cocoyam gari). The pH values of gari containing more than 20% cocoyam were above the recommended range of 3.5 – 4.5 for acid fermented products (Bainbridge *et al*., 1996) while the TTA fall within the recommended standard of 0.6 – 1.2 for cassava-gari (Oduro et al., 2000). The values of TTA recorded in all the gari samples were in agreement with Nigerian Industrial Standard (NIS, 1998) recommendation of less than 1.00% TTA for gari samples. A range of 0.77 and 1.62% TTA was reported by Franklin *et al*. (2009) for cassava gari samples. The codex standard of total acidity for gari is between 0.6 and 1.0%, expressed as percent lactic acid (Codex Alimentarius Commission, 1989). The gari samples had values within the codex standard. This further corroborates the potential of cocoyam in gari making.

Physical and Physicochemical Properties of the Gari Samples

From Table 3, the bulk density of gari samples varied between 0.54g/cm^3 for gari produced from 100% cocoyam and 0.57g/ cm^3 for control. The bulk density result of all the gari samples was desirable and fell within acceptable range of 0.50g/ cm^3 to 0.91g/ cm^3 reported for cassava gari by Adindu and Aprioku (2006). Komolafe and Arawande (2010) gave the bulk density of 0.55 g/cm^3 to 0.82 g/cm^3 for gari samples from cassava. According to Ukpabi and Ndimele (1990), good gari should have bulk density of 0.56 g/cm^3 to 0.908 g/cm^3. Udensi *et al* (2008) reported that high bulk density increases the rate of dispersion of granule in water which is important in the reconstitution of flours in hot water to produce dough. Substituting cassava mash with 10 to 50% cocoyam mash did not result in any significant ($p<0.05$) difference in the bulk density values compared with the control.

The swelling capacity of the samples ranged from 56.28% (control) to 38.78% (100% cocoyam gari). The control sample had the highest swelling capacity of 56.28%, followed by gari produced from cassava mash substituted with 10% cocoyam mash with swelling capacity value of 54.55%, while the gari produced from 100% cocoyam mash had the least swelling capacity

Table 1: Proximate composition (%) of gari produced from cassava substituted with cocoyam.

Sample description	Crude Fibre	Moisture Content	Ash Content	Crude Protein	Crude Fat	CHO by difference
100:0	3.13[a]	7.29[a]	1.13[e]	1.84[f]	1.63[e]	84.99[a]
90:10	2.32[d]	7.07[b]	1.22[d]	1.96[e]	1.52[f]	85.93[a]
80:20	2.49[d]	6.47[e]	1.64[b]	2.07[d]	1.68[d]	85.67[a]
70:30	2.32[d]	6.93[c]	1.68[a]	2.16[c]	1.92[c]	85.01[a]
50:50	2.87[b]	6.32[f]	1.52[c]	4.33[b]	2.08[b]	82.89[b]
0:100	2.57[c]	6.82[d]	1.63[b]	5.21[a]	2.45[a]	82.83[b]

Values are means of four determinations.
Means with the same superscript along the same column are not significantly different ($p<0.05$).

Table 2: Chemical composition of gari produced from cassava substituted with cocoyam

Sample description	HCN (mg/kg)	pH	TTA (%)
100:0	14.22[a]	3.47[f]	0.97[a]
90:10	12.19[b]	4.17[e]	0.80[b]
80:20	10.82[c]	4.45[d]	0.80[b]
70:30	9.15[d]	4.76[c]	0.79[b]
50:50	8.21[e]	5.01[b]	0.64[c]
0:100	0.24[f]	6.03[a]	0.60[d]

Values are means of four determinations.
Means with the same superscript along the same column are not significantly different ($p<0.05$).

Table 3: Physico-chemical and physical properties of gari produced from cassava substituted with cocoyam

Sample description	Swelling Capacity (%)	Water Absorption Capacity (%)	Rec. Index	Syneresis (%)	Bulk density (g/cm^3)
100:0	56.28[a]	54.63[a]	77.00[a]	42.75[a]	0.57[a]
90:10	54.55[a]	50.13[b]	74.75[ab]	42.00[a]	0.56[ab]
80:20	50.46[b]	49.00[b]	69.75[ab]	38.50[b]	0.55[ab]
70:30	47.35[bc]	48.13[bc]	65.50[cd]	34.75[cd]	0.55[ab]
50:50	45.30[c]	46.00[c]	60.50[cd]	33.25[d]	0.55[ab]
0:100	38.78[d]	38.38[d]	55.75[d]	37.25[bc]	0.54[bc]

Values are means of four determinations.
Means with the same superscript along the same column are not significantly different ($p<0.05$).

ISSN: 2226-5899

value of 38.78%. Substituting cassava mash with 10% cocoyam mash did not result in any significant ($p<0.05$) difference in the swelling capacity from the gari produced from 100% cassava. A progressive decrease in the swelling capacity was observed with increase in the substitution level of cocoyam in the mixture. Franklin *et al.* (2009) gave swelling capacity values ranging from 34 to 39% for gari samples produced from four cassava varieties. The observed reduction in the swelling capacity with increasing substitution corroborates the findings of Oluwamukomi and Adeyemi (2013) who also reported a reduction in the swelling capacity of gari with enrichment. According to Udensi *et al* (2008), high swelling capacity was shown to give a greater volume and more feeling of satiety per unit weight of gari. Swelling capacity is one of the important quality criteria as it indicates the degree of gelatinization of the gari. The result suggests that substituting cassava with 10% cocoyam resulted in similar swelling capacity with gari produced from 100% cassava and the gari samples had swelling capacities within the range reported in the literature (Franklin *et al*., 2009).

A similar trend was observed with water absorption capacity (WAC) values where a general decrease was recorded with increase in the level of cocoyam in the gari samples. The maximum value (54.63%) was for the control sample while the minimum value was recorded for 100% cocoyam gari. Water holding property is a term commonly used to describe the ability of a matrix of molecules, usually macromolecules, to entrap large amounts of water in a manner such that exudation is prevented (Chen & Lin, 2002). Water absorbed is usually reported as weight increase in relation to the original dry weight of the sample. It is known to be related to the degree of dryness and porosity. Moorthy *et al.* (1993) observed that a product which restrict access of water into the starch granules, can delay gelatinization. The trend observed in this work is similar to those reported for sweet potato-gari by Sanni *et al.* (2001).

Reconstitution index also follows the same pattern with water absorption capacity as the value reduced from 77.00 for control to 55.75 in 100% cocoyam gari. The lower values recorded in gari samples containing varying levels of cocoyam might not be unconnected to the relatively high fat content of cocoyam compared to cassava and the different starch properties and carbohydrate levels in the samples. This is important in the reconstitution of ‘gari’ in hot water to produce ‘eba’ dough, a commonly form of consuming gari in Nigeria. According to Almazan (1992) lipids lower the swelling power of starch granule as they act as a buffer.

The values of syneresis decreased generally with control having the highest value (42.75%) and gari containing 50% cocoyam had the lowest value (33.25%). All gari samples containing cocoyam had reduced tendency to synerese as compared with pure cassava gari. This observation suggests that substitution of cassava mash with cocoyam mash showed an enhancement of the syneresis tendency of cassava gari. This agrees with work of Chinma *et al.* (2011) who reported reduced syneresis for soy protein-cassava starch. High syneresis tendency has been attributed to variation in setting time, nature and the ratio of the amylase to amylopectin of each starch (Miles *et al*., 1985).

Pasting Properties of the Gari Samples

The results in (Table 4) show the pasting properties of the gari samples. Peak viscosity reflects the ability of starch to swell freely before their physical breakdown (Sanni *et al*., 2004). The peak viscosity, which is the maximum viscosity attained during or soon after the heating portion, ranged from 2054.75 RVU to 2620.75 RVU. The peak viscosity increased generally as the substitution level increased. Gari produced from cassava mash substituted with 30% cocoyam gave the highest value (2620.75RVU) and gari produced from 100% cassava mash had the lowest peak viscosity value (2054.75RVU) indicating that all the samples had higher peak viscosity values than the control sample. It was however surprising that gari containing 50% cocoyam had similar peak viscosity with that of gari from pure cassava. This observation was similar to those reported for five varieties of *Dioscorea* spp yams starches where high peak viscosity was attributed to large granule size of starch (Perez *et al* ., 2013). This is however in contrast with earlier work of Afoakwa *et al* (2010) who observed general decrease in the peak viscosity of the soy-cassava gari with increasing level of fermentation. The increasing level might be a reflection of high starch content (Odedeji and Adeleke, 2010). It has been noted that granules with high peak viscosity have weaker cohesive forces within the granules than those with lower values and would disintegrate more easily (Hoover, 2001).

The holding strength is sometimes called trough or hot paste viscosity. It is the minimum viscosity value in the constant temperature phase of RVA profile which is an index of starch granule stability to heating (Shittu et al, 2007). The least value (1905.25 RVU) was recorded for the control sample while the highest value (2284.25 RVU) was recorded for 100% cocoyam gari. The general increase in the holding strength value of the substituted gari sample was similar to the report of Odedeji and Adeleke (2010) who observed increase in the holding strength value of wheat flour substituted with sweet potato flour.

Breakdown viscosity which is considered a measure of the resistance to heat and shear of the gari samples varied significantly ($p<0.05$) between 149.50 RVU (control) and 566.25 RVU (30% cocoyam- cassava gari). Since breakdown viscosity is an estimation of paste resistance to disintegration in response to heat and shear, lower breakdown viscosity showed greater resistance which would be expected of starches with lower peak viscosities. Generally, the values increased for the substituted samples. The value obtained for control sample in this study is lower than the values reported for cassava gari (202.58 RVU) and values for cocoyam substituted gari are above the range reported

Table 4: Pasting properties of the gari samples

Sample description	Peak viscosity (RVU)	Holding Strength (RVU)	Break Down (RVU)	Final Viscosity (RVU)	Set Back (RVU)	Pasting Temp (°C)	Peak Time (min.)
100:0	2054.80^{c}	1905.30^{b}	149.50^{c}	4331.00^{b}	2425.75^{a}	86.78^{a}	6.98^{a}
90:10	2304.00^{bc}	2132.00^{ab}	172.00^{c}	3429.80^{c}	1297.75^{d}	80.89^{abc}	5.82^{b}
80:20	2504.50^{ab}	2091.00^{ab}	413.50^{ab}	3496.80^{c}	1405.75^{cd}	72.94^{cd}	5.20^{c}
70:30	2620.80^{a}	2054.50^{ab}	566.25^{a}	3616.80^{c}	1562.25^{c}	80.48^{abc}	5.17^{c}
50:50	2169.00^{c}	1988.80^{b}	180.25^{c}	3493.30^{c}	1504.50^{c}	81.86^{abc}	6.05^{b}
0:100	2564.00^{a}	2284.30^{a}	279.75b^{c}	4223.00^{a}	1938.75^{b}	83.94^{ab}	5.83^{b}

Values are means of four determinations.
Means with the same superscript along the same column are not significantly different ($p<0.05$).

Table 5: Sensory scores of gari produced from cassava and cocoyam

Sample	Color	Texture	Taste	Aroma	Appearance	Overall acceptability
100:0	5.34^{a}	4.88^{bc}	5.40^{a}	5.00^{ab}	5.12^{ab}	5.92^{a}
90:10	4.60^{b}	4.60^{bcd}	4.64^{cd}	4.96^{ab}	4.84^{bc}	5.14^{b}
80:20	4.82^{ab}	4.42^{cd}	4.78^{b}	4.18^{cd}	4.82^{bc}	5.12^{b}
70:30	4.64^{b}	4.69^{bcd}	4.14^{cde}	4.80^{bc}	4.94^{bc}	4.96^{b}
50:50	3.20^{c}	3.68^{e}	4.06^{de}	3.92^{d}	3.76^{de}	4.16^{c}
0:100	4.76^{ab}	5.14^{a}	3.62^{e}	5.38^{a}	5.46^{a}	5.50^{ab}

Means with the same subscript along the same column are not significantly different ($p<0.05$).

Plate 1: Photograph of Gari samples from cassava and cocoyam

ISSN: 2226-5899

for enriched gari samples with soybean and melon (136.17-178.42 RVU) by Oluwamukomi and Jolayemi (2012). The control sample is more stable compared to substituted gari.

Final viscosity has been identified as the most commonly used parameter to characterize the ability of starch-based materials to form a viscous gel after cooking and cooling as well the resistance of the paste to shear force during stirring (Adebowale *et al.,* 2005; Maziya-Dixon *et al.,* 2007). This ranged between 3429.75 RVA (gari with 10% cocoyam) and 4331.00 RVU (control). These values are higher than the values reported for pure cassava gari and enriched gari in another study (Oluwamukomi and Jolayemi, 2012). This might be due to varietal and compositional differences.

The highest value for setback was 2425.75 RVU (control) and decreased generally with substitution, while the least value was 1297.75 RVU (gari with 10% cocoyam). This observation shows that setback viscosity which determines the tendency of starch to retrograde was highest in gari with pure cassava indicating that its gel would likely be stiffer than those produced from gari containing cocoyam (Falade and Okafor, 2013). This finding correlates with the earlier observation that gel from 100% cassava gari has greater tendency to synerese than those containing cocoyam. The obtained setback values are higher than those reported for gari from pure cassava by other researchers which might be attributed to the characteristics of the raw materials and processing methods (Oluwamukomi and Jolayemi, 2012).

The substituted gari samples formed pastes at lower temperatures (72.94-83.94 ^{o}C) and took shorter times (5.12-6.05 min.) compared to pasting temperature of 100% cassava gari (86.78^{o}C) with the peak time of 6.98 min. The pasting temperature of the substituted gari is similar to the value reported for the enriched gari (79.20-80.05^{o}C), however, with higher peak time (Oluwamukomi and Jolayemi, 2012). This indicates that the substituted gari formed paste in the same range with the control sample but required more heating to form paste.

Sensory attributes of gari produced from cassava, sweet potato and cocoyam

The mean sensory scores of gari samples are summarised in Table 5. The result showed that gari produced from 100% cassava was the most preferred in terms of colour, taste and appearance which is similar to the report of Sanni *et al*. (2007) in which gari from pure cassava had the best sensory ratings than those produced from sweet potato. However, pure cocoyam gari was best preferred in terms of texture and aroma. The panelists rated the colour of the control sample better than the rest, followed by the gari from cassava mash substituted with 20% cocoyam, while the gari produced from cassava mash substituted with 50% cocoyam was least rated. There were no significant differences ($p<0.05$) in the colour ratings for control sample, gari containing 20% cocoyam and those produced from 100% cocoyam indicating the feasibility of incorporating cocoyam in gari making. The texture of gari produced from 100% cocoyam had the best rating, while the gari from cassava mash substituted with 50% cocoyam was least preferred by the panelists. Statistical analysis of the data showed that all the gari samples containing cocoyam at varying proportions except the one with 50% cocoyam had texture attribute similar to the pure cassava gari. In terms of taste, control sample had the best rating while gari produced from 100% cocoyam was least preferred by the panelists. The panelists preferred the aroma of gari produced from 100% cocoyam, while the gari from cassava mash substituted with 50% cocoyam was least preferred. The panelists preferred the appearance of gari produced from 100% cocoyam to the rest, followed by the control sample, while the gari produced from cassava mash substituted with 50% cocoyam had the lowest score. Control sample was best preferred in terms of overall acceptability, followed by the gari produced from 100% cocoyam, while the gari from cassava mash substituted with 50% cocoyam had the least rating. Statistical analysis of the data indicated that the gari produced from 100% cocoyam had overall acceptability attribute similar to that of the control sample which was best rated by the panelists.

CONCLUSION

Substitution of cassava with varying proportions of cocoyam resulted in gari products with good and in some cases better quality parameters than gari produced from pure cassava indicating the potential of this underutilized tuber in gari processing. Gari produced from 100% cassava consistently had the best ratings in terms of colour, taste and appearance while pure cocoyam gari had the highest scores in texture and aroma. There was no significant difference in the overall acceptability of the control sample and gari from 100% cocoyam. Further studies are underway to establish the best cocoyam variety and produce enhanced sensory quality gari product.

