Quality of Guava Whey Beverage Fortified With *Moringa Oleifera* Leaves Extract

Afaf O. Ali, Wael H. M. Elreffaei, Adel M. Elkarmany and Fatma M. A. Elsheek

Regional Center for Food & Feed, Agriculture Research Center, Giza, Egypt.
waillh@hotmail.com

Abstract: Efficacy of preservation and antioxidant activities of aqueous leaf extract of *Moringa oleifera* in whey beverage was *determined. It is* well known for its nutritional and health benefits and is being recommended for malnourished people all over the world. *Moringa oleifera* leaves, is a rich source of bioactive compounds, as polyphenols. The present investigation aimed to evaluate the stability of bioactive compounds and free radical scavenging abilities of the leaf extracts guava in whey beverages stored under different conditions. *Moringa* leaves were turned into an aqueous extract and fortified to whey guava beverage at different ratios (2.5,5.0, and 7.5%). The total phenolic and flavonoid contents have been measured using colorimetric methods. The antioxidant capacities were evaluated using scavenging assays of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) in aqueous extract. The results showed that extractability of *Moringa* leaves compounds Total Phenolic Compound (TPP) was significantly higher ($p \leq 0.05$) in the phenolic extract in the beverage contains 7.5% (5.560 ±0. 21g/100ml) of *Moringa* leaves extract at them in control without *Moringa* extract (2.083±0.29 g/100ml) and it showed higher antioxidant activities at zero time and up to 10 storage days. Consistent differences ($P \leq 0.05$) in DPPH activity were observed among the different ratio of *Moringa* leaves extract in the beverages about 77 to 99% scavenging activity against free radicals from DPPH at the zero time. Generally, antioxidative potential was expressed at a later stage resulting in higher DPPH scavenging activity values initially (zero time) and a latter (10 days) decrease in the most of prepared beverages except for fortified *Moringa* leaves extract with guava whey at concentration 2.5%, 5.0% and 7.5%. The sensory evaluation of the whey guava beverage fortified with 2.5 or 5% was the acceptable ratios for using *Moringa* leaves extract in such beverage. It is can concluded that, of the stability of the leaf extract of *M. oleifera* as a preservative has been limited up to 10 days when uses in the beverages.
[Afaf O. Ali, Wael H. M. Elreffaei, Adel M. Elkarmany and Fatma M. A. Elsheek. **Quality of Guava Whey Beverage Fortified With *Moringa Oleifera* Leaves Extract.** *Life Sci J* 2015;12(4s):113-122]. (ISSN:1097-8135). http://www.lifesciencesite.com. 1

Key words: *Moringa oleifera,* leaves,total phenolic, DPPH, whey beverages

1. Introduction

The uses of plant and herb extracts as antimicrobial agents in food and soft drinks have also been reported for centuries (Gulmez *et al.,* 2006). Due to potential toxicity of chemical food preservatives, there has been increased demand for food preservatives from natural sources. This has led researchers and food processors to come across natural food additives with a wide range of antimicrobial activities. As a result, today plant antimicrobial products have acquired importance in the food system to retard bacterial and fungal growth (Souza *et al.,* 2005b). Naturally occurring antimicrobial compounds isolated from animal, plant and microbial sources can be used alone or in combination with other approved antimicrobial preservatives (Davidson, 2006). In recent years, it has been shown that a large number of naturally occurring compounds from vegetables and fruits are effective as chemo preventive agents. An advantage of diet – derived products for cancer prevention is that they also have apparent benefit in other chronic diseases (Sporn and Suh, 2000 and Kellof, 2000). Therefore, natural antioxidants can protect the human body from free radicals that may cause some chronic diseases, including cancer, cardiovascular diseases and cataract (Li *et al.,* 2001).

Moringa oleifera Lam, commonly referred to as 'drumstick tree' (describing of its pods) or horseradish tree (describing the taste of its roots) also it named such as Benz olive, Marango and miracle tree, is a member of *Moringaceae* family. It was utilized by the ancient Roman, Greeks and Egyptians (Fahey, 2005). *Moringa oleifera* also have an economical importance in several industrial and medical uses (Nautiyal and Venhatarman, 1987). *Moringa oleifera* Lam. is commonly known in Egypt. Most of the parts of the plant possess antimicrobial activity (Caceres *et al.,* 1991).

Almost all parts of this plant have been used such as seeds, leaves and flowers having as antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypocholesterolemic, hypoglycemic activities (Sreelatha *et al.,* 2011) and cardiovascular and liver diseases, immune boosting agent, regulate blood sugar and cholesterol (Limaye *et al.,* 1995). Jung (2014) has shown that an aqueous extract of *M. oleifera* leaves exhibited significant

antineoplastic activity against a lung cancer cell line and several other types of cancer cells. The extract induced apoptosis, inhibited tumor cell growth, and lowered the internal level of reactive oxygen species in human lung cancer cells.

They are well known for their pharmacological actions too and are used for the traditional treatment of diabetes mellitus (Babu and Chaudhuri, 2005) hepatotoxicity (Ruckmani *et al.,* 1998), rheumatism, and venomous bites and for cardiac stimulation (Chaudhary and Chopra, 1996). WHO has emphasized strongly on the rational use of traditional and natural indigenous medicines, for treating diabetes mellitus (WHO, 1994). The aqueous extract of the leaves has been found to possess antifertility activity (Prakash, 1998) and is very useful in regulating the thyroid hormone status in adult Swiss rats (Tahiliani and Kar, 2000).

M. oleifera is rich in various phytochemicals like carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids, moringine, moringinine, phytoestrogens caffeoylquinic acids and phenolics in flowers, leaves, roots, fruits and seeds (Anwar *et al.,* 2007 and Vongsak *et al.,* 2014). In addition, it contains vitamin B and several amino acids which could be consumption of nutritious vegetable recommended for children to overcome malnutrition in some countries (Fuglie, 2001). *Moringa* leaves are quite rich in minerals; however, their bioavailability is likely to be reduced by the presence of oxalates and phytates at concentrations of 4.1% and 3.1%, respectively (Foidl *et al.,* 2001). The polyphenols have antioxidant properties as they could neutralize or quench oxidants (Pietta, 2000).

