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Utilization Whey in Production of Functional Healthy Beverage "Whey-mango Beverages"

[1]Ahmed S. Gad, [2]Wafaa H. Emam, [2]Gamal F. Mohamed and [1]Ahmed F. Sayd
[1]Department of Dairy Science, [2]Department of Food Technology, National Research Centre, Dokki, Giza, Egypt

Corresponding Author: Ahmed S. Gad, Department of Dairy Science, National Research, Centre, Dokki, Giza, Egypt

ABSTRACT

Beverages can be a refreshing way for consumers to get an increasing range of health-promoting ingredients. The main objective of this investigation was utilizing whey in production of functional beverage as a whey-based fluid. Whey was fortified with mango powder (20.0%), flaxseed oil (0.5%) and (pectin JMJ and monoglyceride, 0.5% w/w), homogenized, pasteurized and then kept cool. Beverages were monitored at specific time intervals over a 15 day storage period. Physical properties and chemical composition was tested. Total Antioxidant Capacity (TAC), were analyzed using three different assays, i.e., DPPH free radical scavenging activity, Trolox Equivalent Antioxidant Capacity (TEAC) and Ferric Reducing-antioxidant Power (FRAP). These assays, based on different chemical mechanisms, were selected to take into account the wide variety and range of action of antioxidant compounds present in beverage. Results showed a new functional beverage with special characteristics has a little sedimentation and low viscosity exhibit excellent flavor derived from mango. This functional beverage increased its nutrient content including omega 3, minerals, vitamins and unique proteins, besides digestible carbohydrates. The polyphenol-rich and carotenoids in beverage displayed high antioxidative capacities. Beverage was rich in polyphenol and carotenoids exhibited high antioxidative capacities. This whey-based beverage is put a wide range of components acting together synergistically that role, maintain and improve of a consumer health.

Key words: Whey, mango powder, functional beverage, antioxidant capacity, nutritional value

INTRODUCTION

Consumers are usually looking for ways to improve their health whether it's changing their diet, lifestyle. Whey is enriched by biologically active ingredients or valuable organic complements gained from nature's resources (not using chemical synthesis) e.g., nutritious protein source which is a high-quality that provides all of the essential amino acids necessary for good health (Kimball and Jefferson, 2001; Layman, 2002). On the other hand, mango fruit contains essential vitamins and dietary minerals besides other phytonutrients, such as antioxidant pigments (Rocha Ribeiro *et al*., 2007; Ajila and Rao, 2008), carotenoids, such as the provitamin A compound, beta-carotene, lutein and alpha-carotene (Gouado *et al*., 2007). Mango powder, free of bitterness, could be successfully prepared starting from the fresh mango pulp juice and used in processing beverage. The process of drying of mango pulps is technology that developed for manufacture of mango with extended shelf life. Addition of mango pulp improves the nutritional value of whey and exhibit excellent flavor derived from mango. Mahattanatawee *et al*. (2006) and Singh *et al*. (2004)

reported that mango also included phytochemicals such as polyphenols (Barreto *et al.*, 2008). Moreover, enrichment of beverage with essential fatty acids gives functional features for a final product for health promotion. It has strong antioxidant capacity (Malcolmson *et al.*, 2000; Przybylski and Daun, 2001) and exhibits potential health benefits that include reducing the risk of cardiovascular disease, cancer and diabetes (Bhatty, 1995; Westcott and Muir, 1997, 2003; Setchell *et al.*, 1980, 1981; Thompson, 1999; Adlercreutz *et al.*, 1986; Thompson *et al.*, 1996; Adlercreutz *et al.*, 1988). The aim of the current study is to utilize whey as a base of beverage supplemented with mango powder and flaxseed oil that adds health promoting value and produce a nutritive and healthy beverage.

MATERIALS AND METHODS

Whey preparation: Dried sweet whey powder resultant from cheese production was purchased from Salon Valley Dairies LTD, (Canada) and was reconstituted in distilled water by dissolving 6 g spray dried whey powder (0.6% protein) per 100 mL of distilled water and left overnight at 4°C to allow full hydration.