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PAK. J. FOOD SCI., 23(3), 2013: 133-138

ISSN: 2226-5899

Assessment of beef meat microbial contamination during skinning, dressing, transportation and marketing at a commercial abattoir in Kigali city, Rwanda

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ABSTRACT

The study was conducted to assess the bacteriological contamination of beef meat in a commercial abattoir at slaughtering stages (skinning and dressing), during transportation from the abattoir to butcheries and during marketing in Kigali City. Twenty four samples were collected (6 samples at each stage) and the total bacterial, total coliforms, *Escherichia coli* and *Staphylococcus aureus* counts enumerated using conventional microbial plate culture methods. The results showed the contamination of carcasses by all tested bacterial groups except *S.aureus*. The level of microbial contamination increased progressively after the slaughtering of cattle to the marketing of carcasses. The contamination by total aerobic bacteria increased from 5.1 to 10.9 log CFU/g. While contamination by total coliforms increased from 3.1 to 4.7 log CFU/g and the contamination by *E. coli* increased from 0.8 to 3.0 log CFU/g. *S.aureus* was not detectedat all the four considered stages. Compared to the European Microbiological Standards for meat, the observed levels of beef carcasses contamination, from the skinning stage to the marketing level, were found to be out of the acceptable range. This could be due to contamination at slaughtering, transportation and marketing stages. In addition, handling meat at ambient temperatures could have lead to increased microbial load during transportation and marketing. Therefore, there is need to improve on hygiene during slaughtering, marketing and transportation in Kigali City.

Keywords: Beef meat, microbiological contamination, slaughtering, transportation, marketing

INTRODUCTION

The beef meat contains 70-73% of water, 20-22% of protein and 4.8% of lipids (Alan *et al.*, 1995). This chemical composition exposes beef meat to the contamination by spoilage and pathogenic bacteria when adequate hygienic measures during the preparation, transport and marketing are not respected (Hudson *et al.*, 1996). In most developing countries, the absence or non respect of the existing hygienic practices in slaughtering, transportation and marketing has been found to be one of the major causes of meat contamination by pathogenic and non pathogenic microorganisms (FAO, 2004). Adzitey *et al.* (2011) reported that in Ghana a number of abattoirs and meat processing units do not meet sanitary standards and operated without adequate quality control systems. In many developing countries, meat is normally transported to markets either in vans, motocycles and at times even using bicycles. In most instances the transportation systems are made of surfaces that are difficult to clean and disinfect. Furthermore, meat are sold in the open markets on tables that are not well cleaned and disinfected. Thus exposing meat to a number of microorganisms which may be pathogenic or non pathogenic.

In Rwanda there is no scientific data available addressing the microbiological quality of meat during the slaughtering and transportation. However a study conducted by Hirwa (2010) showed that beef sold in selected markets of the District of Nyarugenge (Kigali, Rwanda) was out of European Union microbiological standards. All analyzed beef samples were out of acceptable limits for total aerobic bacteria and only 26.6 % and 10 % of the analyzed samples were within the acceptable range respectively for *Escherichia coli* and thermotolerant coliforms. Norman *et al.* (2006) indicated that the contamination of meat at the end consumers level, correspond to the combination of contaminations at different stages of meat preparation including the slaughtering, transportation and marketing. During the slaughtering process the stages of skinning and the dressing were identified to be the critical points for carcasses microbiological contamination (Gill *et al*., 2003). In order to quantify the contribution of different processing stages on the final contamination of meat, the present study was carried out to assess microbiological contamination of beef carcasses at different stages of meat preparation namely; skinning and dressing in slaughterhouse, during the transportation and at carcasses marketing stage. Four microbiological parameters were considered in this study: Total Aerobic Bacteria, Total coliforms, *Escherichia coli* and *Staphylococcus aureus*.

MATERIAL AND METHODS

Description of slaughtering, meat transportation and marketing in Kigali city

The slaughtering, transportation and marketing process were scrutinized in order to identify key stages at which microbiological contamination was likely to occur.

Slaughtering; slaughtering of cattle was carried out as described in the Table 1. Stunning was done by using a captive-bolt pistol (non-penetrating type) which was directly applied to the forehead of the animal. Immediately following stunning the animals were hoisted by one leg and bleed by cutting the major blood vessels of the neck. Carcasses were skinned by using combined horizontal and vertical methods (FAO, 1991). The thoracic viscera were removed from the carcass after sawing the sternum and cutting the trachea.

The stomachs and intestines were removed by cutting respectively between the esophagus and the stomach and the caecum and rectum. No duplicate ties were made at the esophagus – stomach and coecum –rectum junctions. The carcass dressing was done manually by using knives. The facilities for carcass refrigeration were present but during the period of the study beef carcasses were not refrigerated. Concerning sanitation and hygiene, it was noted that in the slaughterhouse there were hand-washing stations with running water but hand washing detergents and disinfectants was not present at all stations. There were no hand-washing stations with running water in the butcheries. The slaughterhouse had knife sterilizing stations but were not functioning at the period of the study. The slaughterhouse had fly screens to protect contamination of meat by flies. In the slaughterhouse the staff was provided with aprons but no head cover.

Table 1: Beef carcasses preparation stages at the Kigali commercial abattoir

Serial number	Processing stages
1.	Stunning
2.	Bleeding
3.	Deheading and Legging
3.	Skinning*
4.	Evisceration
5.	Splitting
6.	Dressing*
7.	Refrigeration
8.	Transportation*
9.	Marketing*

*Stages at which sampling was done

Meat transportation; Carcasses were directly transported to the butcheries by simply heaping once carcass one on another and covering with a plastic sheet. The carcasses were transported in non refrigerated vehicles.

Marketing; In the butcheries, carcasses were suspended on hooks and meat pieces were exposed on tables. The butcheries had fly screens to prevent contamination of the meat by flying insects. In the butcheries, staffs were provided with both aprons and head covers.

Sample collection

A total of twenty four beef round steak samples from twenty four carcasses were randomly collected. Twelve samples were obtained during slaughtering (six samples at the stage of skinning and – six samples at the dressing stage), six samples were collected during transportation and finally six samples from three butcheries located nearby the slaughterhouse (about 500 m from the slaughterhouse). Samples were aseptically collected in sterile polythene bags, sealed and transported in an ice box to prevent microbial growth during sample transportation. The samples were analyzed immediately upon arrival in the laboratory.

Enumeration of bacteria

Sample preparation and serial dilution; a portion of beef (10 g) was minced in 90 ml of peptone water (Biolab – Merck, Wadesville, Hungary). The composition of the peptone water was as follows (g/l); Peptone 10.0, Sodium Chloride 5.0, di-Sodium Hydrogen Phosphate:3.5, Potassium di-Hydrogen Phosphate: 1.5 with the pH of 7.0. Ten-fold serial dilutions of the homogenized meat samples were performed using peptone water as diluent. One hundred micro liters of each dilution was inoculated into Petri dishes.

Aerobic Plate Count

Nutrient agar (Pronadisa, Madrid, Spain) was used for enumeration of total aerobic bacteria in the meat samples. The composition of the nutrient agar was as follows (g/l); Gelatin Peptone: 5.0, Beef Extracts: 3.0, Bacteriological Agar: 15.0 with the pH of 6.8. After sterilization at 121°C for 15 mins, the media was cooled down. About 30 ml of the cooled media was poured into sterile petri dishes and immediately 100 µl was inoculated. The content in the petri dish was gently swirled clockwise and anticlockwise to thoroughly mix the media with the sample. The plates were inverted and then incubated at 30ºC for 48 hours.

Staphylococcus aureus

Baird-Parker agar (Pronadisa, Madrid, Spain) with the following composition (g/l); Glycine: 12.0, Casein Pancreatic Digests: 10.0, Sodium Pyruvate:10.0, Beef Extracts:5.0, Lithium Chloride: 5.0, Yeast Extracts:1.0, Bacteriological Agar: 20.0 with the pH of 6.8; was used for enumeration of *S. aureus*. An amount of 0.1 ml of each dilution of the sample was inoculated to the surface of Baird-Parker agar plates using the spread plate technique. The inoculum was evenly spread on the surface of the agar and allowed to dry for 15 min at room temperature. The plates were inverted and incubated for 24 ± 3h and re-incubated to a total of 48 ±4 h at 37ºC. Typical colonies of *S aureus* were black

or grey, shinning, convex and surrounded by a clear zone.

Total coliforms and *Escherichia coli* count

Mackonkey Agar (Biolab – Merck, Wadesville, Hungary) with the following composition (g/l); Peptone: 20.5; Bile Salts: 1.5; Lactose: 10.0, Sodium Chloride: 5.0, Neutral Red: 0.03, Cristal Violet: 0.001, Bacteriological Agar: 15.0 with the pH of 7.1; was used for enumeration of both total coliforms and *E. coli*. The method of inoculation and plating was the same as described for aerobic plate count. The plates were inverted and then incubated at 37ºC for 24 hours. The suspected colonies of *E. coli* appeared purple on Petri dishes after incubation at 37ºC for 24 hours.

Determination of microbial counts

Colonies on selected plates were counted using a colony counter (Bibby Scientific Limited, Staffordshire, UK). The morphological characteristics of each colony were examined to indicate the shape, size elevation and pigmentation to facilitate the process of grouping and identification. The determination of colony forming units (cfu) was performed by using the following formula (AFNOR,2001):

$$N = \frac{\sum c}{V(n_1 + 0{,}1\, n_2)d}$$

Where, N = total number of microorganisms present in one gram of the product; c = the sum of the colonies counted on all Petri dishes of two successive dilutions, V = the quantity inoculated on each Petri dish in milliliters; n_1 and n_2 = the number of considered Petri dishes respectively at the first and the second dilution and finaly d = the considered first dilution.

RESULTS AND DISCUSSION

The study on microbial contamination of fresh beef carcasses during the slaughtering process, transportation and marketing at a commercial abattoir of Kigali, was conducted mainly to assess the evolution of the microbial contamination of beef during its preparation process and to observe the use of hygienic practices which may reduce incidences of cross contamination in the slaughterhouse and the marketing area. The results of microbial analysis are summarized in the Table 2.

In the four considered stages (skinning, dressing, transportation and marketing) the total aerobic counts ranged from 5.1 to 10.9 log cfu/g. There were significant (p 0.05) increases in total aerobic count during dressing, transportation and marketing. In between skinning and marketing there was a 5.8 log cfu increase in total aerobic bacterial counts indicating that a lot of contamination occurs during meat slaughtering, transportation and marketing in Kigali city.

Table 2: Averages of beef meat microbial contamination at different stages in log cfu/g

Processing stages	Total plate count	Total coliforms	*Escherichia coli*
Skinning	5.1±0.9 ^{a}	3.1±0.5 ^{a}	0.8±3.5 ^{a}
Dressing	8.1±1.5 ^{a}	3.5±1.7 ^{a}	2.1±0.7 ^{a}
Transportation	8.9±1.0 ^{b}	3.9±0.5 ^{a}	2.3±0.7 ^{a}
Marketing	10.9±1.1 ^{c}	4.7±0.8 ^{a}	3.0±0.6 ^{a}

^{a,b,c} Mean values in the same column with different superscript are significantly ($p < 0.05$) different.

Total coliforms ranged from 3.1 to 4.7 log cfu/g representing 1.6 log cfu increase in between slaughtering and marketing of beef. The highest increase (0.8 log cfu) in total coliform counts occurred between transportation and marketing. The contamination by *E. coli* ranged from 0.8 to 3.0 log cfu/g. *S. aureus* was not detected at all stages. Compared to the European Microbiological standards for meat (CE, 2005), the observed levels of beef carcasses contamination, from the skinning stage to the marketing level, were found to be out of the acceptable range. The European Union recommends that the levels of contamination by total aerobic bacteria and total coliforms do not exceed respectively 5.0 and 2.5 log CFU/g. The pathogenic bacteria could be absent from meat. During our study, it was found that for all detected microorganisms except *Staphylococcus aureus*, the level of contamination was low at the skinning stage and increased progressively during the dressing, transportation and marketing stages.

The contamination of carcasses at the skinning stage could be due to the contact between the carcass and the hide. It has been reported that muscle tissue from uneviscerated carcass is sterile (Gill *et al.,* 1978). During skinning, the contact between carcass and hide allows a mixture of micro-organisms to be introduced onto the carcass (Bell, 1997). These contaminating microorganisms derive from the pre-slaughter environment and may be of faecal, soil, water or feed origin. Our results are in agreement with McEvoy *et al.* (2000) who reported that the contamination of beef carcasses in a commercial abattoir is correlated to the cleanliness of hides. Equipments like knives used in dehiding operations have been reported to be responsible for cross contaminations from one carcass to another or from personnel to carcasses especially when the facilities to sterilize knives after being used are lacking or not functioning accordingly. The contamination by coliforms could be due to the lack of hygiene. This is in agreement with Soyiri *et al.* (2008) who reported that the presence of coliforms and *E. coli* was as a result of meat contamination with faecal mater which could be from the environment, air, material used including water. The study conducted by *Elder et al.* (2000) in a cattle slaughtering facility also highlighted the clear correlation of Enterohemorragic *E.coli* (EHEC) O157 prevalence in feces, hides and carcasses. The hands of handlers could also be

implicated. Nel *et al*. (2004) reported that the lack of personal hygiene in an abattoir contributes actively to the contamination of meat especially by coliforms.

At the dressing stage the levels of contamination by all detected microorganisms was higher than the levels of contamination observed at the skinning stage. This could be due to the additional contamination of the carcass by microorganisms from the digestive tract. Our results are in agreement with Soyiri *et al.* (2008) who identified the gastrointestinal tract as a potential source of carcass contamination because of its high microbial load. This contamination was highly accentuated when the stomachs and intestines are punctured during the evisceration process (FAO, 2004; Gill *et al.*, 1996 ; Sheridan, 1998). The high level of coliforms especially *E. coli* are due to the fact that these microorganisms are also found in the animal's digestive tract (McEvoy *et al*., 2003).

A range of carcass intervention treatments have been designed to reduce the contamination of carcasses during the slaughtering process. These include; the low pressure hot water spray, high pressure water spry, steam pasteurization, acetic acid spray, irradiation, amongst others (Chen *et al.*, 2012).These carcass treatments were found to be effective in the reduction of spoilage and pathogenic microorganisms on carcasses in the slaughterhouse (Algino *et al.,* 2007; EFSA, 2011; Spoto *et al*., 2000) but their utilization in most of developing countries is still limited (FAO, 2004). In the studied slaughterhouse there was no antimicrobial treatment applied to carcasses and this could explain the high levels of carcass contamination observed at the transportation and marketing stages.

The levels of carcass contamination observed at the transportation stage were higher compared to the ones at the skinning and dressing stages. The additional contamination could have been due partially to the use of non refrigerated vehicles for transportation of beef carcasses which facilitate the multiplication of pathogenic and spoilage bacteria. Our results are in agreement with Nynchas *et al.* (2008) and the FAO – Good Practices for Meat Industry (2007) who reported that the warm temperatures during transportation encourage the growth of initial microflora and microorganisms got during different slaughtering steps. During the transportation from the abattoir to the butcheries, carcasses were simply heaped one upon another and covered with a plastic sheet. This unhygienic transportation mode encourages cross contamination and could have actively contributed to the observed contamination. The observed high levels of contamination by coliforms and *E. coli* could have been due to the growth of the existing microorganisms encouraged by the warm temperatures and the cross contamination from the transportation vehicle to carcasses or from a carcass to another.

In our study, the highest levels of carcass contamination were observed at the marketing stage. The additional contamination could have been due partially to the contamination of carcasses and meat cuts by undisinfected tables and the handling of meat with unsterilized instruments such as knives. This could be the consequence of the lack of knife-sterilizing equipments observed in the visited butcheries. Our results are in agreement with Adzitey *et al.* (2011) who identified the use of unsterilized instruments as the major source of meat contamination in Ghanaian markets. The hands of butchers could also be considered as contributors to the observed contamination. In the visited butcheries there were no hand-washing stations with running water and this could encourage the transfer of microorganisms from handlers to carcasses or from a carcass to another. Simiarily a study conducted by Nel *et al.* (2004) in South Africa showed that the lack of personal hygiene was the major factor contributing to the contamination of meat especially by coliforms. The contamination by coliforms and *E. coli* could also have been due to the growth of microorganisms that had contaminated the carcasses during the previous processing stages given that there was no decontamination interventions applied to carcasses at the slaughterhouse where the study was conducted. This is supported by the study conducted by McEvoy *et al.* (2003) at a commercial Irish abattoir that showed that the faecal contamination of carcasses by *E. coli* O157:H7 from hides and rumen occurring during hide removal and bung tying, remains during the subsequent washing, chilling and boning operations.

The contamination of carcasses increased progressively during the cattle slaughtering process, transportation and marketing of beef carcasses and meat cuts. The initial contaminating microorganisms both pathogenic and non pathogenic could have originated from the hide and the gastrointestinal tract of slaughtered animals. The high levels of carcass contamination by spoilage microorganisms deteriorate the quality of meat and decrease its shelf life (Soyiri *et al.,* 2008), hence the negative economic effect to meat processors. Also beef has been implicated for a number of meat borne infections and intoxications in several countries (Adzitey *et al*., 2011; Chen *et al*., 2012). Even if the mastery of some sources of meat contamination requires sizeable financial means, the rigid application of good hygienic practices during the slaughtering, transportation and marketing of beef carcasses can considerably reduce the contamination of meat in developing countries (FAO, 2004).