Several low molecular weight bioactive compounds from *Moringa* seeds with bactericidal, fungicidal and immunosuppressive activities were identified (Mahajan and Mehta, 2010). Recent research has noted that synergistic properties between individual bioactive compounds in *M. oleifera* leaves act in broad aspects of physiology, such as nutrient absorption and processing, and antioxidant action that have potential therapeutic effects (Mbikay, 2012).

Whey is typically drain after the cheese curd forms, being what remains of the milk once the cheese or casein is removed. Generally 100 L of milk is produced 87 L of whey is made as a by-product. Whey contains a nutritional component with biological value, such as α-LA, β-LG, and lactoferrin that help reduce health risks (Smithers *et al.,* 1996). Cheese whey also contains appreciable quantities of lactic and citric acids, non-protein nitrogen compounds (like urea and uric acid) and B group vitamins (Panesar *et al.,* 2007). Whey is also the highest natural source of branched-chain amino acids—isoleucine, leucine, and valine and a major source of glutamine (Joseph *et al.,* 2012). Both branched-chain amino acids and glutamine play a vital role in proper cell function, muscle growth, and protein utilization. Glutamine also feeds the white blood cells throughout the body and the cells that line the intestines (a key center of immune health sweet whey with a pH value between 6 and 7 (Bylund, 1995). Moreover, whey proteins have been used as ingredients in infant formulas, sport bars, bakery products, and yoghurt. Whey has been incorporated into fruit drinks, which were favorably received by consumers (Shukla *et al.,* 2004). The protein ratio is about 0.65, lactose 5.2 and 93% moisture.

Therefore, the aim of this study was to (i) investigate the effects of addition different levels of *Moringaoleifera* leaves aqueous extract on the phenolic compounds, antioxidant activity, preservative activity and minerals content of guava whey beverage, and (ii) evaluate physical and sensory properties of guava whey beverage fortified with *Moringa oleifera* leaves aqueous extract.

2. Material and Methods

Fresh sweet whey (7.4% TS and 0.8% protein 0.8%) was obtained from Faculty of Agric., Cairo Univ., Giza, Egypt. High quality guava fruits were obtained from local market, Giza, Egypt. Fresh *Moringa* leaves were kindly obtained from National Research Center, Agronomy Dep., Giza, Egypt.

Methods

Moringa extract:

Fresh *Moringa* leaves were washed in distilled water to remove the dusts, dried in a hot air-blowing oven at 50°C and ground to a fine powder in a mechanical blender. The powders (10 g) were mixed individually with 100 mL distilled water and maintained at 100°C using a water bath with constant stirring (300 rpm) for 2 hr as suggested by Da Silva *et al.,* (2013). The mixtures were cooled to room temperature, stored overnight at 4.0±2°C and then filtered through 40-mesh screen. The filtrate was concentrated to 50.0% of initial volume at 50°C using a rotary vacuum evaporator (ROTAVAPOR R110, Buchi, Switzerland) and stored at -20°C until used.

Guava juice:

Fresh high quality guava fruits was well washed, cut to pieces and then pulped in a blender. The homogenized mixtures of was filtered through a muslin cloth, the resultant juice was filled into polyethylene bags and stored at -18°C until used.

Preparation of whey beverages:

Five percent of sugar and 0.2g of carboxey methyl cellulose (CMC) were added to sweet whey. The mixture were heated to80°C, cooled to 37°C. Then 20% (w/w) of guava juice were added. 2.5, 5.0 and 7.5% *Moringa* extract were added separately to

serve 3 treatment A, B, C, two control samples were conducted :control 1 and control 2. Control 1 was whey beverage with guava juice without moringa extract. Control 2 was whey beverage with 2.5% *Moringa* extract without guava juice. All sample were stored at 4± 1°C for 10 days. All treatment were conducted in three replicates

Chemical composition analysis:

Whey beverage was dried at 60 °C up to weight stable to obtained solid content. Moisture, total nitrogen, fat, ash and fiber contents of whey beverage samples were determined according the method of AOAC (2012). The protein content was obtained by multiplying the percentage of TN by 6.25. Available carbohydrate content was obtained by difference in order to achieve 100 g/100 g of total composition (FAO, 2003). The pH value was measured using digital pH meter (HANNA, Instrument, Portugal) with glass electrode.

Mineral content of the minerals Mn, K, Cu, Fe and Mg were then run through an Atomic Absorption Spectrophotometer (Varian, AA240, Victoria, Australia) using air acetylene flame to determine the mineral content according to AOAC (2012).

Methanol extracts:

In order to separate the phenols in aqueous extracts, the method of Ramey *et al.,* (1986) was used. All dried samples of beverage were milled, 50 g of each dried sample solved in 200 ml of methanol and shaked for continuous 3 days. These extracts were subjected to filtrate and evaporated by rotary at 40-50 °C. Obtained dried phenols were redissolved in methanol, after evaporation of the solvent, for subsequent chromatographic analysis.

GC–MS profile:

The GC–MS instrument used to separate and detect methanol-extracting beverages according to the method described by AOAC (2012) and Boskou (2005). One gram of dried sample was extracted three times with methanol l2 ml. The extracted was combined and methanol evaporated under reduced pressure. The residue was dissolved in acetonitrile (2ml) and washed two times with hexane (3ml). Acetonitrile was evaporated under vacuum and the residue was dissolved in methanol (1ml). Injections of 10µl from this dissolve extracted lipid in methanol, were performed into using a GC/MS (Agilent Technologies 6890N computerized system coupled to an MSD, Agilent 5973B mass spectrometer).

Total phenolic content:

The total phenolic content of the *Moringa* extract and sample of guava juice with and without *Moringa* extract at different storage periods were determined according to Folin Ciocalteu's method (Swain and Hillis, 1959) with some modifications. In a vial, 50 µL of extract, 800 µL distilled water and 25 µL (0.25 N) Folin Ciocalteu's reagent were mixed and incubated at room temperature for 3 min. Then, 100 µL sodium carbonate solution (1 N) was added and further incubated for 2 hr at room temperature. The absorbance was read at 725 nm using spectrophotometer (Analytikjena, Win Aspect plus, Germany). Gallic acid was used in a standard curve and the results were expressed in terms of Gallic acid equivalent (GAE g/100ml).