Drying of mango pulps: Mango pulp was purchased from local markets at Cairo and was slicing into thin and speeded on the clear trays (46 cm wide, 70 cm long and about 5 cm deep) of electric food dehydrator. The dehydration trays were held on stands made of metal (about 82 cm high) set the dehydrator temperature at 50°C. Allow the dehydrator to run until the desired consistency is reached. The dehydrated mango slices were put in an air-tight container or resalable plastic bag inside cartoons boxes and stored at room temperature. The process ensures that the natural flavour and aroma of the dehydrated mango is retained under good hygienic condition and stored away from moisture in air-tight containers (Campbell and Campbell, 1983).

Processing whey-based mango beverage: Reconstitute sweet whey was used as based beverage and 0.5% flaxseed oil (purchased from Fiber Crop Research Center, Agriculture Research Center, Giza, Egypt) was mixed with whey at 45°C, sonicated and blended until the oil droplets broken up into smaller particles that cannot be seen by the naked eye. Mango powder (20.0 g) was blended in whey including Vit C in its salt form (sodium ascorbate-$C_6H_7NaO_6$) to prevent discoloration before any thermal processing steps was undertaken. Hydrophobic and hydrophilic aqueous phases were mixed with adding the mixture of prepared stabilizer-emulsifier (pectin JMJ and monoglyceride, 0.5% w/w) and then made up to 100 mL whey for each sample followed by mild heating to 40°C and blended. The mixture is being mixed again, heated up to 60-70°C, homogenized under pressure of 10-25 MPa (100-250 bar), pasteurized at a temperature of 75°C for 6 min then cooled down to 10-30°C and filled into dark glass bottles then cooled in ice and held at 5°C until analysis. Flavor was adjustment with sucrose (10% w/w). Beverage samples were evaluated fresh and after storage 7°C at one and 2 week old.

Physical properties of the whey-mango beverage

Sensory evaluation: Samples were exposed to a sensor analysis so that the following characteristics were estimated: organoleptic assessment of the beverage samples were carried out by 10 specific stuff members, for appearance (40 points), colour (20 points) and flavor (40 points).

Apparent viscosity of beverage (cP.s): Apparent viscosity was based on measuring resistance to a rotating spindle (Brookfield Model DV III, Programmable rheometer). The instrument was equipped with an 18 measuring head. Rheological behavior depends on time of shearing. Test samples were subjected to shear rate a spindle speed of 50 rpm and spindle rotating velocities, at constant temperature (25°C) for 5 min. Samples were allowed to relax (more than 10 min) prior to measure their viscosity. All apparent viscosity measurements were expressed in centipoise seconds (cP.s) and were performed in triplicate.

Storage stability: The physical stability of the products was analyzed by measuring whey layer and sediment during storage.

Evaluation of storage stability: Whey-mango beverage samples were placed in 5 mL disposable pipettes sealed at both ends with parafilm and samples were stored at 7°C fresh and 2 week old to assess serum separation under gravity. The volume of a layer of clear serum (WL) at the top was recorded as an indication of instability and the percentage volume of serum separated from the total volume was expressed as a percentage of the total fluid height in the bottle according to the following equation (Lucey and Singh, 1997):

$$\text{WL (\%)} = \frac{\text{Whey layer height}}{\text{Total fluid height}} \times 100$$

Sedimentation (phase separation layers): Sedimentation was monitored after prepared beverage according to Towler (1984). Sedimentation was assessed by centrifugation at 3000x g for 20 min at ambient temperature. Sediment was calculated as a percentage of the total fluid weight according to the following equation:

$$\text{Sediment in tube (\%)} = \frac{\text{Sediment after 24 h}}{\text{Fluid total weight}} \times 100$$

Chemical composition of whey-mango beverage: pH, titratable acidity, total protein, total carbohydrate and total solids of whey-mango beverage was determined. The pH was measured using a Jonway 705 pH meter. Titratable acidity was determined by titrating with 0.1 N NaOH using phenolphthalein as an indicator (Laye *et al*., 1993). Total protein was determined spectrophotometrically using a dye-binding assay (Bradford, 1976), total carbohydrate was determined according to Taylor (1995) and glucose standard curve was used for the calculation of carbohydrate content. Total solids were determined by analyzing the formulations, in duplicate, following standard methods (Hooi *et al*., 2004).