CONCLUSION

The study conducted showed that the contamination of beef carcasses increased progressively from the skinning stage in the slaughterhouse to the marketing area. The general sanitary conditions in butcheries in addition to the poor hygienic practices by meat handlers during the slaughtering, transportation and marketing are probable contributors to the microbial contamination on the beef. It is therefore highly recommended to the slaughterhouse and meat sellers to

rigidly enforce standard hygienic practices in the slaughtering, transport and marketing of meat carcasses to assure the quality and the safety of meat. The study looked at only four bacteria and did not address the identification of isolated microorganisms. There is a need for further studies to look at the evolution of the beef carcasses contamination by other pathogenic microorganisms and carrying out their advanced identification.

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PAK. J. FOOD SCI., 23(3), 2013: 139-143
ISSN: 2226-5899

Probiotics: Health Claims, Potential and Realities

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ABSTRACT

Health endorsing micro-organisms (probiotic) have been increasingly incorporated in numerous types of food products, particularly in fermented milks. Most of the probiotics belong to the group of lactic acid producing bacteria. Probiotics may reduce the threat of general childhood diseases such as strep throat, ear infections, Inhibits pathogenic invasion, promotes detoxification of the body, counters allergy, assists in the absorption of vitamins and minerals especially calcium enhancing the immune system, reducing lactose intolerance and risk of certain cancers. Current scientific exploration has reinforced the imperative role of probiotics as a portion of a suitable diet for human. As probiotic are providing a safe, cost operative, and 'natural' approach that enhances a barrier against many microbial infection. Probiotic comprising products have been tremendously successful in Asia, Europe, and other regions of the world. Factors associated to the technological and sensory features of probiotic food production are of extreme significance since only by nourishing the anxieties of the consumer can the food industry adornment in endorsing the consumption of functional probiotic foodstuffs in the future. This review aims to present a current version regarding probiotics role as health promoting agent.

Key words: Probiotics, prebiotics, fermentations, milk proteins

INTRODUCTION

"Probiotic are the live bacteria which when taken in ample quantity give a health advantage on the host" (FAO/WHO, 2009). Another definition of probiotics "these are ingredients mostly fermented and selected that permit precise changes, both in the properties and action in the gastrointestinal tract (GIT). Micro biota that gives advantages upon host welfare and health" (Wang, 2009).

Probiotics are being used in dairy products, mainly in milk powders, ice cream, yoghurts, and frozen dairy desserts. These dairy products have the low viability of probiotic in gastrointestinal tract (GIT) and in food environments. Due to the increasing importance of the dairy products scientists are focusing on increasing the value of certain dairy foods. Products are being produced with beneficial probiotic bacteria, particularly yoghurt. Therefore, probiotic are becoming the integral part of human diet.

The main function of probiotic is balancing the intestinal system and increasing antibiotics against intestinal pathogens. Probiotics are also adding the health benefits like barrier against the colon cancer, improvement in the immune function, improvement in lactose tolerance, decrease the chances of intestinal infections. Now a day's probiotics are being added in the fermented products with respective to prebiotics (Shah, 2007).

Owing to the factors that are increasing the importance of the probiotic have compelled food industry to take keen interest in these beneficial and health promoting bacteria. One of the most important fermented products that contains ample amount of the probiotics is yoghurt. (Reid *et al.,* 2003).

Currently, the importance of the functional foods is increasing. Functional foods are being considered as a significant segment of the growing food marketplace. Now the consumers are considering the health benefits of the food, not focusing only on taste and nutritional values. In this regard nutritional science is updating the consumer with latest knowledge about the food products. The target of the functional foods is to improve the balance and activity of the intestinal milieu (Saarela *et al.,* 2002).

Functional foods are not only providing the health benefits also providing the economic opportunities. These foods have a worldwide attractive market. With the increasing importance of the yoghurt particularly probiotic yoghurt is facing the challenge of probiotics survival. Survival of the probiotic in the yoghurt is lower comparatively other food products. Recommended level for getting the health benefits from the probiotic is 10^6-10^7cfu per gram of the product (Boylston *et al*., 2004)

.The best and extensive way to amplify the numbers of probiotic in the intestinal tract is by consuming the

products that contain the live probiotic. But the stability and viability of the probiotic has become a marketing and technological challenge. Products with good ability to retain the probiotic fulfill the requirements of beneficial effect upon the consumer's health.

The effectiveness of supplementary probiotic bacteria depends on dosage level, viability during the storage and their availability in the gut. Therefore, viability of probiotic bacteria is one of the supreme significance in the dairy products. The reports regarding the survival and viability of the probiotic indicated that the viability of probiotic bacteria is frequently low in yoghurt (Lourens Hattingh and Viljoen, 2001) that results in less than 10^8 –10^9 cells, which is daily recommended intake. Lactic acid bacteria (probiotic bacteria) like Lactobacillus acidophilus and Bifidobacteria have a lot of health benefits good and large population in colon provide excellent intestinal health .On the other hand if there is low population of the probiotics in the colon, it encourages the growth of pathogens.

2.1 Probiotics

These are considered as feed and food supplements that affect the host's health. It is important to know about the particular strains of the bacteria to extract the health benefits (Pineiro and Stanton, 2007). Probiotics include the large number of microorganisms including the bacteria and yeast. As they can stay alive until the intestine and present beneficial effects on the host health, lactic acid bacteria (LAB), non-lactic acid bacteria and yeasts can be considered as probiotics.

The most important sector of the probiotic is Lactic acid bacteria (LAB). These bacteria have very beneficial effect on the human health. These bacteria are considered as most useful for the human gastrointestinal tract (GIT). There characteristics include the following; they are mostly Gram-positive, they prefer to survive as anaerobic. Some studies have shown that they also survive in the aerobic environment and usually survive in a non-aerobic (no oxygen is available) environment but some of these bacteria can also survive under the aerobic conditions. Bifidobacteria that is very important from health point of view are also Gram-positive and they have the ability to reproduce at a pH range of 4.5–8.5. But, the most significant characteristic is that Bifidobacteria are strictly anaerobic (Anal and Singh, 2007; Holzapfel *et al*., 2001).

Some non lactic acid bacteria (e.g. *Escherichia coli*) and some yeast (e.g. *Saccharomyces cerevisiae*) are also taken as probiotics. It has been stated that dead bacteria are also a source of the probiotic bacteria. Their presence could give some health benefits. But they are not alive when controlled they cannot be considered as probiotics.Different strain of probiotic gives different benefits. That is why it is very important to know about the strains of the probiotic for getting the health benefits. Each species of probiotic bacteria is useful for giving the health benefits. The bacteria my provide health advantages due to the production of acid and/or bacteriocins (Sanders, 1998). The literature has shown that 10^6–10^7 CFU/g of product per day is generally recommended (Krasaekoopt *et al.*, 2003). Generally, probiotics are orally taken and are available in different forms such as food products, capsules, and tablets. Probiotics and dairy products provide the essential nutrients. These nutrients are very helpful for maintaining the health. (e.g. calcium, proteins) and the addition of probiotics to these products is a natural way to boost their functionality There are large no of proof that probiotics have the ability to be useful for our health (Weichselbaum, 2009).

Probiotics that we take orally, they have to face very harsh conditions. When they pass through the GIT they face the unfavorable conditions for their survival. Mostly the bacteria that are found in the GIT are of not harmful nature they are mostly useful for the human health. These bacteria that provide large number of health benefits must be included in the diet (Corthésy *et al.*, 2007). Useful probiotic bacteria are specific to enhance the immune system. They also help in reducing the cholesterol level of the humans .These have also anti cancer affects (Sanders *et al*., 2007; Kailasapathy and Chin, 2000).

The probiotics effect has been classified in to three categories. The first is that they attributed for the defense of the host. This is most important to avoid from the different diseases (Collado *et al.,* 2009). The second system can be explained by their direct effect on the other bacteria like useful and pathogenic bacteria. Probiotics have the capability to be competitive with pathogens and therefore allow for preventing their linkage to the intestine (Tuomola *et al*., 1999).

Probiotics have the ability to distress some microbial products like toxins and host products such as bile salts and some food ingredients but, it is imperative to know that mentioned mechanisms of action are strain dependent, and to date the modes of act of probioti bacteria (Good bacteria) are not up till now entirely known (Oelschlaeger, 2010). Severe diarrhea is a health trouble which is well studied, mostly in children, and studies have discovered that selected probiotic strains seem to be effective in reducing the

duration of acute diarrhea (Lomax and Calder, 2009).

A combination of the probiotic and prebiotics commonly called as symbiosis has a positive effect. Usually, products presented in the market that definitely affect the intestinal microflora are mostly probiotics and prebiotics. From this we can say that Prebiotics are 'non-digestible food constituents that, when taken in acceptable amounts, selectively boost the growth and action of one or a limited number of microbes in the colon that is resulting in health assistances (Ouwehand *et al.,* 2007).

When taking into consideration probiotics, viability and dose level are important parameters for their effectiveness. It has been observed that prebiotics have the ability to improve probiotics viability and its survival. These improves the survival not in the in environmental conditions they also helpful in the GIT and its additional attachment and growth in the intestine (Ramchandran and Shah, 2010).

Probiotic bacteria are normally used as the useful ingredient in functional foods. Example include such as bioyoghurt. These are also related to other health related products. The health benefits of probiotics bacteria are classified as either nutritional benefits or therapeutic benefits. Some of the nutritional benefits include: their part in enhancing the bioavailability of calcium, zinc, iron, manganese, copper and phosphorus. These are also helpful in enhancing the digestibility of protein in yoghurt. These are also helpful in the synthesis of vitamins in yoghurt. The therapeutic benefits of probiotics include: resistant against the allergy. Enhancing the immune system, lowering the cholesterol and as anti cancer.

Many probiotic bacteria belong to one of the two genera: Lactobacillus and Bifidobacteria. In order to extract positive health benefits the identification of the strains is very important. There are twelve important criteria for the selection of the strains working as probiotic. The strains selected through these criteria must be safe and health promoting, viable and should be active. It should be active in the gastrointestinal tract in order to exert a beneficial impact on the host. Once a strain has been selected its must be evaluated its effect should be known to get the health benefits. This is very important aspect for the health conscious people (Havenaar *et al.*, 1992).

Beneficial bacteria are that are useful in promoting the health fall in the category of the probiotic bacteria. Dairy products are the excellent source of the probiotic bacteria. Yoghurt and butter milk are the good source for the Probiotic bacteria (Australian Dairy Corporation, 2000).

2.2 How probiotics work?

It is believed that the Probiotics improve health by keeping the balance of intestinal microflora (Shah, 2000). There are many means by which this is attained, including the following:

Repression of harmful bacteria and viruses

- Motivation of local and systemic immunity
- By modifying gut the microbial activities of the microflora
- Through antimicrobial activities

2.3 Probiotic Characteristics

- For benefits to humans, probiotics need certain properties including:
- They should be of human source
- They must be resistant to and viable, when they come into contact with acid bile, intestinal enzymes and oxygen etc.
- They should be stable in the food before their consumption
- They should be stick to the host's intestinal mucosa
- They should Colonies the host's intestinal tract
- They should change and enhance the immune responses
- Be involved in vitamin, lactase and other useful substances production
- They should show the antimicrobial activities
- Probiotic bacteria should be nontoxic and non-pathogenic
- Probiotic bacteria should remain viable in all type of commercial products (Skaaning, 2000).

2.4 Probiotic Bacteria in Dairy Foods

Probiotic bacteria may be built-in into foods. They may also available in dietary supplement form. Most of the probiotic are present in the dairy foods naturally. These probiotic are also added into fermented milk, dairy deserts, ice cream and yoghurt (Sanders, 1998).

2.5 Why Probiotic Viability is Important

As we know that the probiotic bacteria carrying great health benefits that s why there is dire need of their survival. There viability is important until they reached the destination of action (Kailsasapathy and Chin, 2000). Some evidence shows that that non-viable probiotics also have some health benefits. Bacterial components that related to non-viable bacteria are also of great importance. On the other

hand, no association has been made between the effectiveness of viable cells against non-viable probiotic bacteria (Ouwehand et al., 1999).

2.6 Reasons for Low Viability

There are many factors which are responsible for the low viability of the probiotic. Many probiotic show low viability in the dairy products due to different factors, some of the factors responsible for this are enlisted below.

- pH
- Acidity
- Hydrogen peroxide production during fermentation
- Dissolved oxygen content
- Storage temperature
- Species and strains
- Concentration of lactic acid, acetic acids and buffers (e.g. Whey protein concentrates). These factors must be considered during the manufacturing of the probiotic yoghurt (Shah, 2000).

Conclusion

Diet is a main focus of public health policy intended at protecting optimum health throughout life, avoiding early inception of chronic diseases such as osteoporosis, gastrointestinal disorders, cardiovascular disease, cancer, as well as encouraging healthier ageing. Health paybacks of probiotics can merely be possible if the survival of probiotic is optimum in food and human GIT. The food industry has a crucial role in supporting healthier consumption practices through the establishment and advancement of health promoting foods.

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PAK. J. FOOD SCI., 23(3), 2013: 144-147
ISSN: 2226-5899

Nutritional value and antioxidant activity of Fenugreek (*Trigonella foenum-graecum*) from two regions of Pakistan

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ABSTRACT

Plants and plants products have been claimed to have health-promoting effects, which may be related to the antioxidant activity in *vivo*. In the present study the antioxidant potency of fenugreek (*Trigonella foenum-graecum)* extracts from two regions (Kasur and Lahore) of Pakistan were investigated. Total antioxidant activity was determined by DPPH assay. The aqueous methanol extracts of both varieties exhibit potent DPPH scavenging activities towards radical cations. Whereas, the water extracts exhibit a low scavenging activity. In addition, the nutritional values were estimated in these two varieties which showed variant concentration/ proportions of biochemical and other contents.

Key words: Antioxidant activity, DPPH, fenugreek, nutritional value.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* Leguminosae) is a self-pollinating crop, which is a native plant of the Indian subcontinent and the Eastern Mediterranean region. The crop extends to central Asia and North Africa, and more recently it has been successfully grown in Central Europe, UK, and North America. India is the largest producer of fenugreek in the world where Rajistan Gujarat and Uttanchal are producing states. Rajistan produces the lion's share of India's production, accounting for over 80% of the nation's total fenugreek output (Abdul *et. al*., 2008). It belongs to the Papilionaceae section of the family Leguminosae. Fenugreek has been used traditionally to treat diabetes (Yu *et. al*., 2002), coughs, congestion, bronchitis, fever, high blood pressure, headaches/ migraines, diarrhea, anemia (Ng *et. al*., 2007), flatulence, irregular menstrual cycles, analgesic, inflammation and arthritis (Vyas *et. al*., 2008), to ease labor pains and menstruation pain, and as an appetite stimulant.

The oxidative damage caused by reactive oxygen species on lipids, proteins and nucleic acids may trigger various chronic diseases, such as coronary heart disease, atherosclerosis, cancer and ageing (Bhuvaneswari *et. al.,* 2013). Epidemiological studies have demonstrated an inverse association between intake of fruits and vegetables and mortality from age related diseases, such as coronary heart disease and cancer, which may be attributed to their antioxidant activity (Gulçin *et. al.,* 2011). On the other synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used because they are effective and cheaper than natural ones (Vinay *et. al.,* 2010). However, the safety and toxicity of synthetic antioxidants have raised important concerns. Hence, considerable interest has been given to the use of natural antioxidants which may also have nutritional properties (Merinal and Viji, 2012). Now a days search for natural antioxidant source is gaining much importance. Thus, it is important to identify new sources of safe and inexpensive antioxidants of natural origin. Hence, in the present investigation the nutritional values and in-*vitro* antioxidant activity of Fenugreek from two regions of Pakistan were evaluated by DPPH method.

MATERIALS AND METHODS

Materials

The plant materials were collected from two regions of Pakistan, one from district Kasur and other from district Lahore. The fresh plant material was stored in plastic bags and kept frozen until extraction.

Chemicals

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and BHT were purchased from Sigma Chemical Co (St Louis, MO, USA). Hexane, sulphuric acid (H_2SO_4), Hydrochloric acid (HCL), Digestion tablets, Boric acid (H_3BO_3) and sodium hydroxide (NaOH) were purchased from Merck Chemical Suppliers (Darmstadt, Germany). All other chemicals used, including solvents, were of analytical grade. An Agilent 8453 UV/ Vis Spectrophotometer was used for absorbance measurements and spectra recording, using optical or quartz cuvettes of 1 cm optical path.

Preparation of extract

The frozen plant materials of both varieties were dried at 70˚C in cabinet dryer. 100 g of each varieties of dried materials were extracted with methanol, water and

methanol-water (50/50, v/v) at 60-80°C, using a sohxlet apparatus.