Antioxidant activity by DPPH (2, 2-Diphenyl-1-Picrylhydrazyl):

The Antioxidant activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH (Pothitirat *et al.,* 2009). One-milliliter solution of the extract in methanol was added to 0.5 ml of 0.15 mM DPPH solution in methanol. The contents were mixed vigorously and allowed to stand at 20°C for 30 min. The absorbance was measured at 517 nm using spectrophotometer (Analytikjena, Win Aspect plus, Germany). Scavenging activity (SA) of the DPPH free radical was determined from the curve of percent scavenging plotted against the concentration. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect % = $[(A_0 - A_1/A_0) - 100]$,

Where**:** A_0, was the absorbance of the control reaction and A_1, the absorbance in the presence of the sample.

Physical properties:

Viscosity of the whey beverage samples was determined using a Brookfield digital Rheometer (Model Melvern, Kinexus, Germany). Readings were taken at the shear rate 10 s^{-1} using spindle -40 at 200°C. Apparent viscosity measurements were carried out at 20 ^{o}C ± 0.1 and expressed as x 10 $^{-5}m^2/s$.

Sensory evaluation:

Sensory evaluation was carried out on the guava and *Moringa* beverage, using 20 judges selected from their consistency in scoring and the samples were evaluated for flavor, color and appearance using grade of degree 40 (Larmond, 1985).

Statistical analysis:

Analysis of variance (ANOVA) and Duncan's test were conducted using a Statistical Analyses System (SAS, 2004). A probability to $P \leq 0.05$ was used to establish the statistical significance.

3. Results and Discussions

Chemical composition:

The mean values of chemical composition of guava whey beverage fortified with and without *Moringa* leaves aqueous extract are shown in Table 1. The statistical analysis of the five whey beverage either fortified with guava or *Moringa* showed that, there was significant difference at 5% in all beverages according to type of fortified ratio of *Moringa* leaves

extract even though, there were only slight variations among samples, statistical differences were observed (P≤ 0.05). A significant difference was observed (P≤ 0.05) in fiber, moisture, protein, fat and ash, which might be expected for this kind of whey beverages, due to some variations during preparation of whey beverage. Fiber and protein content were increased in whey beverage C and D (major contribution to chemical composition may be attributed to the *Moringa* leaves aqueous derived ingredients in these beverage formulations.

Table (1): Chemical composition of fresh guava whey beverage fortified with and without *Moringa* leaves aqueous extract.

Chemical composition	Guava whey beverages					
	Control 1**	**Control 2*****	**A**	**B**	**C**	**Probability (p≤0.05)**
Fiber (%)	6.84±0.11 [b]	8.09±0.24 [a]	5.91±0.08 [c]	5.36±0.02 [d]	5.84±0.11 [c]	0.001
Moisture (%)	10.10±0.71 [ab]	10.57±0.21 [a]	10.65±0.30 [a]	9.54±0.34 [b]	10.80±0.28 [a]	0.021
Protein (%)	5.95±0.21 [b]	7.15±0.21 [a]	5.95±0.35 [b]	6.25±0.35 [b]	6.85±0.07 [a]	0.000
Fat (%)	2.26±0.00 [d]	2.03±0.07 [e]	3.26±0.11 [c]	3.81±0.13 [b]	4.28±0.11 [a]	0.000
Ash (%)	1.88±0.08 [c]	1.86±0.11 [c]	4.05±0.49 [a]	4.20±0.18 [a]	2.72±0.36 [b]	0.000
Carbohydrates	72.97	70.3	70.18	70.84	69.51	

Mean (n=3± SD) with different letters in the same row imply significant differences at *p≤ 0.05*; Control 1**: control of guava whey without *Moringa* extract, Control 2***: control whey beverage with 2.5% moringa extract and without guava juice; **A**, guava whey beverage fortified with 2.5% *Moringa* extract; **B**, guava whey beverage fortified with 5.0% *Moringa* extract; **C**, guava whey beverage fortified with 7.5% *Moringa* extract.

Minerals content:

Table 2 shows the minerals content in guava whey beverages fortified with and without *Moringa* leaves aqueous extract. A positive relation between addition of aqueous *Moringa* extract to guava whey beverage and their mineral content was noted. Whey beverage containing 2.5% *Moringa extract without* guava juice was higher in Mn, Cu and Fe when compared to the control guava whey beverage without *Moringa* extract. Meanwhile, fortified whey beverage without *Moringa* without guava (D) had a lower than control beverage in potassium content. Regards results, *Moringa* extract is contains a higher ratio of macro nutrient elements, which remain in their fortified whey beverages. These results also indicated by Gowrishankar *et al.,* (2010) that the therapeutic effects of *Moringa* leaves have been attributed to the combined actions of various bioactive components found in the plant that include trace metal ions, especially K, Ca, P, Zn, Mg and Fe.

Table (2): Minerals content in fresh guava whey beverage fortified with and without *Moringa* leaves aqueous extract

Guava whey beverage	Nutrients elements				
	Mn	K	Cu	Fe	Mg
	---------- (mg/kg) ----------				
Control 1**	15.49	8554	11.10	53.05	813
Control 2***	88.31	7661	12.63	113.5	1052
(A)	17.49	9739	7.5	65.13	1252
(B)	61.68	8715	9.42	93.35	1004
(C)	74.91	9587	9.52	108.7	1490

Control 1**: control of guava whey without *Moringa* extract, Contol 2***: control whey beverage with 2.5% moringa extract and without guava juice; **A**, guava whey beverage fortified with 2.5% *Moringa* extract; **B**, guava whey beverage fortified with 5.0% *Moringa* extract; **C**, guava whey beverage fortified with 7.5% *Moringa* extract

Phenolic compounds profile:

Moringa leaves are used as herbal medicines and food supplements because of their natural antioxidant compounds such as phenolic, flavonoids and vitamin C (Sahakitpichan *et al.,* 2011). The results of phenolic compound in the controls and beverage samples are reported in Table 3.