Determination of omega 3 concentration of whey-mango beverage: Omega 3 concentration was determined according to the method described by Park and Goins (1994). The upper phase which has fatty acid methyl esters was transferred to a clean test tube and stored at 4°C until analyzed. Fatty acid methyl esters were separated to fatty acid profile by Gas Chromatography (GC) with a flame ionization detector on 50 m capillary columns. The carrier gas used was helium set at a flow rate of 1.8 mL min^{-1} and the split-ratio was 20:1. Identification of compounds was

based on the retention time of known standards including Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA). Internal standard (25 mg mL^{-1}) was added (1 mL) to lipid samples prior to quantification following (AOCS, 2004).

Determination total soluble solids (TSS) of whey-mango beverage (Brix°): Total soluble solids content in the whey-mango beverage was determined (°Bx readings) using an Abbe Mark II digital refractometer (Lecia Inc., Buffalo, NY) by placing 0.5 g beverage on the lens and reading the sample for temperature corrected Brix. The equipment was calibrated with deionized water (refraction index = 1.3330 and 0° Brix at 20°C) and the readings of the samples were performed (°Brix or g/100 mL) (Cavalcanti *et al.*, 2008).

Determination of HCI-soluble mineral (K, Ca and P) concentrations in whey-mango beverage by atomic absorption spectrophotometer (Varian spectr AA 220): Minerals (K, Ca and P) concentration in beverage was determined by atomic absorption Spectrophotometer (Varian spectra AA 220) as described by Saadatu and Mshelia (2013). Beverage samples were stored at 7°C fresh and 2 week old were evaluated. Samples in aqueous media are aspirated into a high temperature flame. The amount of light absorbed by the element or metal atom is proportional to the concentration of the element and this relationship is known as Beer-Lambert's Law (Elmer, 1993).

Determination of water-soluble vitamin (ascorbic acid-vit. C) concentration in whey-mango beverage: Ascorbic Acid content was estimated by Iodine titration method as described by Helmenstine (2008). Standard ascorbic acid solution was prepared by taking 50 mg ascorbic acid and making up its volume to 50 mL with 3% HPO_3 solution; an aliquot of 5 mL from this solution was made up to 50 mL with 3% HPO_3 solution. 42 mg of $NaHCO_3$ was dissolved in 150 mL hot distilled water. Fifty mg of the dye, 2, 6-dichlorophenol indophenol were added and the volume was made up to 200 mL with distilled water to prepare the Dye solution. Five mL of standard ascorbic acid solution was taken and mixed with 5 mL of 3% HPO_3 solution. Five mL of sample was blended with 50 mL of 3% HPO_3 solution and filtered. Two mL was taken from this solution and titrated against the dye. Dye Factor was 0.5/Titre and the calculations were done according to Ranganna (2001) using the following equation:

$$\text{Ascorbic acid (mg/100)} = \frac{\text{Titre} \times \text{dye factor} \times \text{volume made up} \times 100}{\text{Aliquot of extract taken} \times \text{Weight of sample taken}}$$

Determination of fat-soluble vitamin concentration (vitamin E-α-Tocopherol) by HPLC: Analysis was performed by high-performance liquid chromatography (HPLC) using a ProStar 363 (Varian) equipped with a ProStar fluorescent detector and a Restek Pinnacle II silica column (15×4.6 mm, 5 μm) using ISO method (EN ISO, 2006). Detection of tocopherols was performed at an excitation wavelength of 295 nm and emission wavelength of 330 nm. Isocratic chromatography at room temperature was used with a mobile phase of 0.7% propan-2-ol in n-hexane at a flow rate of 0.6 mL min^{-1}. Quantification of tocopherols was performed using standard calibration curves of α-and γ-tocopherol covering the mass fraction range of 5-750 mg kg^{-1}.