Nutritional analysis

The proximate analysis of each varieties were carried out for moisture content, total ash, crude fat, crude fiber, carbohydrate, crude protein and energy value (AOAC, 2012).

Antioxidant activity (DPPH radical scavenging assay)

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple-coloured methanol solution of DPPH. This spectrophotometric assay uses the stable radical 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Brand-Williams *et. al.,* 1995). Hundred microlitres of various concentrations of the extracts in methanol were added to 3 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The percentage inhibition of free radical (DPPH) was calculated as under:
Inhibition % (DPPH) = (A_{blank} -A_{sample}/ A_{blank}) x 100
where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.
The purple colored DPPH is a stable free radical, which is reduced to 2, 2-diphenyl-1-picrylhydrazine (yellow colored) by reacting with an antioxidant (Ivanisova *et. al*., 2013). The decrease in absorbance at 517 nm was being recorded in a digital Spectrophotometer.

Statistical analyses

The determinations of nutritional facts and radical-scavenging activity of *Trigonella foenum-graecum* were carried out in triplicate, and results are presented as means ± standard deviation (SD).

RESULTS AND DISCUSSION

Nutritional analysis

Fruits and vegetables are primary food sources providing essential nutrients for sustaining life. The proximate analysis of edible fruit and vegetables plays a crucial role in assessing their nutritional significance (Saeed *et. al.*, 2010). The considerable use of vegetable species by the local people in their diet motivated to carry out the present proximate and nutrient analysis. The result of nutritional analysis showed variant concentration/ proportions of biochemical and other contents. The moisture content of *Trigonella foenum-graecum* from Lahore was 7.5%, while *Trigonella foenum-graecum* from Kasur contains 8.1% moisture which was also in the range of many fenugreek varieties. Ash contents of *T. foenum-graecum* varieties from Lahore and Kasur were 3.10 and 3.90%. Fat was 6.47% in *Trigonella foenum-graecum* from Lahore which is very near to the standard fat content of fenugreek (6.4%), while Kasur's variety of fenugreek contains 6.4% fat. Fiber content of Lahori *Trigonella foenum-graecum* is 7.6%, while *T. foenum-graecum* of Kasur contains 8.5% fiber. Protein content of *Trigonella foenum-graecum* from Lahore was 22.50%, while *Trigonella foenum-graecum* from Kasur contains 25.0% fiber. *Trigonella foenum-graecum* of Kasur had maximum values of ash, protein and carbohydrates as compared to *Trigonella foenum-graecum* from Lahore (Table 1) and had slightly low amount of energy (Fig. 1).

Antioxidant activity

Antioxidant capacity DPPH radical was used as a stable free radical to determined antioxidant activity of natural compounds. The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals (Stoilova *et. al.,* 2007. Thus, the purple color of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) will reduce to , -diphenyl- -picrylhydrazine (yellow colored) (Maizura *et. al*., 2011). According to Suhaj (2006) scavenging of the stable radical (DPPH) is considered a valid and easy assay to evaluate scavenging activity of antioxidants.
Results of the activity of free radical scavenging of plants (fenugreek) (*Trigonella foenum-graecum*) extracts are presented in **Fig. 2.** Results showed that Kasuri fenugreek (methanol + water extract) contained the highest DPPH radical scavenging activity (19.01 ± 0.7%), followed by Kasuri fenugreek water extract (16.49 ± 0.6%). The DPPH radical scavenging activity Lahori fenugreek methanol + water extract and water extract were 18.36 and 14.59%, respectively at same concentration i.e. 1 mg/ ml. These results are in similar as described by Priya *et. al*., 2011. The results did not show significant difference (P > 0.05) between Kasuri and Lahori fenugreek in respect of antioxidant activity.

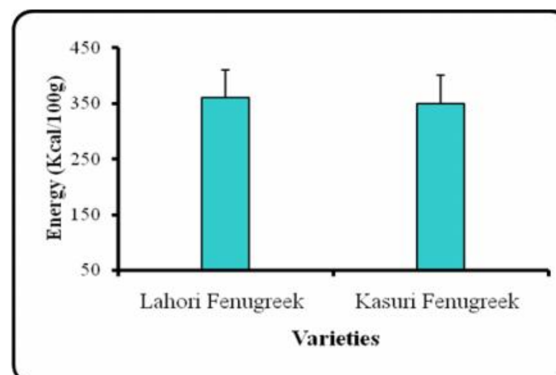

Fig. 1: Energy value (Kcal/100g) of Fenugreek.

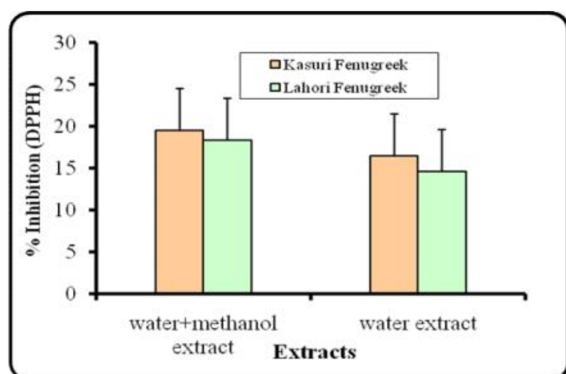

Fig. 2: Inhibition % (DPPH) of Fenugreek.

T Table 1: Nutritional values (%) of Fenugreek (*Trigonella foenum-graecum*).

Sr. No.	Constituents	Lahori Fenugreek	Kasuri Fenugreek
1	Moisture	7.50 ± 0.7	8.1 ± 0.7
2	Ash	3.10 ± 0.3	3.9 ± 0.4
3	Fat	6.47 ± 0.5	6.4 ± 0.5
4	Fiber	7.60± 0.7	8.5 ± 0.8
5	Protein	22.50 ± 1.5	25.0 ± 1.6
6	Carbohydrate	52.83 ± 2.4	48.1 ± 2.1

CONCLUSION

In this study it was found that the aqueous methanolic extracts of both varieties of fenugreek showed the highest Scavenging effect, whereas H_2O extracts of fenugreek exhibited the lowest activity. The present investigation evaluates the antioxidant activity of fenugreek in order to find potential new source of natural antioxidants.

ACKNOWLEDGMENT

The authors wish to acknowledge the Food and Biotechnology Research Center, PCSIR Laboratories Complex, Ferozepur Road Lahore, Pakistan, for providing laboratory facilities to conduct the research work.

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PAK. J. FOOD SCI., 23(3), 2013: 148-152
ISSN: 2226-5899

Nutritional evaluation and stabilization studies of wheat germ

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Abstract

Wheat germ was collected from commercial source and subjected to nutritional evaluation. Physicochemical characteristics were determined as moisture (8.50% $\pm$ 0.168), ash (3.30% $\pm$ 0.1), fat (8.75% $\pm$ 0.15), fiber (2.33% $\pm$ 0.025), protein (22.2% $\pm$ 0.3), carbohydrates (54.68% $\pm$ 0.36) and energy (388.60 ± 0.664 Kcal/100g). Brown colored oil was subjected to determine refractive index (1.47 $\pm$ 0.1 at 20°C), neutralization value (7.93 $\pm$ 0.146), sponification value (186.50 $\pm$ 0.081), ester value (178.57 $\pm$ 0.045) and peroxide value (5.00 $\pm$ 0.081). Fatty acid profile was determined by using gas chromatography. Content of Capric acid, Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Oleic acid, Linoleic acid and Linolenic acid were 0.019%, 0.022%, 0.084%, 18.580%, 0.439%, 14.030%, 57.920%, and 8.340% respectively. Wheat germ oil is rich in essential fatty acids; wheat germ oil can become a marketable product after improving its storage quality. As wheat germ has very poor keeping quality so procedures like toasting and defatting has been done for its stabilization.

Key words: nutritive value, wheat germ, characterization, stabilization, fatty acid, sensory evaluation

INTRODUCTION

Agriculture is the backbone of Pakistan economy. One-fourth of total revenue is generated from agriculture (Economic survey, Government of Pakistan 2001-2002). Wheat is grown on more land than any other food crop (F. A. O. 2001) being the staple diet of the people, wheat grain occupies a central position in agricultural policies. The approximate composition of wheat grain is 85% endosperm, 2% embryo/germ and 13% husk (Kent-Jone, and Amos 1957). To make cereals more palatable and thus more desirable as food, milling is undertaken. It generally involves removal of bran i.e., pericarp, seed coat, nucleus, epiderm, aleurone layer and germ. The germ of wheat comprises 2.5 – 3.5 % of the kernel germ which is composed of two important parts: the embryonic axis and 32 % the scutellum, which functions as a storage organ. The germ is relatively high in protein (25%), sugar (18%), oil (16% of the embryonic axis and 32% of the scutellum are oil) and ash (5%). Wheat germ is unique in being one of the most highly concentrated, natural sources of nutrients. It is of interest to note that wheat germ contains 3 times as much protein, 7 times as much fat with high percentage of unsaturated fatty acids, 15 times as much sugars, 6 times as much thiamine and 15 times as much tocopherol, as compared to wheat flour . For determination of stability, wheat germ oil obtained by ethanolysis reactants was characterized by measuring the acid value, peroxide value, free fatty acid contents, thiocyanate method, DPPH radical scavenging effect and rancimat test. The optimized condition of 40°C shows the highest oil stability among the various conditions (Sim et al. 2013). In milling process usually germ is removed although it is rich in protein, B vitamins, minerals and fats. In contrast to its nutritional potential, wheat germ has a very poor storage quality and this is the major constraint in it being separated and utilized for augmenting the supply of nutritious foods. This is due to very high activities of enzymes as well as large amount of unsaturated fatty acids present in germ. As a result it develops off-odor, bitter taste and become unfit edibly with in a few days of its separation from the grain, during which it is subjected to physical damage (Kalpana and Vali 2009). Wheat oil is more unsaturated than other cereal oils. In the United States, wheat oil is sold mainly in health food stores as a nutritional supplement (Megahed et al. 2011). Present study is undertaken for nutritional evaluation of wheat germ, its fatty acid profile by gas chromatography. This study is also carried out to stabilize wheat germ using treatment methods with least effects on the nutritional value of wheat germ.

Materials and Methods

Wheat germ was collected from commercial source. Moisture was determined in a drying oven at 100°C. Determination of ash was carried out at 550°C in muffle furnace. Fat was determined by Soxhelt extraction using hexane (B. P. 40°C). Fiber content was determined by digestion with acid and alkali using Fiber Tech System-M (Tecator) and protein (N = 6.25) value found

by micro kjeldhal method and total carbohydrates were calculated by difference.

Physiochemical Characteristics of Wheat Germ Oil

200 grams of wheat germ sample was used for extracting oil by Soxhelt apparatus using hexane (B. P. 40°C) as solvent for six hours. The solvent was evaporated by rotary evaporator to get oil. Physiochemical characteristics of wheat germ oil such as color, refractive index, neutralization value, sponification number, ester value and peroxide value were determined.

Free Fatty Acids Analysis

To prepare methyl esters of fatty acids oil extracted from wheat germ with hexane was treated with boron-trifloride methanol reagent in a test tube with Teflon lined screw cap for half an hour. The methyl ester so formed was extracted with n-hexane and solvent was removed by distillation to get pure methyl esters for gas liquid chromatography (Raie et al. 1983).

Methyl esters of fatty acids were analyzed on Schimadzu GC -14A chromatography with flame ionization detector using 1.6 m, 3 mm (i.d.) glass column packed with GP 15% OVR-275 coated on chrome R-P.AW–DMCS (100/120 mesh) column. Temperature was kept isothermal at 200 °C. Injector and detector temperature were 220 °C and 250 °C, respectively. Nitrogen was used as carrier gas with flow rate 40 ml per minute. The methyl esters were identified by comparing their retention times with those of authentic methyl fatty esters under the same conditions. The percentage of various acids was identified by comparing their retention times with those of authentic methyl fatty esters under the same conditions. The percentage of various acids was determined by Schimdzu-CR4, chromatopack commuting integrator.

Processing of Wheat Germ for Stabilization

Toasting and defatting was carried out for stabilization of wheat germ.

1. **Toasting in oven**

The raw wheat germ was toasted at 150 °C in oven by spreading on aluminum trays. The sample was accurately weighed before toasting. The thickness of wheat germ layer varied between 0.5, 1.0, 2.0 cm for toasting from 10-40 minutes each.

2. **Defatting of wheat germ**

Wheat germ was extract with hexane by using Soxhelt apparatus for 8 – 10 hours. All the fat extracted in to hexane. After the extraction process the defatted sample was spread on tray and expose in sun with periodic aeration to ensure the complete evaporation of the residual solvent.

3. **Sensory evaluation of raw and processed wheat germ sample**

The raw and processed wheat germ sample was evaluated subjectively for sensory parameters, such as colour, aroma and taste. A panel of seven judges evaluated the samples and scores were recorded according to Hedonic scale. The mean values of the scores for taste, colour and aroma of raw and processed wheat germ samples are recorded.

Results and Discussion

Proximate analysis (Table 1) for potential nutrients was performed in triplicate in accordance with AOAC (2000). Physiochemical characteristics of wheat germ were determined as moisture, ash, fat, fiber, protein and carbohydrates and the values were 8.5%, 3.3%, 8.75%, 2.33%, 22.2% and 54.68% respectively. All the experimental values reported lie with in the range (Gopalan et al. 2007).

Table 1: Proximate Analysis of Wheat Germ

Sr. No.	Parameter	Composition of Experimental value (%)
1	Moisture	8.50±0.168
2	Ash	3.30± 0.1
3	Fat	8.75±0.15
4	Protein	22.20±0.3
5	Fiber	2.34± 0.025
6	Carbohydrates	54.68±0.36
7	Energy	388.60±0.664Kcal/100g

The variation / range of moisture value may be due to the some environmental changes or due to the conditioning treatments. The variation / range of fiber content may be due to the contamination with bran which contains high fiber content than germ. Wheat oil is more unsaturated than other cereal oils. Germ is high in oil content. Wheat germ oil was obtained and subjected to study for parameters (Table 2). Color of oil was brown. Refractive index at 20 °C was 1.47. Acid value was 7.93. Sponification number, ester value and peroxide value were 186.5, 178.57 and 5 respectively.

Sr. No.	Parameter	Wheat germ oil
1	Colour	Brown
2	Refractive index (20°C)	1.47
3	Neutralization value	7.93
4	Specification value	186.50
5	Ester value	178.57
6	Peroxide value	5.00

Table 2: Characterization of wheat germ oil

The fatty acid profile (Table 3) of wheat germ oil showed the high contents of linoleic and linolenic acid i .e; 57.92 and 8.34% respectively. These two fatty acids are essential for human beings (Mycek et al. 1997). Results showed that wheat germ oil contains appreciable amount of both of these essential fatty acids. Palmitic, stearic, oleic, linoleic and linolenic acid contents were found to

be 18.580%, 0.439%, 14.030%, 57.920% and 8.340% respectively. These values were 11.76%, 3.06%, 28.14%, 52.31% and 3.55% found by Sullivan (1937). Difference in fatty acid content showed that wheat germ oil under study is richer in fatty acids due to different environmental and geographical conditions of wheat. It contains good amount of essential fatty acids and can be brought to market after treatments to avoid rapid rancidity. Palmetic acid makes more than 80.0% of the saturated fatty acids of wheat germ oil. While linoleic acid makes more than 62.0% of the unsaturated fatty acids of wheat germ oil. These results are in a good agreement with the previous results (Lancas et al. 1994, Eisenmenger and Dunford 2006 and Eisenmenger and Dunford 2008).

Table: 3 Fatty Acid composition of wheat germ oil.

Sr. No.	Fatty Acids	Percentage (%)
1	Capric acid	0.019
2	Lauric acid	0.022
3	Myristic acid	0.084
4	Palmitic acid	18.580
5	Stearic acid	0.439
6	Oleic acid	14.030
7	Linoleic acid	57.920
8	Linolenic acid	8.340

The data regarding moisture content of wheat germ toasted in oven at 150°C by varying the germ layer thickness between 0.5, 1.0, 2.0 cm and time from 10 – 40 minutes for each layer represented in Table 4. It was observed that the moisture content decreased with increasing the time for toasting from 10 – 40 minutes. The decrease in moisture content was may be due to excessive drying of wheat germ samples with increase in time of toasting. It was also observed that the moisture content decreased more in thin layers rather than thick layers. Less the moisture content more the stability of wheat germ. Finally the moisture content decreased by increasing the time of toasting and decreasing the thickness of layer. Hence, less the moisture content more the stability (Sullivan 1937).

The data regarding proximate composition of raw and defatted wheat germ sample is presented in Table 5. It was observed that defatted wheat germ contained 8.90% moisture, 4.60% ash, no fat, 29.03% protein, 2.33% fiber, 55.13% carbohydrates and 336.66 Kcal /100g energy. It was observed that the moisture content of defatted sample increased slightly, which was 8.9% while the raw wheat germ contained 8.5% moisture content. The protein and ash contents also increased after defatting. The ash was 4.6% and protein 29.03% while the raw wheat germ contained 3.3% ash and 22.2% protein content.