Considering both control beverage were contains 18-nanadecenoic acid (27.99%), p-menthane-1, 2, 3-triol (12.55%) and octadecanoic acid, 4-methyl (11.55%) as the most three abundant compounds (table 3). The lignoceric acid, pentadecanedioic acid, 6-octadecenoic acid, 6-Octadecenoic acid and L-Ascorbic acid, 6-stearate were the second abundant GC-MS identified compound in control beverage containing guava and milk whey. Most of these acids were responsible to decrease pH of control beverage. Other compound less than 1% are responsible to flavor of beverage were identified in control beverage such as lactic acid ethyl ester; nonanoic acid ethyl ester; and propionic acid, 3 nitro. Also, phenol, 4-methyl-2-[5-2-thienyl) pyrazol-3-yl] (3.55%) can identified in the control beverage as phenolic antioxidant material. Consider to fortified the control beverage with 2.5% Moringa extract (control 2) phenol, 4-methyl-2-[5-2-thienyl) pyrazol-3-yl] was defiantly increased up to 20.25%. This increased in phenolic compound in beverage A may attributed to the content of phenolic compounds in *Moringa* extract. Vitamin D2 and Vitamin A were identified in

equal ratio (4.52%) in the 2.5% *Moringa* beverage (A). Both of Butyric acid, 2-hydroxy-3-mthyl, methyl ester (17.47%) and Propanoic acid, 3-nitro (2.53%) were identified in beverage (A) which consider as acids of flavoring material and preserve the product. Another types of acids could identified in 5.0% Moringa beverage with guava, acetic acid and propionic acids were the most abundant acids in beverage (B) raged 7 up to about 21%.The major monoterpene hydrocarbons of volatile oil in amounts less than 3% were Limonene in beverage B.

Table 3 shows about 10 major volatile compounds as identified in the beverage C. These compounds include different chemical classes, such as alcohols, ketones, aldehydes and one heterocyclic compound. In particular, the identified volatile compounds were acetic acid, 10 aroma volatiles. Among these identified compounds2-hexyldecanoic acid, Dodecanoic acid and Docanoic acid, 2-hexyl were the most abundant volatile acids in the beverage C, this is attribute to the increase ratio of aqueous *Moringa* extract in whey guava beverage. Corresponding to add *Moringa oleifera* extract in beverage, type of natural volatile antioxidants was obvious in GC-MS profile. Most of these volatile is concern in 2-hexyldecanoic acid, 2-octadecoxyethanol, p-Methane-1, 2-diol, and Geranylisovalerate. These compounds were similar to identify by Mukunzi, *et al.,* (2011) in *Moringa* leaves and Leon-Rodriguez *et al.,* (2008). Control 2 sample, it is the interested of propionic acid, 3 nitro (41.02%); hexanoic acid (19.84%), and –ethyl cyclobutanol (14.70%) were contained as most predominate identify compounds (Table 3). A wide variety of essential oils are known to possess antimicrobial properties and in many cases this activity is due to the presence of active constituents, mainly attributable to isoprenes such as monoterpenes, sesquiterpenes and related alcohols, other hydrocarbons and phenols (Berger, 2007). In this study, the *Moringa oleifera* extract contains hydrocarbon and alcoholic as well as phenolic, might be consider as microbial inhibition and increased preservative activity of fortified beverage by *Moringa*. The compounds also are increase self-life of beverage contains *Moringa* leaves extract. In addition, it can be deduced that there is a relationship between the chemical components of the essential oils and the antimicrobial activity of aqueous *Moringa* leaves.

Total phenol content (TP)

Table 4 considerable antioxidant capacity presents the analytical data for total phenolic of the studied samples of fortified guava whey with *Moringa* extract beverages. The total phenolic compounds of the extracts were expressed as Gallic acid Equivalent in g/100ml. Considerable antioxidant capacity was also detected in five beverages along storage period 5 and 10 days. The fortified beverage with 7.5% *Moringa* leaves extract, significantly ($P \leq 0.05$) affected the amount of TP in the (C) (5.56 g/100ml) at zero time of prepare of beverage. Meanwhile, there is non- significantly affected between control1 and 2 at the zero time. At the storage period more than 5 days the beverage C has a considerable amount of TP and significantly higher than control1 beverage.

Whereas, TP was significantly ($P \leq 0.05$) decreased after 10 days of storage in beverages C and control 2. However, the beverage C, that was contained 5.0% *Moringa* leaves extract significantly stable and consider as higher in TP at different time of storage period and other beverages samples. The obtained results are very consistent with many previously reported results indicating that phenolic compounds are generally less soluble in water (Wanga *et al.,* 2009). It could be concluded that, the total phenolic contents indicate that most of the potent antioxidant and phenolic compounds in the beverage C.

Antioxidant activity:

Consistent differences ($P \leq 0.05$) in DPPH activity were observed among the different ratio of *Moringa* leaves extract in the whey beverages (Table 5). About 77 to 99% scavenging activity against free radicals from DPPH in the zero time were observed. The radical-scavenging capacities of *Moringa* aqueous extracts have been previously observed in different model systems (Razis *et al*., 2014 and Ndhlala *et al.,* 2014).

The *Moringa* leaves extracts was show a higher scavenging activity in the fortified beverages with 5.0 and 7.5% *Moringa* in guava whey beverages especially a long of storage period (10 days). Meanwhile, the 2.5% *Moringa* beverage extract was quite similar to control in zero and after 5 days of storage. The positive anti-oxidation and preservation of *Moringa* in beverage B and C, its might attributed to the synergistic role of natural compound and vitamins were found in these beverages. The scavenging potential of fortified *Moringa* leaves extract was twice greater in B and C than original beverage without *Moringa* extract, followed by in beverage A. Furthermore, the presence of a negligible concentration of ascorbic and acetic acids in the respective extracts contributed to the effectiveness of phenolic-induced reducing power. Moreover, the hydrophilic antioxidant and ascorbic acid were less effective in an oil-in-water emulsion system, whereas the opposite trend was found for the hydrophobic antioxidants (phenolic acids, flavanones, flavonols, etc.).