Determination of total antioxidant capacity (TAC) of whey-mango beverage

Determination of total phenols content: The method of Zheng and Wang (2001) was followed for the determination of total phenol compounds in beverage using Folin Ciocalteu Reagent (FCR) and gallic acid as a standard solution. This assay measures the change in color when metal oxides are reduced by polyphenolic antioxidants such as gallic acid and catechin, resulting in a blue solution with maximal absorption at 765 nm. The total phenol contents were calculated from a standard curve of diluted gallic acid solution and expressed as Gallic Acid Equivalent in (GAE) mg/100 mL beverage.

Measurement of free radical-scavenging activity on α, α,-diphenyl-β picrylhydrazyl (DPPH): The DPPH free radical scavenging activity of whey-mango beverage was assessed according to the method of Larrauri *et al.* (1998) with some modifications. Briefly, 40 μL of beverage sample was mixed with 2.9 mL of 0.1 mM DPPH solution in methanol and the absorbance was measured at 517 nm. A standard curve was prepared for the reaction between 40 μL of Trolox solutions (0.5 mM) and DPPH the same as the samples. The scavenging activity of samples were measured from the prepared standard curve and expressed as μmoles Trolox equivalents/100 mL sample (TE).

Trolox equivalent antioxidant capacity (TEAC) assay: ABTS stock solution was prepared by dissolving ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) in water to a 7 mM concentration. ABTS radical cation ($ABTS^{+1}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (di-potassium peroxdisulfate-Sigma-Aldrich) (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The radical is stable in this form for more than two days when stored in the dark at room temperature. For determination the antioxidant in beverage, 40 mL of the beverage and 1.96 mL of the radical solution were mixed in a cuvette. After 6 min, the absorption was measured at 734 nm (Van den Berg *et al.*, 1999, 2000; Schilling *et al.* 2007; Maier *et al.*, 2009). To calculate the TEAC value (or Trolox equivalents) is assigned by comparing the scavenging capacity of an antioxidant to that of Trolox the standard reference compound. Results of test compounds were expressed relative to Trolox, as TEAC in umol of Trolox equivalents per 100 mL of beverages The TEAC assay is often used to measure the antioxidant capacity of foods, beverages and nutritional supplements (Huang *et al.*, 2005).

Measurement of the ferric reducing antioxidant power (FRAP): The method of Benzie and Strain (1996) was followed for the determination of the reducing capacity of beverage that measured as Ferric Reducing Antioxidant Power (FRAP). Aliquots of 100 μL of blended beverage sample was mixed with 3 mL FRAP reagent and the absorbance of reaction mixture was measured at 593 nm after incubation at 37°C for 10 min. FRAP value (the antioxidant capacity) of beverage was obtained from a standard curve prepared created by Trolox based on the abihty to reduce ferric ions and expressed as umol of Trolox equivalents per 100 mL of beverage (Reihani and Azhar, 2012).

Determination of total carotenoid content (TC) in lipophofilic fraction of beverage by absorption UV-VIS spectrophotometry: Carotenoids are expected to be dissolved in fat phase and were evaluated by the determination of total carotenoid content (TC) by absorption SP-2000UV

UV-VIS spectrophotometry according to Lachman *et al.* (2003). Absorbance of organic extracts was then measured in 1 cm cuvettes at λ = 444 nm and total carotenoid content in mg kg^{-1} fw of sample was expressed as lutein equivalent according to the following equation:

$$(K+X)L = \frac{A444.\ 25.\ 15\ (mg\ kg^{-1}\ fw)}{0.259 \times m}$$

where, $(K+X)_L$ is total carotenoid content (carotenes and xanthophylls), A_{444} is absorbance of acetone extract at λ = 444 nm, m is sample weight (g).

Statistical analysis: Statistical analysis was performed by using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS, 1988). The Least Significant Difference test (LSD) was used to test differences between means (p = 0.05). Standard errors of the means of 3 replicates were derived from the error mean square term of the ANOVA. Correlation between phenol content and antioxidant activity was also determined.