Table 4: Moisture (%) of Wheat Germ Samples Toasted at 150°C

Wheat germ sample	Moisture (%)	Germ Layer Thickness (cm)	Time of roasting (min)
S1	3.2	0.5	10
	2.1	0.5	25
	0.6	0.5	40
S2	3.8	1.0	10
	2.8	1.0	25
	0.7	1.0	40
S3	5.5	2.0	10
	4.0	2.0	25
	2.0	2.0	40

Table 5: Proximate composition of raw and defatted wheat germ

Sr. No.	Parameter	Raw Wheat Germ (%)	Defatted Wheat Germ (%)
1	Moisture	8.50	8.90
2	Ash	3.30	4.60
3	Fat	8.75	Nil
4	Protein	22.20	29.03
5	Fiber	2.33	2.33
6	Carbohydrates	54.68	55.13
7	Energy	388.60 Kcal/100g	336.66 Kcal/100g

As the fat extracted ultimately, the ash and protein contents increased. After defatting the wheat germ will be stable, but due to the absence of fat contents the nutritional value of wheat germ may decrease because the wheat germ fat contains vitamin E which is very essential against heart diseases and thought to protect immune system. However, fat can also be used as such as a rich source of vitamin E. Defatted wheat germ can also be used as rich source of protein, minerals and fiber.

The data regarding free fatty acid content of raw and processed wheat germ sample is presented in Table 6. In processed samples, one was defatted wheat germ sample

and other was toasted at 150°C for 10, 25 and 40 minutes with germ layer thickness 0.5 cm.

It was observed that raw wheat germ contained highest free fatty acid content that is 53.9. In toasted samples the free fatty acid content was decreased slightly as the heating time increases. This slight decrease in free fatty acid value was may be due to the evaporation of volatiles during the process of toasting. Overall there was no major change in free fatty acid content during toasting period at 150 °C. The defatted wheat germ sample contained no free fatty acid content due to the absence of fat content.

Table 6: Free fatty acid content of raw and processed wheat germ sample

Sr. No.	Wheat germ Sample	Germ Layer Thickness (cm)	Time for Toasting (min)	Free Fatty Acid Value
1	Raw	--	--	53.00
2	Defatted	--	--	Nil
3	T_1	0.5	10	50.60
4	T_2	0.5	25	48.05
5	T_3	0.5	40	47.81

Table 7: Sensory evaluations of raw and processed samples

Sr. No.	Wheat germ Sample	Color	Aroma	Taste
1	Raw	8.00	6.00	8.00
2	Defatted	6.00	5.00	6.2
3	S_1a (10min.)	7.5	6.8	6.8
4	S_1b (25min.)	6.8	7.5	7.5
5	S_1c (40min.)	3.0	3.2	3.0
6	S_2a (10min.)	7.6	6.7	6.7
7	S_2b (25min.)	4.0	8.0	7.5
8	S_2c (40min.)	3.5	3.9	4.0
9	S_3a (10min.)	7.8	6.5	6.9
10	S_3b (25min.)	7.1	7.6	7.0
11	S_3c (40min.)	4.0	4.3	4.5

Sensory evaluation of raw and processed wheat germ samples was carried out by external characteristics such as color and aroma and internal characteristics. The results are given in Table 7. It was observed that the colour of processed wheat germ samples changed in case of toasted samples more intense the colour change as toasting time increase. In case of defatted samples, the change in colour was may be due to the extraction of oil from sample because most of the colour was due to the oil content.

The S_2b samples which was toasted for 25 minutes with germ layer thickness 1.0 cm showed highest scores for aroma. S_1c sample which was toasted for 40 minutes with germ layer thickness 0.5 cm showed least score. The sample S_1b and S_2b also showed good results. The toasted sample S_1c showed the least score for taste. This was may be due to the less thickness of germ layer and highest toasting time which burnt the sample due to which the sample gave the burnt taste and showed least score. The sample S_1b and S_2b showed a valuable nutty flavor after toasting. The defatted sample showed the average taste not too good not too bad.

CONCLUSION

Results showed that wheat germ contained 8.50% moisture, 3.30% ash (mineral content), 8.70% fat, 22.20% protein, 2.33% fiber, 54.68% carbohydrates and 388.6 Kcal/ 100g energy. Low moisture content of wheat germ helps in stabilization of wheat germ for longer period. Due to less water content deterioration of fat will be decreased. Results indicated that toasting at temperature 150°C with 0.5 cm thick layer decreased moisture content but appearance and Organoleptic quality was affected. The toasting at 150°C with 2.0 cm thick layer did not significantly lower the moisture content and at longer toasting period its appearance and Organoleptic quality was affected while in toasting at 150°C with 1.0cm thick layer for 25 minutes, moisture content decreased and appearance and Organoleptic quality or sensory characteristics maintained. In defatting process the fat was separated out due to which nutritional factors such as protein and mineral contents were increased as compared to raw wheat germ. While comparing two methods i.e. toasting in oven and defatting, toasting in oven was better than defatting because the toasted sample was nutritionally dense and its sensory characteristics were also better than defatted one.

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PAK. J. FOOD SCI., 23(3), 2013: 153-162
ISSN: 2226-5899

Effect of anti-browning agents in extended the storage life of fresh-cut guava slices by delaying enzymatic activity

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ABSTRACT

This study was undertaken to extend the shelf-life of fresh-cut guava slices of Surahi variety at lower temperature by delaying enzymatic activity. Freshly harvested guavas were subjected to wash with sterilized water, cut into uniform sized slices and removed there seeds. Then slices were packed in plastic boxes after dipping in anti-browning solutions of ascorbic acid (0.9%, 1.8% or 2.7%) and cysteine (0.4%, 0.8% or 1.2%) for eight minutes. Physico-chemical and sensory attributes were evaluated during 24 days of storage at 5°C + 2°C. Dip with 1.8% ascorbic acid treatment prevented product browning and colour properties till 8 days of storage. Firmness and sensory attributes presented the maximum changes. Maximum activity of 1.8% ascorbic acid against enzymatic browning showed that fresh-cut guava slices remain acceptable till 8 days of storage 5°C.

Kew words: guavas, storage life, safe food, minimal processing

INTRODUCTION

Production of minimally processed fruits is becoming an important task in food industry because of their ready-to-serve/consume/ process property as well as for the health point of view. Fresh-cut fruits, as living tissues, undergo enzymatic browning and softening, microbial contamination, and undesirable volatile production which result in shorter shelf life than the whole produce (Soliva-Fortuny and Martin-Belloso, 2003). Fresh-cut fruit slices have a faster rate of browning and softening because of wounding (Bico *et al.,* 2009).

Any fruit or vegetable that has been washed, peeled, trimmed, sliced into required sizes/slices, treated with suitable method and packed in sterilized packaging materials made up of plastic or glass and finally they are subjected to store in refrigerated condition is to be known as fresh-cut produce (IFPA, 2004). This technology is also being known as minimally processed, partially/lightly processed and value added foods. Main objective of adopting the fresh-cut technology is to increase its availability in market for longer time in fresh form.

The demand for fresh-cut fruit and vegetables is growing with the likeness for pure nutritional source as fresh fruit. In fresh-cut processing through cutting, the fruit tissues many undergo physiological disorders or may initiate the nutrient retention as compared with whole fruits during storage (James and Ngarmsak, 2010).

In a study, Gil *et al.* (2006) concluded that the quality changes and nutrient retention occur in fresh-cut fruits like pineapples, mangoes, cantaloupes, watermelons, strawberries and kiwifruits versus whole fruits by increasing higher losses of carotenoids in pineapple up to 25% followed by 10-15% in cantaloupe, mango, and strawberries pieces till 6 days of storage at 5°C. Light exposure was a factor that enhances browning in pineapple pieces and decreased vitamin C content in kiwifruit slices. No significant loss of total phenolics was observed in fresh-cut fruits up to 6 days at 5°C whereas whole fruits visually spoiled prior to significant loss of nutrient.

Ascorbic acid exists mostly in reduced form in leaves and chloroplasts and well known antioxidant or anti-browning agents, acidic in nature, soluble in water and forms neutral salts (Alzamora and Lopez-Malo, 2000; Soliva-Fortuny and Martin-Belloso, 2003). Ascorbic acid is also considered as a nutritive agent (vitamin C). All known physiological and biochemical actions of vitamin C are due to its ability to act as a reducing agent. Ascorbic acid donates two electrons from a double bond between the second and third carbons of the 6-carbon molecule. However, during this reaction, vitamin C itself is oxidized (Padayatty et al., 2003).

Gil *et al.* (2006) found that 2% ascorbic acid was effective in diminishing the browning of fresh-cut Fuji apple slices. Esparza et al. (2005) found that overall acceptability and flavor quality of green leaf

lettuce was highest when treated in a 1% ascorbic acid solution for two minutes and stored at 5°C for up to 14 days in sealed polyethylene bags.

Oms-Oliu *et al.* (2006) used combinations of N-Acetyl-L-cysteine, reduced glutathione, ascorbic acid and 4- Hexylresorcinol and concluded that 0.75% of N-Acetyl-L cysteine was effective to prevent browning of fresh-cut pears up to 28 days at temperature of 4°C and 0.7% glutathione at same temperature up to 21 days. Similarly Rojas-Grau et al. (2006) compared the browning inhibition of N-acetyl cysteine, glutathione, ascorbic acid and 4-Hexylresorcinol through Fuji variety of apples while storing at 4°C temperature up to 14 days and found best treatment of 0.75% of N-acetyl cysteine, 0.60% of N-acetyl cysteine with 0.60% of glutathione than other. Moreover Gonzalez-Aguilar et al. (2005) compared N-Acetyl cysteine with ascorbic acid and iso-ascorbic acid as anti-browning agents for fresh-cut pineapple stored at 10°C up to 14 days and found 0.05M N-acetyl-cysteine more effective in minimizing browning problem with superior look.

Guava is one of the most important tropical and subtropical fruits, due to its high nutritional level. Its vitamin C content can reach 400 mg. 100 g–1 of fruit, which exceeds recommendations for daily intake, surpassing the vitamin C content of oranges, limes and pineapples. Also found in guava are high levels treatments of carotene, pro-vitamin A, thiamine, calcium, iron and phosphorous compounds (Lima et al., 2010; Nadeem et al., 2011). Guava is considered an important commodity in the local economy of tropical countries as well as in the international economy, probably due to both, its minimal agricultural requirements and its high nutritional value (Hui, 2004).

Moreover, guava has ability to produce a variety of food products like puree and paste, candies, juice concentrates, jams, jellies and squash. By virtue of its commercial and nutritional value, guava is considered a common man's fruit and can rightly be termed as "apple of the tropics" (Hui, 2004).

The purpose of the essay is to extend the shelf-life and to test the level of consumer acceptance of chemically treated and packed fresh-cut guava slices, as well as to evaluate the ability of anti-browning agents applied after cutting on the sensorial characteristics of the product.

MATERIALS AND METHODS

Raw material selection

Surahi, variety of guava was collected from Saloni Jhall, Samundri Road, Sunandri, Pakistan. These guava fruits were harvested at the stage when they just started to change their colour from light green to yellow (Hui, 2004; Sing and Pal, 2008a). Harvested guava fruits were processed freshly.

Raw material preparation

Guava fruits were sorted out into different categories, washed, sliced and removed the central seed portion of fruit.

Fresh-cut/minimally processing

Cleaned and uniform sized slices of guava fruits were then dipped for 8 minutes (Moretti et al., 2002) in the solutions of anti-browning agents ascorbic acid (0.9%, 1.8% or 2.7%) and cysteine (0.4%, 0.8% or 1.2%). Treated slices were then air dried, packed in plastic boxes and stored at $5^oC \pm 2^oC$. The samples were analyzed on 0, 6, 12, 18 and 24 days (Lamikanra and Watson, 2007) after storage for firmness, browning, colour tests and organoleptic attributes including taste, flavour, colour and texture. Samples were stored and analyzed in triplicate using CRD with factorial arrangement.

Firmness

Fresh-cut slices of each treatment were analyzed with the help of TA.XT Plus texture analyzer (Texture Analyzer of Stable Micro-System, UK) by using needle probe/puncture (2mm needle) instead of TA-212-cylinder probe following the method of Pereira et al. (2006) and Wang et al. (2007). Before each test the probe was cleaned with wet cloth to remove adhering material from probe to avoid from any error chance. As probe touched the guava slice surface the graph start to represent the applied force on slice and showing the distance covering in a specific time. Triplicate replications were taken from each treatment.

Browning of samples

Sample preparation (Homogenization)

Fresh-cut guava slices (20g) of each treatment in triplicate replicate were taken and homogenized (ULTRA TURRAX IKA T18 Basic) in the presence of 20ml sterilized water instead of deionized water. The speed of homogenizer was adjusted at "5" for 50 seconds.

Aliquot preparation

That homogenized mixtures were then taken in centrifuge tubes up to level and centrifuged in a centrifuged machine at 27600 g and at 5°C temperature for 15 minutes. Aliquot was separated by filtering the centrifuged material through Whatman paper #4.

Spectrophotometer absorption

Absorption of each sample was taken with the help of spectrophotometer (VARIAN Spectrophotometer AA240) at 340 nm (Moretti et al., 2002).

Colour measurement

The colour determination of guava fruit slices by objective mean in three replications were recorded on 6 day interval up to 24 days of total storage study, using Hunter colourimeter, Color Tech Bench, PCM/PSM (USA). For practical comparison, three readings per fruit slice were taken from same points around the equatorial axis throughout the experimental duration. The corresponding colour equivalents (L* and b*) were calculated automatically. The lightness coefficient (L*) ranges from black = 0 to white = 100 and colour coordinates b* represents yellow colour when values are positive, grey when zero, blue when values are negative (Pereira et al., 2006).

Sensory evaluation of fresh cut guavas

Sensory evaluation (taste, flavour, colour and texture) of fresh-cut guava was conducted according to 9-point hedonic scale (Meilgaard et al., 2007) through rating the samples from dislike extremelty (1) to like extremelty (9). The sensory evaluation panel was composed of five trained members from the faculty of National Institute of Food Science and Technologist (Appendax-I). Results were analyzed using one way ANOVA and LSD test was applied to the data to ascertain the statistical differences among mean values of different treatments.

RESULTS AND DISCUSSIONS

A significant ($P < 0.01$) influence of chemicals (calcium chloride and calcium lactate), storage days and their interaction on the firmness, browning and colour tests were observed during statistical evaluation.

Firmness of fresh cut guava

Regarding firmness (Table 1), application of 1.8% ascorbic acid maintained the highest fruit firmness (71.20g) followed by 2.7% ascorbic acid (69.43g) and 0.8% cysteine (62.79g), while the control samples exhibited the lowest fruit firmness. The interaction of treatments and storage days also showed a decreasing trend in firmness during storage. But the lowest firmness (20.67g and 20.80g) was recorded in untreated treatment and 0.4% cysteine on 24th days of storage followed by 1.2% cysteine (22.30g) and 0.9% ascorbic acid (22.40g); while the highest firmness (103.67g and 103.60g) was noted in slices treated with 1.8% ascorbic acid and 2.7% ascorbic acid at 0 day of storage. Similarly, through the study of Danyen et al. (2008) it was found that firmness in fresh-cut banana increases by using 2% ascorbic acid in combination with same concentration of calcium chloride. Moreover, It was observed from different studies (Martınez-Romero, 2003; Bassetto et al., 2005; Hershkovitz et al., 2005; Guillen et al., 2006; Manenoi et al., 2007; Bico et al., 2009) that with the reduction of respiration and ripening processes, the firmness in fresh-cut banana was delayed for greater time along treating with calcium chloride, ascorbic acid and cysteine as a combination (Bico et al., 2009) than untreated treatment.

Browning

The result of browning (Table 2) showed the more absorption intensity in control (1.62) followed by 0.4% cysteine (1.56) while the lowest browning intensity (1.37 and 1.40) was found non-significantly in T_2 and T_3 which contain 1.8% ascorbic acid and 2.7% ascorbic acid. The interaction of treatments and storage days also showed an increasing trend in browning of guava slices during storage. But the maximum increase in browning (0.60) was recorded in untreated treatment followed by 0.4% cysteine (0.56); while the lowest rise in browning (0.42) was noted in slices treated with 1.8% ascorbic acid followed by 0.8% cysteine (0.46).