Table (3): Comparison of phenolic compound in guava whey beverages fortified with and without *Moringa* leaves aqueous extract

Phenolic compounds	Control 1**		A		B		C		Control 2***	
	Area %	RT	Area %	RT	Area %	RT	Area %	RT	Area %	RT
Propionic acid,3 nitro	0.97	4.6							41.02	4.6
Acetic acid, 2-benzolthio. 2-oxo-2-phenylester	3.47	4.73							9.66	4.73
Lactic acid, ethyl ester	0.89	5.75								
Cyclohexene, 4-isopropenyl-1-methoxymethoxymethyl	0.85	6.69								
B-Asarone	0.69	8.31								
Nonanoic acid, ethyl ester	0.77	8.52								
Ocimene	0.77	9.08								
Heptadecanoic acid	1.74	10.21								
Aracdeic acid	0.97	11.58	2.53	11.6						
Fumaric acid, 2,2-dichloroethyl pentadecyl ester	2.51	12.23								
Eurcic acid	2.12	12.65	24.05	12.65						
9-Hexadecenoic acid	1.74	13.66	5.06	13.66						
Phenol, 4—methyl-2-[5-2—thienyl) pyrazol-3-yl]	3.55	15.53	20.25	15.3						
6-Octadecenoic acid	6.76	16.16								
L-Ascorbic acid, 6-stearate	4.63	17.37								
Pentadecanedioic acid	7.72	17.53								
Lignoceric acid	7.72	18.08								
Octadecanoic acid, 4-methyl	11.58	18.15								
p-Menthane-1,2,3-triol	12.55	18.93								
18-Nanadecenoic acid	27.99	19.57								
Butyric acid, 2-hydroxy-3-mthyl, methyl ester			17.47	4.08						
Acetic acid, ethoxy, ethylester			6.33	4.15	6.56	4.15				
Propioic acid, 2-hydroxy, ethyl ester			3.80	4.38	3.28	4.38				
Propanoic acid, 3-nitro			2.53	4.79	1.09	4.79	1.59	4.79		
Limonene			1.27	6.75	2.84	6.75				
Vitamin A aldehyde			4.56	8.24	0.00					
-himachaleneα			3.80	10.70	1.09	10.70				
Patchoulene			3.80	10.87	3.94	10.87				
Vitamine D2			4.53	11.41	0.00					
2,4-Di-tert-butylphenyl benzoate					5.03	3.62				
Propanoic acid, 2,3-dihydroxy-2-methyl					18.60	4.06				
Acetic acid, 2,2-[oxybis (2,1-ethanediyloxy)]bid					12.04	4.26				
Propionic acid					20.79	14.70				
Pentanoic acid					17.51	15.80				
Acetic acid, 2,2-[oxybis (ethanediyloxy)]bis					7.22	15.9	1.88	5.32		
2-Propenoic acid, tetradecyl ester							3.10	12.76		
p-Methane-1,2-diol							6.38	13.78		
Undecanoic acid							5.91	15.14		
Methol, 1-(butyn-3-one-1-yl),(1R,2S,5R)							5.44	15.45		
Dodecanoic acid							7.32	16.39		
2-octadecoxyethanol							2.81	16.64		
14-pentadecenoic acid							5.16	16.80		
Geranylisovalerate							3.75	17.04		
o-Anisic acid, 3.6 –dichloro-5hydroxy							1.41	17.21		
9-hexadecenic acid							4.69	17.46		
1-hexadecsnol,2-methyl							5.63	17.70		
z-11-tetradecenoic acid							5.16	17.81		
2-methyl-E,E-3-13-Octadecadien-1-ol							5.16	17.98		
Pentadecanoic acid							1.88	18.07		
2-hexyldecanoic acid							9.29	18.64		
n-hexadecanoic acid							3.28	19.27		
Docanoic acid, 2-hexyl							7.97	19.55		
Liolenin, 1-mono							7.04	19.92		
-2Ethyl cyclobutano									14.70	8.0
)Z)-3-Octenol									0.48	13.9
3-Methylbutanoic acid									1.61	16.04
Hexanoic acid									19.84	17.71
4-Hexenoic acid									0.36	18.38
E-beta-Ionone									2.20	18.74
2-Hexenoic acid									2.11	18.81
Phenyl acetonitrile									0.8	18.61
Octanoic acid									1.15	19.60
beta-Ionone epoxide									1.77	19.21

Control 1**: control of guava whey without *Moringa* extract, Control 2***: control whey beverage with 2.5% *Moringa* extract and without guava juice. **A**: guava whey beverage fortified with 2.5% *Moringa* extract, **B**: guava whey beverage fortified with 5.0% *Moringa* extract and **C**: beverage fortified with7.5% *Moringa* extract.

Generally, antioxidative potential was expressed at a later stage resulting in higher DPPH scavenging activity values initially (zero time) and a latter (10 days) decrease in the most of prepared beverages except for fortified *Moringa* leaves extract with guava whey at concentration 2.5%, 5.0% and 7.5%. On the other hand Vongsak *et al*. (2013), the investigation of chemical stability of the leaf extract of *M. oleifera* has been limited.

Table (4): Total phenol content (g/100ml) and relation of progressive stability in guava whey fortified with *Moringa* extract

Guava whey beverages	Total phenol content at different storage periods*		
	Zero time	**5 days**	**10 days**
Control 1	2.083±0.29 [C]	4.670±0.01 [c]	2.717±0.04 [c]
Control 2	2.450±0.06 [c]	2.363±0.12 [d]	1.853±0.06 [d]
A	3.020±0.04 [b]	4.547±0.15 [c]	4.337±0.08 [b]
B	3.490±0.11 [b]	5.257±0.02 [b]	5.353±0.17 [a]
C	5.560±0.21 [a]	5.747±0.12 [a]	1.520±0.05 [e]
Probability	0.0001	0.0001	0.0001

Mean (n=3± SD) with different letters in the same row imply significant differences at $P \leq 0.05$**;** Control 1**: control of guava whey without *Moringa* extract, Control 2***: control whey beverage with 2.5% *Moringa* extract and without guava juice. **A**: guava whey beverage fortified with 2.5% *Moringa* extract, **B**: guava whey beverage fortified with 5.0% *Moringa* extract and **C**: beverage fortified with 7.5% *Moringa* extract.