RESULTS AND DISCUSSION

Physical properties of the whey-mango beverage

Sensory evaluation: The main sensory evaluation test for a whey-mango beverage involved 10 specific stuff members. The values of appearance showed none significantly decreasing trend with the passage of storage at 15 days period that recorded values 35, 34, 34, respectively (Table 1). Color has been historically the first sensory attribute by consumers to judge the acceptability and overall quality of food (Clydesdale, 1984). The compounds responsible for color during storage of beverage are produced by the Maillard reaction (Berg and van Boekel, 1994; Pellegrino *et al.*, 1995). Sucrose present in the mango pulp as well as added sucrose will likely be hydrolyzed in to produce glucose and fructose and reduction in color scores although presence of Vit C, but generally color consider in stability state with mean values as 19.0, 18.0 and 17.0 during 15 days of storage (Table 1). The values for flavour were decreased for beverage during storage at 15 days of storage. Mango pulp taste and sucrose were masked the flaxseed oil taste in the beverage. Participants were not informed that beverage contains flaxseed rather than few, but when they informed that flax beverage is a new beverage in the market that may provide a positive health benefit when consumed they welcome. Generally, the samples were acceptable flavour and the values recorded 35, 35 and 33 during fresh, 7 and 15 days of storage at 7°C, respectively. This sensory evaluation studies suggests that whey-mango beverage retain the over all acceptability with good sensory quality. These sensory evaluation help manufacturers in the evaluation of beverage quality and marketing success. Sensory evaluation, the treatment stored at refrigeration

Table 1: The average scores of sensory evaluation of whey-mango beverage

Storage period (days)	Appearance (40)	Colour (20)	Flavour (40)	Total (100)
1	35±1.34	19±0.87	35±2.14	86±3.21
7	34±1.22	18±0.64	35±2.11	82±3.22
15	34±1.37	17±0.31	33±2.09	76±2.98

Appearance: Homogeneity, precipitate and clearness, Flavour: Taste, odor and feeling, Each value is expressed as Mean±SD, n = 3

temperature remained most acceptable and no significant change occurred in it during the storage period. It was found that whey-mango beverage can successfully be stored at refrigeration temperature on commercial scale manufacturing due to significant stability in colour, flavour and taste.

Apparent viscosity of beverage η_{app} (cP.s): Viscosity is a primary factor in the prevention of settling and the aggregation of solids suspended in beverage. It is an important quality factor for providing beverage with quantifiable attributes. Viscosity at a single shear rate value is presented to show the influence of storage on viscosity. The current result showed that storage period affected the apparent viscosity which decreased from 20.7-18.36 cP.s then 17.0 after 1, 7 and 15 days of storage, respectively (Table 2). The statistical results revealed that the components (sweeteners, mango powder and stabilizer) significantly ($p<0.05$) affected on the apparent viscosity of beverage. In this concern, Zhao *et al.* (2003) reported that apparent viscosity varies quadric ally with the ultrasound velocity.

Storage stability

Evaluation of storage stability (serum separation): The analysis of variance for results in Table 3 showed that time had no significant ($p<0.05$) effect on the volume of serum produced. The quality of a beverage is often directly correlated to the stability that keeps maintaining appearance which depends on the size of the particles of which it is composed. Beverage showed good stability due to both the thick adsorbed layer and the high viscosity of the system.

Sedimentation (phase separation layers): The current results presented in Table 3 showed that time has a significant ($p<0.05$) effect on sedimentation. It recorded 14% after 1 week then became 17% at the end of 2nd week. This is likely due to the activation of reaction between phenolics in mango and the protein in whey to form large aggregates. Glahn (1982) reported that large particles were more difficult to stabilize because repulsive forces are inadequate to prevent sedimentation. The partitioning of whey protein and phenolics in the pellet is likely a result of protein-phenolic interactions. Mango pulp contains polyphenols that impart astringency in products. The phenolic and protein contents of the sediment collected were higher than those of the supernatant. The results showed that combination of pectin JMJ and monoglyceride gave beverage without settling

Table 2: Apparent viscosity of whey-mango beverage η_{app} (cP.s)

Storage period (days)	Apparent viscosity (cP.s)
1	20.7±0.9960
7	18.36±0.559
15	17.0±1.5900

The components significantly ($p<0.05$) affected on the apparent viscosity of beverage, Each value is expressed as Mean±SD, n = 3