The results obtained in the present study are in line with the findings of Moretti et al. (2002) who found that the higher absorption is the indication of higher contents of phenolic contents, whereas it was also observed by Lee and Whitaker (1995) that the cut surface browning in fruit slices is caused by the action of polyphenol oxidase (PPO) in the presence of phenolic compounds and environmental oxygen. These findings indicate reduction in browning in fresh-cut banana slices as observed by Bico et al. (2009) along with Vilas-Boas and Kader (2006) than untreated samples in which browning problem

Table 1: Effect of ascorbic acid and cysteine on firmness (g) of fresh-cut guava slices by different treatments and storage days

Treatments	Storage (Days)					
	0	6	12	18	24	Mean
To	99.43 c	83.30 i	53.27 p	34.50 v	20.67 z	**58.23 g**
T1	101.20 b	89.50 g	58.80 n	41.40 r	22.40 y	**62.66 d**
T2	103.67 a	94.87 d	69.70 j	53.40 p	34.37 v	**71.20 a**
T3	103.60 a	94.70 e	69.13 k	50.30 q	29.40 w	**69.43 b**
T4	99.50 c	89.20 h	57.30 o	35.23 u	20.80 z	**60.41 f**
T5	101.30 b	90.40 f	59.47 l	38.20 s	24.60 x	**62.79 c**
T6	101.27 b	90.33 f	59.13 m	37.80 t	22.30 y	**62.17 e**
Mean	**101.42 a**	**90.33 b**	**60.97 c**	**41.55 d**	**24.93 e**	

Different letters in the same column show the means significantly different ($P \leq 0.05$)

Table 2: Effect of ascorbic acid and cysteine on browning of fresh-cut guava slices by different treatments and storage days

Treatments	Storage (Days)					
	0	6	12	18	24	Mean
To	1.33 nop	1.43 lm	1.62 fg	1.79 c	1.93 a	**1.62 a**
T1	1.27 pqr	1.33 nop	1.45 kl	1.60 gh	1.78 c	**1.49 c**
T2	1.20 s	1.23 rs	1.34 no	1.4817 i-l	1.62 fg	**1.37 e**
T3	1.19 s	1.23 rs	1.36 no	1.52 ij	1.70 de	**1.40 de**
T4	1.30 opq	1.38 mn	1.53 hi	1.70 de	1.86 b	**1.56 b**
T5	1.24 qrs	1.30 opq	1.38 mn	1.52 ijk	1.70 de	**1.43 d**
T6	1.24 qrs	1.34 no	1.46 jkl	1.59 gh	1.76 cd	**1.48 c**
Mean	**1.25 e**	**1.32 d**	**1.45 c**	**1.60 b**	**1.77 a**	

Different letters in the same column show the means significantly different ($P \leq 0.05$)

Table3: Effect of ascorbic acid and cysteine on taste of fresh-cut guava slices by different treatments and storage days

Treatments	Storage (Days)					
	0	6	12	18	24	Mean
To	7.20 b	4.80 e	1.00 g	1.00 g	1.00 g	**3.00 e**
T1	7.60 ab	5.60 d	3.60 f	1.00 g	1.00 g	**3.76 c**
T2	7.80 a	7.40 ab	5.40 d	1.00 g	1.00 g	**4.52 a**
T3	6.60 c	5.80 d	3.60 f	1.00 g	1.00 g	**3.60 cd**
T4	7.40 ab	5.40 d	3.40 f	1.00 g	1.00 g	**3.64 cd**
T5	7.40 ab	6.40 c	4.60 e	1.00 g	1.00 g	**4.08 b**
T6	7.20 b	4.60 e	3.40 f	1.00 g	1.00 g	**3.44 d**
Mean	**7.31 a**	**5.71 b**	**3.57 c**	**1.00 d**	**1.00 d**	

Different letters in the same column show the means significantly different ($P \leq 0.05$)

Table4: Effect of ascorbic acid and cysteine on flavor of fresh-cut guava slices by different treatments and storage days

Treatments	Storage (Days)					
	0	**6**	**12**	**18**	**24**	**Mean**
To	7.00 c	4.00 f	1.00 h	1.00 h	1.00 h	**2.88 d**
T1	7.60 ab	5.20 e	3.60 g	1.00 h	1.00 h	**3.68 c**
T2	8.00 a	7.00 c	5.20 e	1.00 h	1.00 h	**4.44 a**
T3	7.60 ab	5.60 e	4.60 f	1.00 h	1.00 h	**3.96 b**
T4	7.20 bc	4.60 f	3.60 g	1.00 h	1.00 h	**3.48 c**
T5	7.60 ab	6.40 d	4.60 f	1.00 h	1.00 h	**4.12 b**
T6	7.20 bc	5.20 e	3.40 g	1.00 h	1.00 h	**3.56 c**
Mean	**7.46 a**	**5.48 b**	**3.71 c**	**1.00 d**	**1.00 d**	

Different letters in the same column show the means significantly different ($P \leq 0.05$)

Table5: Effect of ascorbic acid and cysteine on colour of fresh-cut guava slices by different treatments and storage days

Treatments	Storage (Days)					
	0	**6**	**12**	**18**	**24**	**Mean**
To	6.60 d	3.60 hi	2.40 kl	1.00 m	1.00 m	**2.92 e**
T1	7.40 bc	7.20 bcd	4.20 fgh	3.20 ij	1.80 l	**4.76 c**
T2	8.80 a	7.60 b	5.40 e	4.60 f	2.60 jk	**5.80 a**
T3	8.60 a	7.40 bc	4.40 fg	3.40 i	2.40 kl	**5.24 b**
T4	6.60 d	6.80 cd	3.60 hi	2.60 jk	2.20 kl	**4.36 d**
T5	7.80 b	7.20 bcd	4.40 fg	3.80 ghi	2.40 kl	**5.12 b**
T6	7.40 bc	6.80 cd	3.40 i	2.60 jk	2.40 kl	**4.52 cd**
Mean	**7.60 a**	**6.66 b**	**3.97 c**	**3.03 d**	**2.11 e**	

Different letters in the same column show the means significantly different ($P \leq 0.05$)

Table 6: Effect of ascorbic acid and cysteine on texture of fresh-cut guava slices by different treatments and storage days

Treatments	Storage (Days)					
	0	**6**	**12**	**18**	**24**	**Mean**
To	6.40 c	3.60 fg	2.20 hi	1.00 k	1.00 k	**2.84 d**
T1	6.60 bc	5.40 e	3.40 g	1.40 jk	1.00 k	**3.56 c**
T2	7.40 a	6.60 bc	4.20 f	2.60 h	1.40 jk	**4.44 a**
T3	7.20 ab	6.20 cd	3.60 fg	1.60 ijk	1.20 jk	**3.96 b**
T4	6.80 abc	5.20 e	3.40 g	1.80 ij	1.20 jk	**3.68 bc**
T5	7.20 ab	5.60 de	3.60 fg	1.40 jk	1.20 jk	**3.80 b**
T6	6.80 abc	5.40 e	3.60 fg	1.40 jk	1.20 jk	**3.68 bc**
Mean	**6.91 a**	**5.43 b**	**3.43 c**	**1.60 d**	**1.17 e**	

Different letters in the same column show the means significantly different ($P \leq 0.05$)

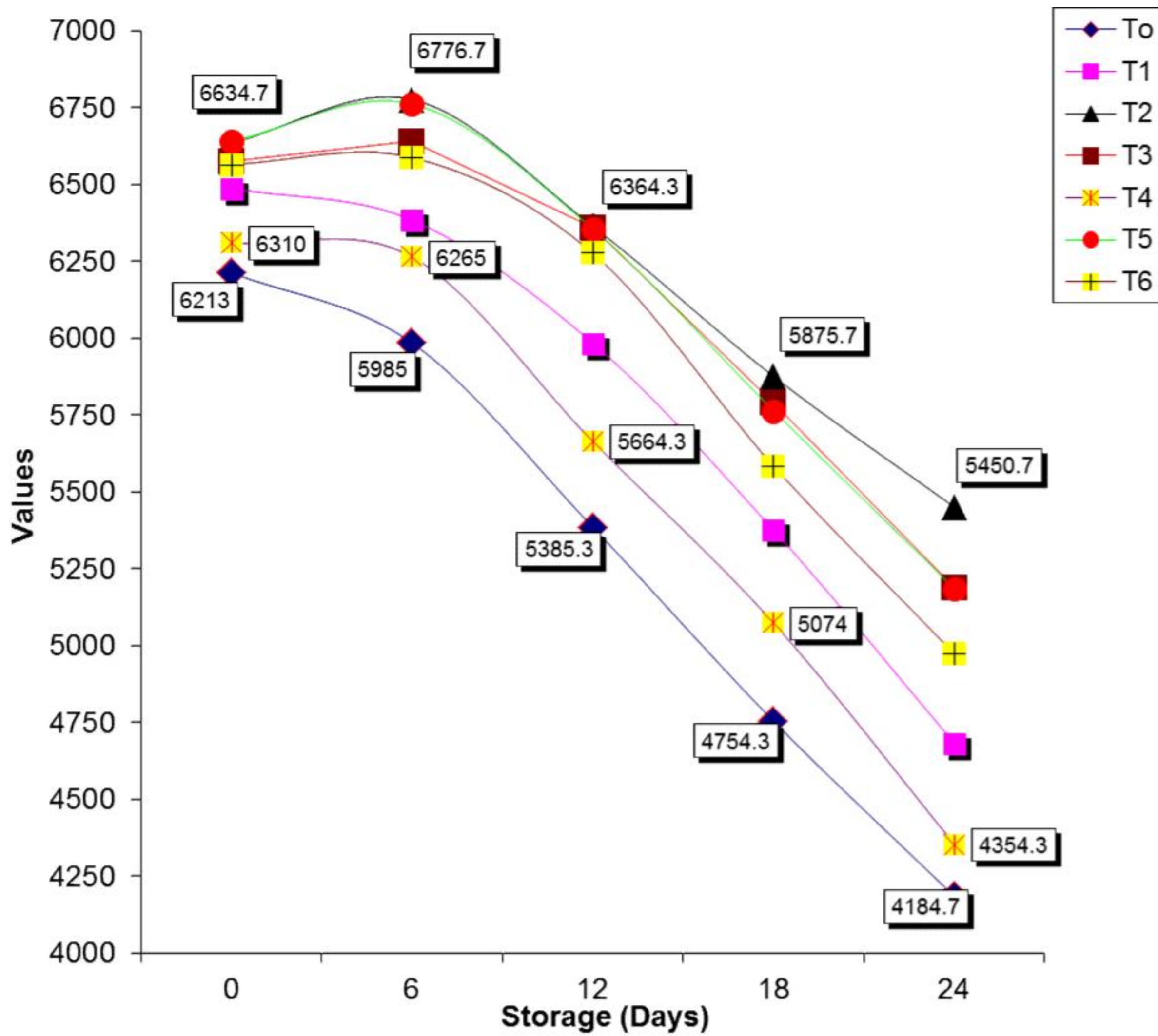

Figure 1: Effect of anti-browning agents on colour L* by different treatments and storage days

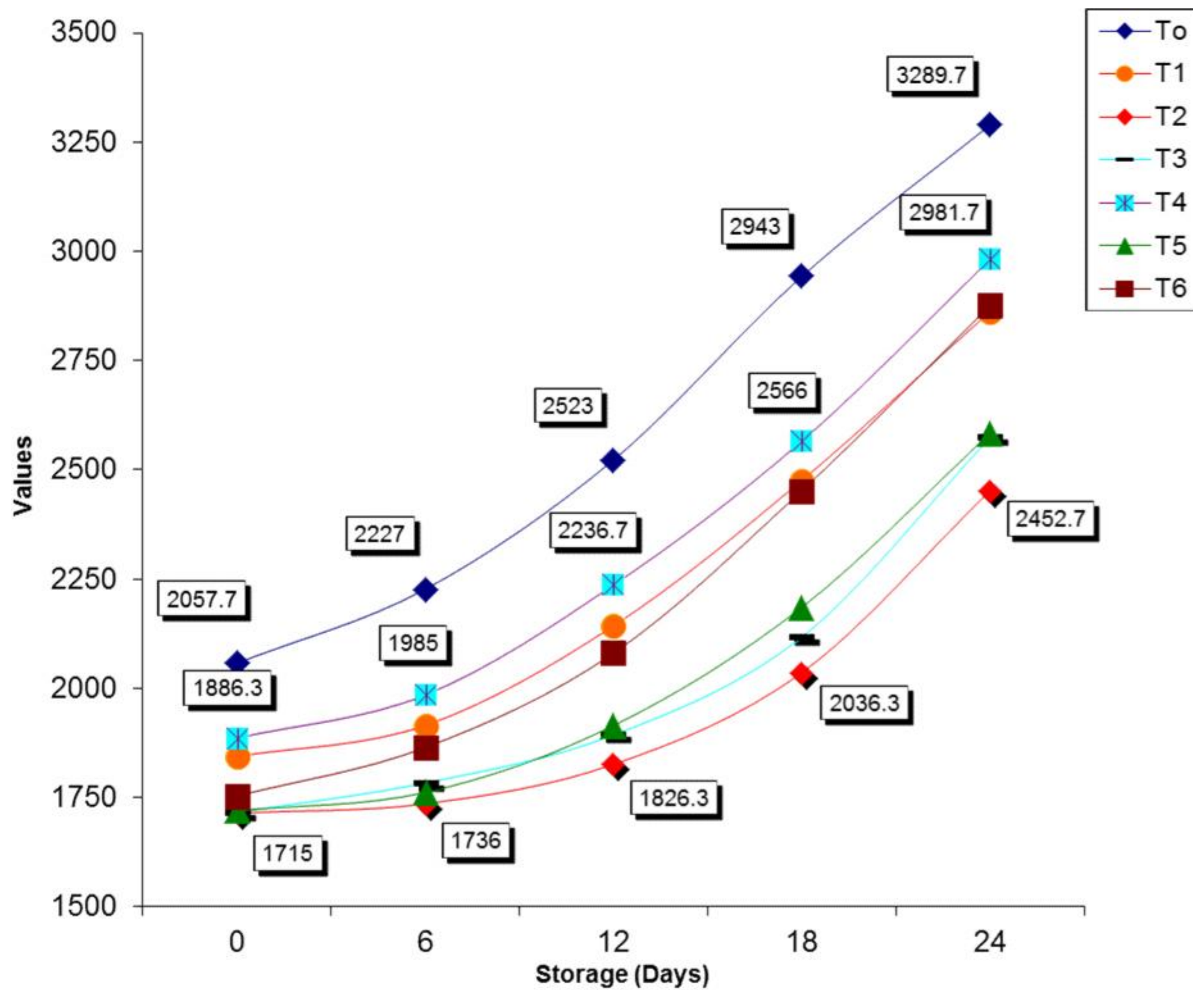

Figure 2: Effect of anti-browning agents on colour b* by different treatments and storage days

enhanced with the passage of time. Similarly, Pongsakul *et al.* (2006) recorded that L-cysteine is the most competent agent in reducing the problem of browning in the solution of pure chlorogenic acid solution as well as in the juice of loquat.

Colour:

L* and b* values for colour are most suitable parameters for observing enzymatic browning at cut surfaces of fruits (Campos-Vargas et al., 2008. Browning is highlighted through reducing the L* values of fruits increasing of b* values (Danyen et al., 2008). The results regarding the colour attributes like L* and b* were significantly influenced by the chemical treatments and storage period. For L*, the values assigned to the guava samples have been presented in Fig. 1. Graph show a decreasing trend in all of the samples during storage except 1.8% ascorbic acid in which increasing trend in L* values have been observed till 6th day of storage followed by 0.8% cysteine and 2.7% ascorbic acid. T_2 (1.8% ascorbic acid) was only treatment which showed slow decreasing trend till 24th day of storage followed by T_6 and T_3, while the least values were given to the T_o (control). Similarly, Thommohaway et al. (2007) found that L* value of fresh-cut guava peel reduced in all treatments throughout the storage time but on 7th day of storage L* value of chemically treated fresh-cut guava peel was significantly higher than uncoated. Furthermore, Campos-Vargas et al. (2008) found that the difference of L* (lightness) values between lower concentrations of cysteine and control was not much different during storage. But when the concentrations of cysteine reached at 0.5% or exceed, then L* values start to rise as compare to the control during storage.

The line graph (Fig 2) showed the increasing trend in all treatments throughout the storage period, while T2 was the only treatment which showed slowest increase in b* values during whole storage intervals followed by T5 and T3. Whereas To showed highest increasing trend in b* values of guava slices followed by T4 and T1. The results are in accordance with the results of other authors (Campos-Vargas et al., 2008; Danyen et al., 2008). They found that the b* values was decreased while increasing the concentration of cysteine from 0.125-0.5% and ascorbic acid from 0-2%. Moreover, Ndiaye et al. (2009) observed that b* values increase with the increase of ripening criteria in mango fruits.