Table (5): Antioxidant activity (scavenging activity SA) in guava whey fortified with and without *Moringa* leaves aqueous extract

Items	**Storage period (day)**	**Treatments**					
		Cont	**Control 2*** **	**A**	**B**	**C**	**Probability p≤0.05**
SA_{DPPH}• (%)	**zero**	79.33±0.35 [c]	77.02 ±0.07 [d]	76.50 ±0.40 [d]	99.19 ±0.42 [a]	98.27 ±0.34 [b]	0.0001
	5	73.48 ±0.91 [d]	78.50 ±1.04 [c]	85.07 ±1.18 [b]	94.77 ±0.81 [a]	94.09±1.07 [a]	0.0001
	10	65.93 ±0.48 [e]	76.41 ±0.57 [d]	86.58 ±1.75 [d]	99.05 ±0.67 [a]	96.27 ±0.44 [b]	0.0001

Mean (n=3± SD) with different letters in the same row imply significant differences at $P \leq 0.05$**;** Control 1**: control of guava whey without *Moringa* extract, Control 2***: control whey beverage with 2.5% *Moringa* extract and without guava juice **A**: guava whey beverage fortified with 2.5% *Moringa* extract, **B**: guava whey beverage fortified with 5.0% *Moringa* extract and **C**: beverage fortified with7.5% *Moringa* extract

pH of bevarages fortified guava whey with Moringa extract:

Fig 1 shows that a gradual increase in pH value was observed with increasing the *Moringa* extract level, while as storage period increased, the pH value of guava whey beverages fortified *Moringa* extract decreased. These positive correlation between concentration of used *Moringa* leaves extract in beverage and pH values according to period zero, 5 and 10 days of storage beverages, was found R^2 = +0.517, +0.384 and +0.457, respectively. Considerably a significant positive correlation between concentration of *Moringa* in the fortified beverage and pH during storage periods has been indicated, that aqueous *Moringa* extract containing preservative agents to increase in the first period of storage and it decreased according to storage period. The fortified guava why beverage with *Moringa* aqueous extract was considered as not stable (Fig 2). pH variations was regular in the fortified *Moringa* beverages during storage. After stored 10 days, the beverage showed small variations in the values of pH.

Viscosity of fortified guava whey with Moringa extract:

As shown in Table 6, viscosity value was higher in guava whey fortified with *Moringa* leaves extract that non fortified. In particular, the viscosity of guava whey beverages fortified with *Moringa* extract ranged from 200 to 240 (x $10^{-5}m^2/s$) at fresh time and ranged from 190 to 500 (x $10^{-5}m^2/s$) at 10 day. The average of viscosity in beverage C (7.5% *Moringa* with guava whey beverage) was the highest viscosity values at the different storage periods. Viscosity also tends to increase during refrigerated storage, which could be attributed to the behavior of the beverage during cooling can discussed based exclusively on the characteristics of its carboxy methyl cellulose (CMC), but also on the influence of protein of sweet whey on the beverage.

The protein in the beverage resulted increase of viscosity depend on molecular structure of whey protein in the beverages. This is in agreement by Oszvald *et al.* (2007). According to the characteristics of *Moringa* leaves extract the forecast of behavior was an increase in viscosity due to CMC and flocculation activity of *Moringa* leaves extracts. These results could be accompanied with the binding of the protein particles in an emulsion is the result of three types of forces, i.e. attractive, repulsive, and steric interactions. The van der Waals attractive interactions are universal in all food emulsions and are desirable because they lead to the formation of a network-like structure to stabilize emulsion products and increase viscosity (Logaraj *et al.,* 2008).

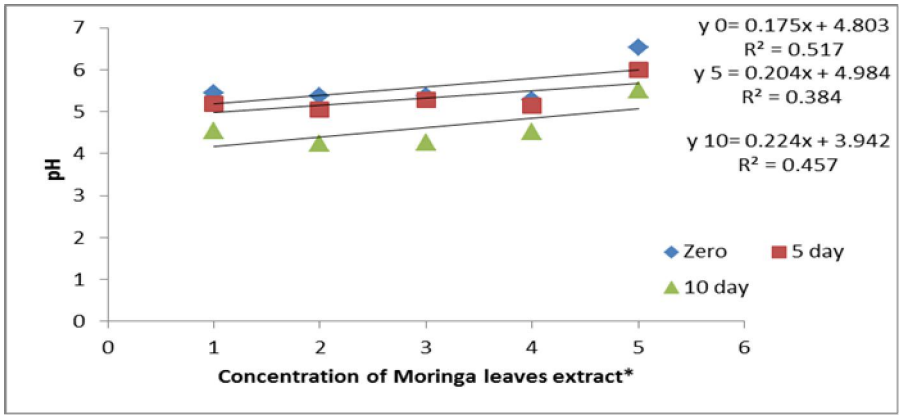

Figure (1): Correlation between concentrations of fortified guava whey with *Moringa*leaves extract on PH value.