Table 3: % Sedimentation of whey-mango beverage stored for two weeks at 7°C

Storage period (days) at 7°C	Serum separation (%v/v)	Sedimentation (%)
1	$0.50±0.02^{ns}$	13±1.587*
7	$0.55±0.07^{ns}$	14±1.978*
15	$0.55±0.06^{ns}$	17±2.758*

ns: Not significant, *Significant at $p<0.05$, Each value is expressed as Mean±SD, n = 3

or phase separation. Whey proteins will be bit more heat sensitive in the 3.5 to 4.2 pH range, so they often need some protection. The presence of hydrocolloid stabilizers is important to stabilize beverage system to sedimentation. Moreover, addition of an emulsifier also prevented fat ringing and gave a nice homogeneous product. Utilize stabilizer system with emulsifier to assure stability through the entire shelf life, prevent whey proteins to aggregate, precipitate or become insoluble.

Total soluble solids (TSS) of whey-mango beverage (Brix°): The whey-mango beverage recorded total soluble solids TSS (23.5°Brix) at temperature 30±2°C. Brix, is measure of total content of soluble solids (proteins, lipids, glucides, mineral salts, vitamins, organic acids, pigments and other substances) in a sample (Chaves *et al*., 2004).

Chemical composition of whey-mango beverage: Means for chemical composition (pH, acidity, protein, carbohydrate and total solids) of mango-whey beverage are presented in Table 4. Whey-mango beverage had a lower acidity 0.25 and pH 4.23. The chemical composition of beverage is presented in Table 4 showed that protein content beverage was 1.2 g that is including whey protein and mango protein. Whey protein is the only protein type that cannot be obtained from regular food in significant amounts. Whey proteins have also all essential amino acids (of Branched-chain Amino Acids (BCAAs)-leucine, isoleucine and valine) in higher concentrations (Walzem *et al*., 2002). Previous reports suggested that it supports healthy body composition, retention of lean muscle mass, glucose metabolism, satiety and gastrointestinal health (Hayes and Cribb, 2008; Luhovyy *et al*., 2007; Marshall, 2004; Sousa *et al*., 2012; Pal and Ellis, 2010). Its roles in the maintenance of blood pressure and blood lipid levels already within the normal range are also areas of interest (Marshall, 2004; Pal and Ellis, 2010). As a rich source of the sulfur-containing amino acids cysteine and methionine, whey protein can enhance immune function through intracellular conversion to glutathione (Marshall, 2004). On the other hand, carbohydrate is a major component of beverage followed by vitamin C had recorded 19.0 g carbohydrate which complimented the low carbohydrate content of the beverage in the blends. Beverage also contains lactose that provides calories in an easily available form with slower digestion and absorption (compared to glucose). The addition of flaxseed oil as a source of essential oils to the beverage increased the total percentage of omega-3 fatty acids which resulted 45 mg of omega-3 fatty acids per 100 mL. Incorporation flaxseed oil in the beverage is particularly attractive for development

Table 4: Composition of whey-mango beverage

Composition	Amount in 100 mL beverage
Chemical composition	
TS	21.0±1.08
Protein (g)	1.2±.060
Total carbohydrate, CHO (g)	19.0±0.95
omega-3 fatty acid "eicosapentaenoic acid (EPA)" (mg)	45.0±2.03
Minerals	
Calcium (mg)	37.0±1.47
Phosphorus (mg)	55.7±2.62
Potassium (mg)	270.0±3.54
Vitamins	
Vitamin C (Ascorbic acid)	250.0±2.58
Vitamin E (α-tocopherol)	200.0±3.54