Organoleptic evaluation of guavas:

The results regarding the organoleptic evaluation indicate that the sensory attributes like taste, flavour, colour and texture were significantly influenced by the chemical treatments and storage period.

Taste and flavor

The result regarding taste and flavour (Table 3 and 4) of guava slices showed highest scores (4.52 and 4.44) of 1.8% ascorbic acid followed by 0.8% cysteine (4.08 and 4.12), while the control samples exhibited the lowest taste and flavor properties. The interaction of treatments and storage days also showed higher stability in taste and flavor of guava slices regarding to 1.2% ascorbic acid up to 12th day of storage (5.40 and 5.20) than other treatments. Moreover, a decreasing trend regarding both sensory characteristics taste and flavor was simultaneously observed during whole storage intervals. But the lowest score of 1.00 of taste and flavor was recorded in untreated treatment on 12th day of storage followed by same score in other treatments on 18th day; while the highest score concerning taste (7.80) and flavor (8.00) were noted in slices treated with 1.8% ascorbic acid at 0 day of storage. Similarly, Mahajan et al. (2011) and Aguayo et al. (2006) found that the flavoring properties of guava and taste attribute of fresh-cut strawberries reduces with the passage of storage time.

Colour and texture

The result of colour and texture (Table 5 and 6) showed the lowest stability in control (2.92 and 2.84) followed by 0.4% cysteine (4.36 and 3.68) while the highest stability in colour (5.80) and texture (4.44) was in T_2 (1.8% ascorbic acid) than other treatments. The interaction of treatments and storage days also showed a decreasing trend in colour and texture of guava slices during storage.

The minimum decrease in colour (4.60) and texture (2.60) was recorded in T_2 (1.8% ascorbic acid) at 18th day of storage; while untreated guava slices showed 1.00 scores at the same day of storage. Similarly, Gorny et al. (2002) found that the flesh colour decrease as the storage interval passed at both temperatures (0°C and 10°C) but the rate of depletion in colour of pear was greater at 10°C than 0°C. Moreover, calcium (Ca+2) ions formed the cross bridge cross linked with COO- groups from the pectin contents of the vegetables and fruits decreasing pectin solubalization to form calcium pactate which stabilize the membrane system and

enhance the firmness of cell wall and middle lamella portion of fruits (Lamikanra, 2002).

CONCLUSION

1.8% ascorbic acid presented the lowest rate of firmness loss, browning and colour (L* and b*) along with preserving the sensory attributes of fresh-cut guava slices up to 8 days of total shelf-life at 5°C. Chemical dip might have reduced the respiration and ripening processes in fruits by controlling the activity of responsible enzymes and strengthening the cell-wall structure. From the result of present study, it was observed that the taste of fresh-cut guava slices reduced steadily with increase in storage period.

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PAK. J. FOOD SCI., 23(3), 2013: 153-162
ISSN: 2226-5899

Effect of anti-browning agents in extended the storage life of fresh-cut guava slices by delaying enzymatic activity

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ABSTRACT

This study was undertaken to extend the shelf-life of fresh-cut guava slices of Surahi variety at lower temperature by delaying enzymatic activity. Freshly harvested guavas were subjected to wash with sterilized water, cut into uniform sized slices and removed there seeds. Then slices were packed in plastic boxes after dipping in anti-browning solutions of ascorbic acid (0.9%, 1.8% or 2.7%) and cysteine (0.4%, 0.8% or 1.2%) for eight minutes. Physico-chemical and sensory attributes were evaluated during 24 days of storage at 5°C + 2°C. Dip with 1.8% ascorbic acid treatment prevented product browning and colour properties till 8 days of storage. Firmness and sensory attributes presented the maximum changes. Maximum activity of 1.8% ascorbic acid against enzymatic browning showed that fresh-cut guava slices remain acceptable till 8 days of storage 5°C.

Kew words: guavas, storage life, safe food, minimal processing

INTRODUCTION

Production of minimally processed fruits is becoming an important task in food industry because of their ready-to-serve/consume/ process property as well as for the health point of view. Fresh-cut fruits, as living tissues, undergo enzymatic browning and softening, microbial contamination, and undesirable volatile production which result in shorter shelf life than the whole produce (Soliva-Fortuny and Martin-Belloso, 2003). Fresh-cut fruit slices have a faster rate of browning and softening because of wounding (Bico *et al.,* 2009).

Any fruit or vegetable that has been washed, peeled, trimmed, sliced into required sizes/slices, treated with suitable method and packed in sterilized packaging materials made up of plastic or glass and finally they are subjected to store in refrigerated condition is to be known as fresh-cut produce (IFPA, 2004). This technology is also being known as minimally processed, partially/lightly processed and value added foods. Main objective of adopting the fresh-cut technology is to increase its availability in market for longer time in fresh form.

The demand for fresh-cut fruit and vegetables is growing with the likeness for pure nutritional source as fresh fruit. In fresh-cut processing through cutting, the fruit tissues many undergo physiological disorders or may initiate the nutrient retention as compared with whole fruits during storage (James and Ngarmsak, 2010).

In a study, Gil *et al.* (2006) concluded that the quality changes and nutrient retention occur in fresh-cut fruits like pineapples, mangoes, cantaloupes, watermelons, strawberries and kiwifruits versus whole fruits by increasing higher losses of carotenoids in pineapple up to 25% followed by 10-15% in cantaloupe, mango, and strawberries pieces till 6 days of storage at 5°C. Light exposure was a factor that enhances browning in pineapple pieces and decreased vitamin C content in kiwifruit slices. No significant loss of total phenolics was observed in fresh-cut fruits up to 6 days at 5°C whereas whole fruits visually spoiled prior to significant loss of nutrient.

Ascorbic acid exists mostly in reduced form in leaves and chloroplasts and well known antioxidant or anti-browning agents, acidic in nature, soluble in water and forms neutral salts (Alzamora and Lopez-Malo, 2000; Soliva-Fortuny and Martin-Belloso, 2003). Ascorbic acid is also considered as a nutritive agent (vitamin C). All known physiological and biochemical actions of vitamin C are due to its ability to act as a reducing agent. Ascorbic acid donates two electrons from a double bond between the second and third carbons of the 6-carbon molecule. However, during this reaction, vitamin C itself is oxidized (Padayatty et al., 2003).

Gil *et al.* (2006) found that 2% ascorbic acid was effective in diminishing the browning of fresh-cut Fuji apple slices. Esparza et al. (2005) found that overall acceptability and flavor quality of green leaf

lettuce was highest when treated in a 1% ascorbic acid solution for two minutes and stored at 5°C for up to 14 days in sealed polyethylene bags.

Oms-Oliu *et al.* (2006) used combinations of N-Acetyl-L-cysteine, reduced glutathione, ascorbic acid and 4- Hexylresorcinol and concluded that 0.75% of N-Acetyl-L cysteine was effective to prevent browning of fresh-cut pears up to 28 days at temperature of 4°C and 0.7% glutathione at same temperature up to 21 days. Similarly Rojas-Grau et al. (2006) compared the browning inhibition of N-acetyl cysteine, glutathione, ascorbic acid and 4-Hexylresorcinol through Fuji variety of apples while storing at 4°C temperature up to 14 days and found best treatment of 0.75% of N-acetyl cysteine, 0.60% of N-acetyl cysteine with 0.60% of glutathione than other. Moreover Gonzalez-Aguilar et al. (2005) compared N-Acetyl cysteine with ascorbic acid and iso-ascorbic acid as anti-browning agents for fresh-cut pineapple stored at 10°C up to 14 days and found 0.05M N-acetyl-cysteine more effective in minimizing browning problem with superior look.

Guava is one of the most important tropical and subtropical fruits, due to its high nutritional level. Its vitamin C content can reach 400 mg. 100 g–1 of fruit, which exceeds recommendations for daily intake, surpassing the vitamin C content of oranges, limes and pineapples. Also found in guava are high levels treatments of carotene, pro-vitamin A, thiamine, calcium, iron and phosphorous compounds (Lima et al., 2010; Nadeem et al., 2011). Guava is considered an important commodity in the local economy of tropical countries as well as in the international economy, probably due to both, its minimal agricultural requirements and its high nutritional value (Hui, 2004).

Moreover, guava has ability to produce a variety of food products like puree and paste, candies, juice concentrates, jams, jellies and squash. By virtue of its commercial and nutritional value, guava is considered a common man's fruit and can rightly be termed as "apple of the tropics" (Hui, 2004).

The purpose of the essay is to extend the shelf-life and to test the level of consumer acceptance of chemically treated and packed fresh-cut guava slices, as well as to evaluate the ability of anti-browning agents applied after cutting on the sensorial characteristics of the product.

MATERIALS AND METHODS

Raw material selection

Surahi, variety of guava was collected from Saloni Jhall, Samundri Road, Sunandri, Pakistan. These guava fruits were harvested at the stage when they just started to change their colour from light green to yellow (Hui, 2004; Sing and Pal, 2008a). Harvested guava fruits were processed freshly.

Raw material preparation

Guava fruits were sorted out into different categories, washed, sliced and removed the central seed portion of fruit.

Fresh-cut/minimally processing

Cleaned and uniform sized slices of guava fruits were then dipped for 8 minutes (Moretti et al., 2002) in the solutions of anti-browning agents ascorbic acid (0.9%, 1.8% or 2.7%) and cysteine (0.4%, 0.8% or 1.2%). Treated slices were then air dried, packed in plastic boxes and stored at 5°C $\pm$ 2°C. The samples were analyzed on 0, 6, 12, 18 and 24 days (Lamikanra and Watson, 2007) after storage for firmness, browning, colour tests and organoleptic attributes including taste, flavour, colour and texture. Samples were stored and analyzed in triplicate using CRD with factorial arrangement.

Firmness

Fresh-cut slices of each treatment were analyzed with the help of TA.XT Plus texture analyzer (Texture Analyzer of Stable Micro-System, UK) by using needle probe/puncture (2mm needle) instead of TA-212-cylinder probe following the method of Pereira et al. (2006) and Wang et al. (2007). Before each test the probe was cleaned with wet cloth to remove adhering material from probe to avoid from any error chance. As probe touched the guava slice surface the graph start to represent the applied force on slice and showing the distance covering in a specific time. Triplicate replications were taken from each treatment.

Browning of samples

Sample preparation (Homogenization)

Fresh-cut guava slices (20g) of each treatment in triplicate replicate were taken and homogenized (ULTRA TURRAX IKA T18 Basic) in the presence of 20ml sterilized water instead of deionized water. The speed of homogenizer was adjusted at "5" for 50 seconds.

Aliquot preparation

That homogenized mixtures were then taken in centrifuge tubes up to level and centrifuged in a centrifuged machine at 27600 g and at 5°C temperature for 15 minutes. Aliquot was separated by filtering the centrifuged material through Whatman paper #4.

Spectrophotometer absorption

Absorption of each sample was taken with the help of spectrophotometer (VARIAN Spectrophotometer AA240) at 340 nm (Moretti et al., 2002).

Colour measurement

The colour determination of guava fruit slices by objective mean in three replications were recorded on 6 day interval up to 24 days of total storage study, using Hunter colourimeter, Color Tech Bench, PCM/PSM (USA). For practical comparison, three readings per fruit slice were taken from same points around the equatorial axis throughout the experimental duration. The corresponding colour equivalents (L* and b*) were calculated automatically. The lightness coefficient (L*) ranges from black = 0 to white = 100 and colour coordinates b* represents yellow colour when values are positive, grey when zero, blue when values are negative (Pereira et al., 2006).

Sensory evaluation of fresh cut guavas

Sensory evaluation (taste, flavour, colour and texture) of fresh-cut guava was conducted according to 9-point hedonic scale (Meilgaard et al., 2007) through rating the samples from dislike extremelty (1) to like extremelty (9). The sensory evaluation panel was composed of five trained members from the faculty of National Institute of Food Science and Technologist (Appendax-I). Results were analyzed using one way ANOVA and LSD test was applied to the data to ascertain the statistical differences among mean values of different treatments.

RESULTS AND DISCUSSIONS

A significant ($P < 0.01$) influence of chemicals (calcium chloride and calcium lactate), storage days and their interaction on the firmness, browning and colour tests were observed during statistical evaluation.

Firmness of fresh cut guava

Regarding firmness (Table 1), application of 1.8% ascorbic acid maintained the highest fruit firmness (71.20g) followed by 2.7% ascorbic acid (69.43g) and 0.8% cysteine (62.79g), while the control samples exhibited the lowest fruit firmness. The interaction of treatments and storage days also showed a decreasing trend in firmness during storage. But the lowest firmness (20.67g and 20.80g) was recorded in untreated treatment and 0.4% cysteine on 24th days of storage followed by 1.2% cysteine (22.30g) and 0.9% ascorbic acid (22.40g); while the highest firmness (103.67g and 103.60g) was noted in slices treated with 1.8% ascorbic acid and 2.7% ascorbic acid at 0 day of storage. Similarly, through the study of Danyen et al. (2008) it was found that firmness in fresh-cut banana increases by using 2% ascorbic acid in combination with same concentration of calcium chloride. Moreover, It was observed from different studies (Martınez-Romero, 2003; Bassetto et al., 2005; Hershkovitz et al., 2005; Guillen et al., 2006; Manenoi et al., 2007; Bico et al., 2009) that with the reduction of respiration and ripening processes, the firmness in fresh-cut banana was delayed for greater time along treating with calcium chloride, ascorbic acid and cysteine as a combination (Bico et al., 2009) than untreated treatment.

Browning

The result of browning (Table 2) showed the more absorption intensity in control (1.62) followed by 0.4% cysteine (1.56) while the lowest browning intensity (1.37 and 1.40) was found non-significantly in T_2 and T_3 which contain 1.8% ascorbic acid and 2.7% ascorbic acid. The interaction of treatments and storage days also showed an increasing trend in browning of guava slices during storage. But the maximum increase in browning (0.60) was recorded in untreated treatment followed by 0.4% cysteine (0.56); while the lowest rise in browning (0.42) was noted in slices treated with 1.8% ascorbic acid followed by 0.8% cysteine (0.46).

The results obtained in the present study are in line with the findings of Moretti et al. (2002) who found that the higher absorption is the indication of higher contents of phenolic contents, whereas it was also observed by Lee and Whitaker (1995) that the cut surface browning in fruit slices is caused by the action of polyphenol oxidase (PPO) in the presence of phenolic compounds and environmental oxygen. These findings indicate reduction in browning in fresh-cut banana slices as observed by Bico et al. (2009) along with Vilas-Boas and Kader (2006) than untreated samples in which browning problem

Table 1: Effect of ascorbic acid and cysteine on firmness (g) of fresh-cut guava slices by different treatments and storage days

Treatments	Storage (Days)					
	0	6	12	18	24	Mean
To	99.43 c	83.30 i	53.27 p	34.50 v	20.67 z	**58.23 g**
T1	101.20 b	89.50 g	58.80 n	41.40 r	22.40 y	**62.66 d**
T2	103.67 a	94.87 d	69.70 j	53.40 p	34.37 v	**71.20 a**
T3	103.60 a	94.70 e	69.13 k	50.30 q	29.40 w	**69.43 b**
T4	99.50 c	89.20 h	57.30 o	35.23 u	20.80 z	**60.41 f**
T5	101.30 b	90.40 f	59.47 l	38.20 s	24.60 x	**62.79 c**
T6	101.27 b	90.33 f	59.13 m	37.80 t	22.30 y	**62.17 e**
Mean	**101.42 a**	**90.33 b**	**60.97 c**	**41.55 d**	**24.93 e**	

Different letters in the same column show the means significantly different ($P \leq 0.05$)

Table 2: Effect of ascorbic acid and cysteine on browning of fresh-cut guava slices by different treatments and storage days

Treatments	Storage (Days)					
	0	6	12	18	24	Mean
To	1.33 nop	1.43 lm	1.62 fg	1.79 c	1.93 a	**1.62 a**
T1	1.27 pqr	1.33 nop	1.45 kl	1.60 gh	1.78 c	**1.49 c**
T2	1.20 s	1.23 rs	1.34 no	1.4817 i-l	1.62 fg	**1.37 e**
T3	1.19 s	1.23 rs	1.36 no	1.52 ij	1.70 de	**1.40 de**
T4	1.30 opq	1.38 mn	1.53 hi	1.70 de	1.86 b	**1.56 b**
T5	1.24 qrs	1.30 opq	1.38 mn	1.52 ijk	1.70 de	**1.43 d**
T6	1.24 qrs	1.34 no	1.46 jkl	1.59 gh	1.76 cd	**1.48 c**
Mean	**1.25 e**	**1.32 d**	**1.45 c**	**1.60 b**	**1.77 a**	

Different letters in the same column show the means significantly different ($P \leq 0.05$)

Table3: Effect of ascorbic acid and cysteine on taste of fresh-cut guava slices by different treatments and storage days