Table (6): Viscosity of guava whey fortified with and without *Moringa* leaves aqueous extract

Items	Storage period (day)	Treatments				
		Control 1	Control 2***	A	B	B
Viscosity (x 10 $^{-5}$m^2/s)	zero	190	40	240	220	220
	5	170	60	360	180	180
	10	190	110	190	250	250

Control 1**: control of guava whey without *Moringa* extract, Control 2***: control whey beverage with 2.5% *Moringa* extract and without guava juice **A**: guava whey beverage fortified with 2.5% *Moringa* extract, **B**: guava whey beverage fortified with 5.0% *Moringa* extract and **C**: beverage fortified with7.5% *Moringa* extract

Table (7): Sensory evaluation of guava whey fortified with and without *Moringa* leaves aqueous extract

Sensory properties	Storage period (days)	Treatments				
		Cont	Control 2 ***	A	B	C
Flavour (40)	zero	36	30	38	35	35
	5	30	30	38	35	35
	10	30	30	36	35	35
Colour (40)	zero	36	32	36	36	32
	5	32	30	34	36	30
	10	32	30	32	32	30
Appearance (40)	zero	38	30	38	35	35
	5	35	30	35	35	32
	10	35	30	35	34	32

Control 1**: control of guava whey without *Moringa* extract, Control 2***: control whey beverage with 2.5% *Moringa* extract and without guava juice **A**: guava whey beverage fortified with 2.5% *Moringa* extract, **B**: guava whey beverage fortified with 5.0% *Moringa* extract and **C**: beverage fortified with7.5% *Moringa* extract.

Sensory evaluation:

Sensory evaluation in guava whey beverages fortified with and without *Moringa* leaves aqueous extract is shown in Table 7. After preparation of the beverages, no major changes occurred in flavor of all five beverages except for fortified beverage with 7.5% *Moringa* extract that recorded decrease in color and appearance scores compared to control beverage. Control 2 beverage has lower in the flavor and appearance at zero time, 5 and 10 days. Both beverage B and C had shown a little decrease in flavor, color and appearance in zero time up to 10 days. Beverages A and B gained higher scores in flavor, color and appearance throughout storage period. This result indicated that, the fortification of whey guava beverage with 2.5 or 5% was the fit ratios for using *Moringa* leaves extract in such beverage.

Conclusion

The *Moringa oleifera* extract contains hydrocarbon and alcoholic as well as phenolic, might be consider as microbial inhibition and increased preservative activity of fortified beverage by *Moringa.* These compounds also, are increase self-life of beverage containing *Moringa* leaves extract. Fortification of whey with guava juice and *Moringa* leaves aqueous extract produced beverage with higher nutrients like protein, fiber and mineral contents. Also, the guava whey beverage fortified with 5.0% *Moringa* extract was more stable and consider as

higher in TP and antioxidant activity at different time of storage period.

References

1. Anwar, F., Latif, S., Ashraf, M. and Gilani, A.H. (2007). *Moringa oleifera*: a food plant with multiple uses. Phytother. Res., 21: 17–25.
2. AOAC, (2012). Official Methods of Analysis. 19 thed. Gaithersburg, MD: AOAC International.
3. Babu, R. and Chaudhuri, M. (2005). Home water treatment by direct filtration with natural Coagulant. Journal of Water and Health,3: 27–30.
4. Berger, R.G. (2007). Bioactivity of essential oils and their components. In: Flavors and fragrances: Chemistry, bioprocessing, and sustainability. Germany: Springer; p.88-90.
5. Boskou, D., Blekas, G., and Andtsimidou, *M.* (2005). Phenolic compounds in olive and olives current topics in nutaceutiacl Research., 3:125-136.
6. Bylund, G. (1995). Dairy Processing Handbook; Tetra Pak Processing Systems: Lund, Sweden.
7. Caceres, A., Cabrera, O., Morales, O., Mollinedo, P., Mendia, P., (1991). Pharmacological properties of Moringaoleifera 1: preliminary screening for antimicrobial activity. Journal of Ethno pharmacology 33, 213–216.
8. Chaudhary, R.D., Chopra, R.D., (1996). Herbal Drug Industry: A Practical Approach to Industrial Pharmacognosy. Eastern Publishers, New Delhi, pp58.
9. Da Silva J. K., C. B. B. Cazarin, T.C. Colomeu, Â. G. Batista, L.M.M. Meletti, J. A. R. Paschoal, S. B. Júnior, M. F.Furlan, F. G. R. Reyes, F. Augusto, M.R. M. Júnior,R. D. L. Zollner (2013). Antioxidant activity of aqueous extract of passion fruit (Passifloraedulis) leaves:In vitro and in vivo study. Food Research International 53 882–890.
10. Davidson, P. M., (2006). Proceedings of Ist international symposium on natural preserve in food systems. Acta Horticulturae 709, 29–33.
11. Fahey, J. W., (2005). Moringa *oleifera* : a review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Part 1 Trees for Life Journals, 5, www. TFL journal. Org/johns Hopkins School Medicine, Dept of Pharma and Molecular Sciences.
12. FAO. (2003).Food energy Methods of analysis and conversion factors. Report of a technical workshop. FAO food and nutrition paper 77. Rome: Food and Agriculture Organization of the United Nations. http://www.fao.org/DOCREP/006/Y5022E/Y5022E00.HTM.
13. Foidl, N., Makkar, H.P.S., and Becker, K., (2001). The potential of *Moringaoleifera* for agricultural and industrial uses. In: Fuglie, Lowell J. (Ed.), The Miracle Tree: The Multiple Uses of *Moringa*, CTA, Wageningen, The Netherlands, pp. 45–76.
14. Gowrishankar R, Kumar M, Menon V, Divi SM, Saravanan M, Magu-dapathy P, et al. (2010). Trace element studies on Tinosporacordifolia (Menispermaceae), Ocimum sanctum (Lamiaceae), *Moringa oleifera* (Moringaceae) and Phyllanthusniruri (Euphorbiaceae) using PIXE. Biol Trace Elem Res; 133:357–63.
15. Gulmez, M., Oral, N., Guven, A., Vatansever, L., and Baz, E., (2006). Antibacterial activity of oregano tea and a commercial oregano water against Escherichia coli O157:H7, Listeria monocytogenes 4b, Staphylococcus aureus and Yersinia enterocolitica 03. Int. J. Food Sci. 8: 7–13.
16. Joseph P., Pizzorno Jr and Murray M., (2012). For complete discussion of glutamine, branched-chained amino acids, and whey's nutritional profile, see Textbook of Natural Medicine.
17. Jung, I. L. (2014). Soluble extract of Moringa oleifera leaves with a new anticancer activity. PLOSO ne 9.
18. Kauer, C., and Kapoor H. C., (2001). Antioxidants in fruits and vegetables — The millenium's health. International Journal of Food Science and Technology, 36, 703–725.
19. Kellof G. J. (2000). Perspective on cancer chemoprevention research and drug development. Advences in Cancer Res., 78:199-334.
20. Larmond, E. (1985). Laboratory methods for sensory evaluation of foods. Canada Agriculture Canada.
21. Leon-Rodriguez, A.D., Escalante-Minakata, P. Jiménez-Garcia, M.I. Ordoñez-Acevedo, L.G. Flores J.L.F and Rosa, A.P.B.D.L. (2008). Characterization of volatile compounds from ethnic agave alcoholic beverages by gas chromatography-mass spectrometry. Food Technol. Biotechnol., 46: 448-455.
22. Limaye, D.A., Nimbkar, A.Y., Jain, R., and Ahmed, M., (1995). Cardiovascular effects of Moringa pterygosperma. Phytother. Res. 9: 37–40.
23. Logaraj T V, Bhattacharya S, Sankar K U, and Venkateswaran G. (2008). Rheological behavior of emulsions of avocado and watermelon oils during storage. Food Chem, 106: 937–943.