Each value is the average of three Measurements±Standard deviation

of beverage with specific health advantage. This oil was reported to decrease cancer incidence and reduced susceptibility to cardiovascular diseases (Breslow, 2006), hypertension, arthritis and improving of visual acuity (Wright *et al*., 1998; Ruxton *et al*., 2004). The current results also indicated that HCl-soluble minerals content (calcium, phosphours, potassium) in beverage was 37, 55.7 and 270 mg, respectively (Table 4). Adequate intake of calcium and other nutrients from beverage has also been demonstrated to help reduce the risk of high blood pressure (Griffith *et al*., 1999; Miller *et al*., 2000; Appel *et al*., 1997). The high levels of phosphorus and calcium in beverage protect the body against bone weakness and help reduce such disorders. Potassium is not stored in the body and much is lost in perspiration, it must be continually replenished. This beverage is consider rich with potassium which is regulating the water balance in the body (Lindinger, 1995), provides the appropriate alkaloidal features for body fluids and stimulates the kidneys to expel toxic metabolites. Analysis of the produced beverage presents a challenge due to the difference in solubility limits of the two classes of vitamins (water-and fat-soluble vitamins). This beverage is a great source of vitamin C 250 ug/100 g from mango (Pudmini and Prabha, 1997) and also is a rich source of vitamin E (200 ug/100 g) as shown in Table 4. Vitamin C and E are considering antioxidant vitamins that are required to prevent deficiency diseases. Vitamin E might help to prevent heart disease (Glynn *et al*., 2007; Traber, 2007) because of its antioxidant properties (free radicals are beheved to be a factor in atherosclerosis). Vitamin C is an antioxidant which has led to its endorsement by some researchers as a complementary therapy for improving quality of life (Yeom *et al*., 2007).

Polyphenolics content and total antioxidant capacity of whey-mango beverage: The Total Polyphenols Content (TPC) was determined using the Folin-Ciocalteu method. Gallic acid was used as calibration standard and the results (expressed as gallic acid equivalents) were expressed as Mean±SD of triplicate analysis. The average soluble phenolic content in whey-mango beverage was 348 mg GAE/100mL. Various health-promoting effects of phenolic compounds have mainly been ascribed to their antioxidant activity. Interactions between phenolic compounds and tocopherols may increase the bioavailability of vitamin E and decrease cholesterol (Kamal-Eldin *et al*., 2000). Mango extracts are used widely in traditional medicines for treating a number of conditions including diarrhoea, diabetes and skin infections (Selles *et al*., 2002) and mangiferin is reported to inhibit bowel carcinogenesis in rats (Yoshimi *et al*., 2001). Since the phenolic compounds in dietary sources exhibit potent free radical-scavenging properties, their main role was thought to be as antioxidants involved in protection against lipid peroxidation (Masella *et al*., 2005; Halliwell *et al*., 2005; Romier *et al*. 2009; D'Archivio *et al*., 2008).

Three common antioxidant activity methods, TEAC, FRAP and DPPH•, based on the reaction with electron donating (single electron transfer) were used to measure the total antioxidant capacity of the beverage and the values were expressed as a the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent (TE) (as the reference standard) because it will offer the benefit to compare them as shown in Table 5.

Table 5: Total antioxidant capacity of whey-mango beverage

Total antioxidant capacity	DPPH free radical scavenging activity	Trolox equivalent antioxidant capacity (TEAC)	Ferric reducing antioxidant power (FRAP)
Values umol Trolox/100 mL	443±46	885±6	361±6

Each value is the average of three Measurements±SD

DPPH free radical scavenging activity: The stable radical DPPH (α,α-diphenyl-2-picrylhydrazyl hydrate) has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging with pure antioxidant compounds by hydrogen donation. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound (Koleva *et al*., 2002). DPPH is a nitrogen centered radical (its reduction by an antioxidant) having maximum absorbance at 517 nm which gets converted to 1,1, diphenyl-2-picryl hydrazine on reacting with hydrogen donating species (Jayaprakasha *et al*., 2008). The DPPH free radical scavenging activity for beverage is presented in Table 5. The ability of beverage to scavenge the DPPH radical (DPPH value) was 443±46 μmoles Trolox Equivalents (TE)/100 mL beverage.

Trolox equivalent antioxidant capacity (TEAC): Trolox Equivalent Antioxidant Capacity (TEAC) assay portrays the ability of antioxidants to scavenge $ABTS^{\bullet+}$ long life radicals cation (Perez-Jimenez and Saura-Calixto, 2006) and it is one of the most commonly employed methods for determining antioxidant capacity. TEAC is also defined as the milimolar concentration of a Trolox solution whose antioxidant capacity is equivalent to a 1.0 mM solution of the substance under study (Antolovich *et al*., 2002). For TEAC evaluation, the method reported by Iqbal *et al*. (2007), Trolox equivalent antioxidant activity of beverage was assessed. TEAC value depends mainly on their polyphenol content (Ghszczynska-Swiglo and Tyrakowska, 2006). The TEAC value of a beverage is obtained by interpolating the decrease in absorbance (corresponding to a diluted sample) on the calibration curves, thus giving a concentration of Trolox (Van den Berg *et al*., 1999). The current result showed that the average antioxidant activity values of the beverage determined by TEAC method was 885±6 umol Trolox/100 mL as presented in Table 5.