Treatments	Storage (Days)					
	0	6	12	18	24	Mean
To	7.20 b	4.80 e	1.00 g	1.00 g	1.00 g	**3.00 e**
T1	7.60 ab	5.60 d	3.60 f	1.00 g	1.00 g	**3.76 c**
T2	7.80 a	7.40 ab	5.40 d	1.00 g	1.00 g	**4.52 a**
T3	6.60 c	5.80 d	3.60 f	1.00 g	1.00 g	**3.60 cd**
T4	7.40 ab	5.40 d	3.40 f	1.00 g	1.00 g	**3.64 cd**
T5	7.40 ab	6.40 c	4.60 e	1.00 g	1.00 g	**4.08 b**
T6	7.20 b	4.60 e	3.40 f	1.00 g	1.00 g	**3.44 d**
Mean	**7.31 a**	**5.71 b**	**3.57 c**	**1.00 d**	**1.00 d**	

Different letters in the same column show the means significantly different ($P \leq 0.05$)

Table4: Effect of ascorbic acid and cysteine on flavor of fresh-cut guava slices by different treatments and storage days

Treatments	Storage (Days)					
	0	**6**	**12**	**18**	**24**	**Mean**
To	7.00 c	4.00 f	1.00 h	1.00 h	1.00 h	**2.88 d**
T1	7.60 ab	5.20 e	3.60 g	1.00 h	1.00 h	**3.68 c**
T2	8.00 a	7.00 c	5.20 e	1.00 h	1.00 h	**4.44 a**
T3	7.60 ab	5.60 e	4.60 f	1.00 h	1.00 h	**3.96 b**
T4	7.20 bc	4.60 f	3.60 g	1.00 h	1.00 h	**3.48 c**
T5	7.60 ab	6.40 d	4.60 f	1.00 h	1.00 h	**4.12 b**
T6	7.20 bc	5.20 e	3.40 g	1.00 h	1.00 h	**3.56 c**
Mean	**7.46 a**	**5.48 b**	**3.71 c**	**1.00 d**	**1.00 d**	

Different letters in the same column show the means significantly different ($P \leq 0.05$)

Table5: Effect of ascorbic acid and cysteine on colour of fresh-cut guava slices by different treatments and storage days

Treatments	Storage (Days)					
	0	**6**	**12**	**18**	**24**	**Mean**
To	6.60 d	3.60 hi	2.40 kl	1.00 m	1.00 m	**2.92 e**
T1	7.40 bc	7.20 bcd	4.20 fgh	3.20 ij	1.80 l	**4.76 c**
T2	8.80 a	7.60 b	5.40 e	4.60 f	2.60 jk	**5.80 a**
T3	8.60 a	7.40 bc	4.40 fg	3.40 i	2.40 kl	**5.24 b**
T4	6.60 d	6.80 cd	3.60 hi	2.60 jk	2.20 kl	**4.36 d**
T5	7.80 b	7.20 bcd	4.40 fg	3.80 ghi	2.40 kl	**5.12 b**
T6	7.40 bc	6.80 cd	3.40 i	2.60 jk	2.40 kl	**4.52 cd**
Mean	**7.60 a**	**6.66 b**	**3.97 c**	**3.03 d**	**2.11 e**	

Different letters in the same column show the means significantly different ($P \leq 0.05$)

Table 6: Effect of ascorbic acid and cysteine on texture of fresh-cut guava slices by different treatments and storage days

Treatments	Storage (Days)					
	0	**6**	**12**	**18**	**24**	**Mean**
To	6.40 c	3.60 fg	2.20 hi	1.00 k	1.00 k	**2.84 d**
T1	6.60 bc	5.40 e	3.40 g	1.40 jk	1.00 k	**3.56 c**
T2	7.40 a	6.60 bc	4.20 f	2.60 h	1.40 jk	**4.44 a**
T3	7.20 ab	6.20 cd	3.60 fg	1.60 ijk	1.20 jk	**3.96 b**
T4	6.80 abc	5.20 e	3.40 g	1.80 ij	1.20 jk	**3.68 bc**
T5	7.20 ab	5.60 de	3.60 fg	1.40 jk	1.20 jk	**3.80 b**
T6	6.80 abc	5.40 e	3.60 fg	1.40 jk	1.20 jk	**3.68 bc**
Mean	**6.91 a**	**5.43 b**	**3.43 c**	**1.60 d**	**1.17 e**	

Different letters in the same column show the means significantly different ($P \leq 0.05$)

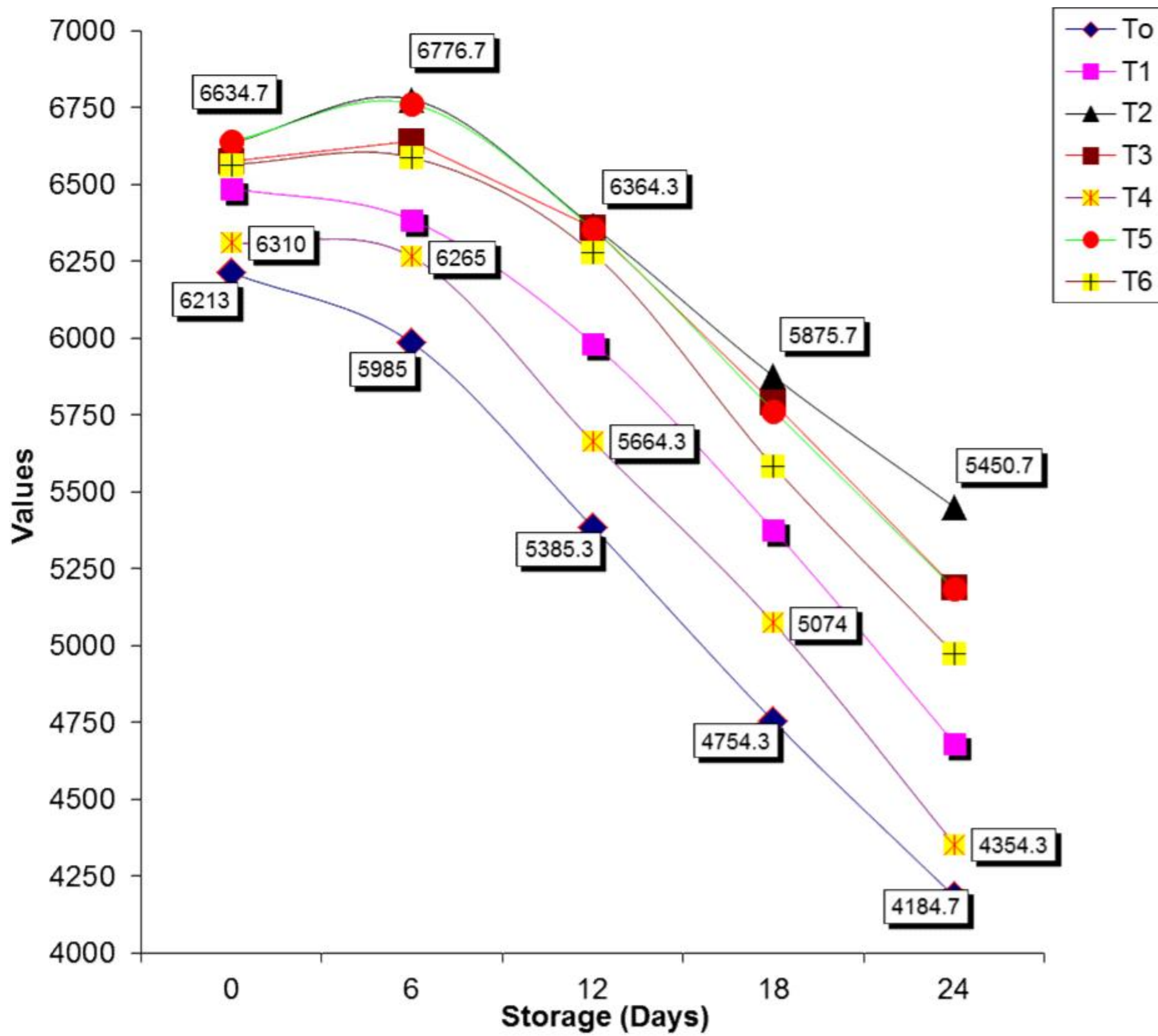

Figure 1: Effect of anti-browning agents on colour L* by different treatments and storage days

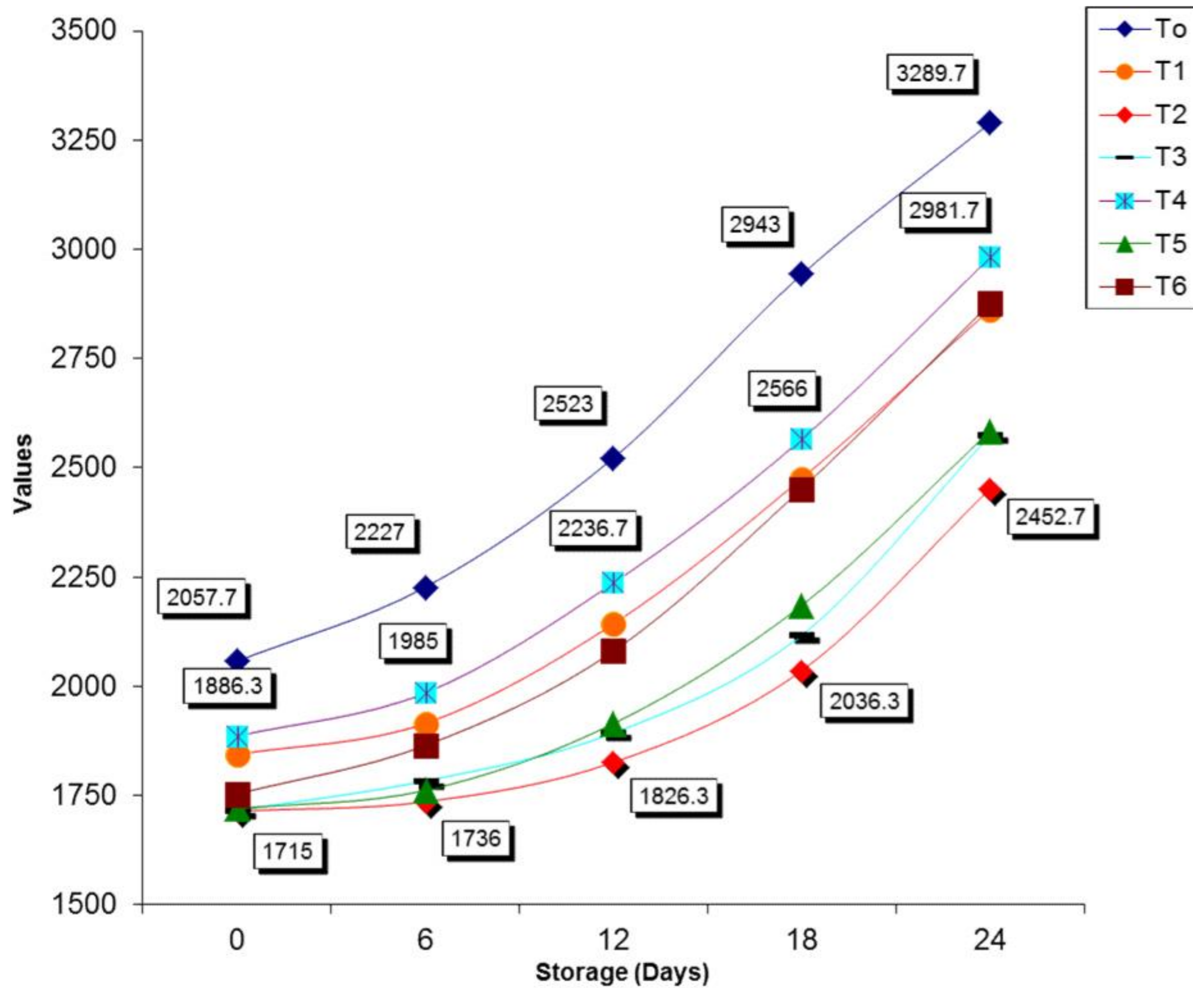

Figure 2: Effect of anti-browning agents on colour b* by different treatments and storage days

enhanced with the passage of time. Similarly, Pongsakul *et al.* (2006) recorded that L-cysteine is the most competent agent in reducing the problem of browning in the solution of pure chlorogenic acid solution as well as in the juice of loquat.

Colour:

L* and b* values for colour are most suitable parameters for observing enzymatic browning at cut surfaces of fruits (Campos-Vargas et al., 2008. Browning is highlighted through reducing the L* values of fruits increasing of b* values (Danyen et al., 2008). The results regarding the colour attributes like L* and b* were significantly influenced by the chemical treatments and storage period. For L*, the values assigned to the guava samples have been presented in Fig. 1. Graph show a decreasing trend in all of the samples during storage except 1.8% ascorbic acid in which increasing trend in L* values have been observed till 6th day of storage followed by 0.8% cysteine and 2.7% ascorbic acid. T_2 (1.8% ascorbic acid) was only treatment which showed slow decreasing trend till 24th day of storage followed by T_6 and T_3, while the least values were given to the T_o (control). Similarly, Thommohaway et al. (2007) found that L* value of fresh-cut guava peel reduced in all treatments throughout the storage time but on 7th day of storage L* value of chemically treated fresh-cut guava peel was significantly higher than uncoated. Furthermore, Campos-Vargas et al. (2008) found that the difference of L* (lightness) values between lower concentrations of cysteine and control was not much different during storage. But when the concentrations of cysteine reached at 0.5% or exceed, then L* values start to rise as compare to the control during storage.

The line graph (Fig 2) showed the increasing trend in all treatments throughout the storage period, while T2 was the only treatment which showed slowest increase in b* values during whole storage intervals followed by T5 and T3. Whereas To showed highest increasing trend in b* values of guava slices followed by T4 and T1. The results are in accordance with the results of other authors (Campos-Vargas et al., 2008; Danyen et al., 2008). They found that the b* values was decreased while increasing the concentration of cysteine from 0.125-0.5% and ascorbic acid from 0-2%. Moreover, Ndiaye et al. (2009) observed that b* values increase with the increase of ripening criteria in mango fruits.

Organoleptic evaluation of guavas:

The results regarding the organoleptic evaluation indicate that the sensory attributes like taste, flavour, colour and texture were significantly influenced by the chemical treatments and storage period.

Taste and flavor

The result regarding taste and flavour (Table 3 and 4) of guava slices showed highest scores (4.52 and 4.44) of 1.8% ascorbic acid followed by 0.8% cysteine (4.08 and 4.12), while the control samples exhibited the lowest taste and flavor properties. The interaction of treatments and storage days also showed higher stability in taste and flavor of guava slices regarding to 1.2% ascorbic acid up to 12th day of storage (5.40 and 5.20) than other treatments. Moreover, a decreasing trend regarding both sensory characteristics taste and flavor was simultaneously observed during whole storage intervals. But the lowest score of 1.00 of taste and flavor was recorded in untreated treatment on 12th day of storage followed by same score in other treatments on 18th day; while the highest score concerning taste (7.80) and flavor (8.00) were noted in slices treated with 1.8% ascorbic acid at 0 day of storage. Similarly, Mahajan et al. (2011) and Aguayo et al. (2006) found that the flavoring properties of guava and taste attribute of fresh-cut strawberries reduces with the passage of storage time.

Colour and texture

The result of colour and texture (Table 5 and 6) showed the lowest stability in control (2.92 and 2.84) followed by 0.4% cysteine (4.36 and 3.68) while the highest stability in colour (5.80) and texture (4.44) was in T_2 (1.8% ascorbic acid) than other treatments. The interaction of treatments and storage days also showed a decreasing trend in colour and texture of guava slices during storage.

The minimum decrease in colour (4.60) and texture (2.60) was recorded in T_2 (1.8% ascorbic acid) at 18th day of storage; while untreated guava slices showed 1.00 scores at the same day of storage. Similarly, Gorny et al. (2002) found that the flesh colour decrease as the storage interval passed at both temperatures (0^oC and 10^oC) but the rate of depletion in colour of pear was greater at 10^oC than 0^oC. Moreover, calcium (Ca+2) ions formed the cross bridge cross linked with COO- groups from the pectin contents of the vegetables and fruits decreasing pectin solubalization to form calcium pactate which stabilize the membrane system and

enhance the firmness of cell wall and middle lamella portion of fruits (Lamikanra, 2002).

CONCLUSION

1.8% ascorbic acid presented the lowest rate of firmness loss, browning and colour (L* and b*) along with preserving the sensory attributes of fresh-cut guava slices up to 8 days of total shelf-life at 5°C. Chemical dip might have reduced the respiration and ripening processes in fruits by controlling the activity of responsible enzymes and strengthening the cell-wall structure. From the result of present study, it was observed that the taste of fresh-cut guava slices reduced steadily with increase in storage period.

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