24. Maubois, J. L., A. Pierre, J. Fauquant, and M. Piot. (1987). Industrial fractionation of main whey proteins. Dairy Research Laboratory, Rennes Cedex, France.
25. Mbikay, M., (2012). Therapeutic potential of Moringaoleifera leaves in chronic hyperglycemia and dyslipidemia: a review. Front. Pharmacol. 3: 1–12.
26. Mleko, S., Kristinsson, H. G., Liang, Y., and Gustaw, W. (2007). Rheological properties of foams generated from egg albumin after pH treatment. *LWT*-Food Science and Technology, 40:908-914.
27. Mukunzi, D., Nsor-Atindana, J. Xiaoming, Z. Gahungu, A. Karangwa E. and Mukamurezi G. (2011). Comparison of Volatile Profile of *Moringa oleifera* Leaves from Rwanda and China Using HS-SPME. Pakistan Journal of Nutrition 10 (7): 602-608.
28. Nautiyal, B. P. and Venharaman K. G., (1987). *Moringa* (drumstick) an ideal for social forestry: growing conditions and uses. Part 1, My Forest 23:53.
29. Ndhlala A, Mulauudzi R, and Ncube B, (2014). Antioxidant, antimicrobial and phytochemical variations in thirteen *Moringa oleifera* Lam. Cultivars. Molecules19: 10,480–10,494.
30. Oszvald M, Tömösközi S, Larroque O, Kereszténvi E, Tamás L, and Békés F. (2007). Characterization of rice storage proteins by SE-HPLC and micro z-arm mixer. J Cereal Sci,, 48(1): 68–76.
31. Panesar, P.S.; Kennedy, J.F.; Gandhi, D.N.; and Bunko, K. (2007). Bio utilization of whey for lactic acid production. Food Chem. 105: 1–14.
32. Pietta, P. G. (2000). Flavonoids as antioxidants. Journal of Natural Products, 63(7): 1035–1042.
33. Pothitirat, W., Chomnawang, M.T., Supabphol, R., and Gritsanapan, W., (2009). Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity. Fitoterapia 80: 442–447.
34. Prakash, A.,(1998). Ovarian response to aqueous extract of *Moringa oleifera*. Fitoterapia 59, 89–91.
35. Ramey, D., Bertrand, A., Oough, C. S., Singleton, V. L., and Sanders, E. (1986). Effects of skin contact temperature on chardonnay must and wine composition. American Journal of Enology and Viticulture, 37, 99-106.
36. Ruck, J. A. (1969). Chemical methods for analysis of fruits and vegetable product Summerland B.C 14-33.
37. Ruckmani, K., Kavimani, S., Anandan, R., Jaykar, B., (1998). Effect of *Moringa oleifera Lam*. on paracetomol induced hepatotoxicity. Indian Journal of Pharmaceutical Science 60, 33–35.
38. Shukla, F. C., Sharma, A. and Singh. B. (2004). Studies on the preparation of fruit beverages using whey and buttermilk. J. Food Sci. 41:102–105.
39. Sithisarn, B., Mangmool, P., Thongpraditchote, S., Wongkrajang, S., and Gritsanapan W., (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringaoleifera leaf extract by the appropriate extraction method. Ind. Crop Prod. 44:566–571.
40. Smithers, G. W., Ballard, J. Copeland, A. D. De Silva, K. J. Dionysius, D. A. Francis, G. L. Goddard C., Grieve, P. A. McIntosh, G. H. Mitchell, I. R. Pearce, R. J. and Regester. G. O. (1996). New opportunities from the isolation and utilization of whey proteins. J. Dairy Sci. 79:1454–1459.
41. Souza, E.L., Lima, E.O., Freire, K.R.L.C.P., Sousa, K.R.L., (2005b). Inhibition action of some essential oils and phytochemicals on the growth of moulds isolated from foods. Brazil. Arch. Biol. Technol. 2: 245–250.
42. Sreelatha, S., Jeyachitra, A. and Padma, P. R. (2011). Anti pro*leifera*tion and induction of apoptosis by *Moringa oleifera* leaf extract on human cancer cells. Food and Chemical Toxicology 49, 1270-1275.
43. Swain, T., and Hillis, W. E. (1959). The phenolic constituents of Prunusdomestica. I.—the quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture, 10(1), 63–68.
44. Tahiliani, P., and Kar,A., (2000). Role of *Moringa oleifera* leaf extract in regulation of thyroid hormone status in adult male and female rats. Pharmacological Research 41, 319–323.
45. Vongsak, B, Sithisam P, Gritsanapan W. (2014). Simultaneous HPLC quantitative analysis of active compounds in leaves of *Moringa oleifera* Lam.J Chromatogr Sci 52: 641–645.
46. Wanga, T., Jonsdottir, R., Lafsdottir, G.,(2009). Food Chem. 116, 240– 248.
47. WHO (1994). Study group on diabetes mellitus,. Technical Report Series No. 844, World Health Organization, Geneva, pp. 78–79.