Ferric reducing antioxidant power (FRAP): In this study, the Ferric Reducing Antioxidant Power (FRAP) assay was used because it is quick and simple to measure the antioxidant capacity of pure compounds of donating a single electron. Antioxidant can reduce ferric ions (Fe^{3+}) in ferric-TPTZ (Fe (III)-TPTZ) complex to the blue ferrous form (Fe^{2+})-TPTZ (Fe (II)-TPTZ) complex which absorbs strongly at 593 nm (Benzie and Strain, 1996). The range of FRAP values of beverage in the present study was 361±6 umol of Trolox equivalents per 100 mL of beverage as shown in Table 5.

All determinations were performed in triplicates and the values of all three methods were expressed as a Trolox Equivalent (TE). As such the results provide a direct comparison of the antioxidant activity with Trolox. The results indicated that the beverage exhibited higher capacity in scavenge long life radicals cation $ABTS^{\bullet+}$ than others activity. In same time capacity in reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) was higher than to scavenging free radicals. The results also showed that the polyphenol-rich in beverage displayed high antioxidative capacities. These findings indicate a correlation between the amount of phenolic compounds and the antioxidative capacity. Study showed a highly significantly correlation between values for antioxidant activity measured with TEAC, DPPH and FRAP and total phenolic content. Correlations were $r^2 = 0.8753$, 0.8352 and 0.9100, respectively. This correlation means that most antioxidant activity in beverage contribute to phenolic compounds that have ability be donors of hydrogen atoms or electrons and to capture the free radicals (Stoilova *et al*., 2007). Whereas, the other activity come from the presence of other antioxidant secondary metabolites, such as carotenoids and vitamins. Consequently, the beverage with these antioxidant capacity can protect against diseases and degenerative processes caused by oxidative stress.

Total carotenoid content (TC): The results showed that beverage contained carotenoids in concentration of 15.5 μg g^{-1} beverage. It is well documented that carotenoids are a group of lipophilic molecules have nine or more conjugated double bonds including C = C and C = O to contribute the quenching activity that can transfer energy from excited species, such as triplet oxygen, peroxyl radicals and singlet molecular oxygen ($^1O_2$), to the carotenoid very efficiently and thus prevent harmful radical damage to other compounds (Beutner *et al*., 2001; Lowe and Young, 2001; Matos *et al*., 2000). This structural characteristic contributes largely to carotenoid antioxidant functions. Carotenoids and polyphenols as the best known antioxidants has specific health benefits that help to maintain or improve the body's overall health and ability to function to its fullest capacity on a daily basis. The antioxidant potential of whey-mango beverage could be due to synergistic actions of bioactive compounds present in it. Whey-mango beverage is believed to have health benefits due to their antioxidant activity and polyphenols which can reduce the damage caused by free radicals and slacken ageing, to cure asthma, heart diseases constipation, indigestion, fatigue, kidney disorders and alzheimer's disease (Atak *et al*., 2011).

This study shows promising results that mango pulp powder; flaxseed can be incorporated into a whey base to produce a beverage that provides both nutrients inherent in whey. This beverage is considering functional product due to its inherent nutrient content (Boland *et al*., 2001; Huth *et al*., 2006).

CONCLUSION

The main objective of this investigation was fortification the whey with mango fruit to elevate its nutritional value and growing interest in new functional beverage with special characteristics and health properties. Chemical composition of whey based mango beverage showed that beverage is a rich source of many vitamins, minerals, digestible carbohydrates. Fortification of beverage with flaxseed oil (i.e., short chain omega-3) may provide the required components for optimum health and could further enhance the healthful perception. Whey mango beverage can be stored best at refrigeration temperature with the minimum alteration in their physicochemical and sensory quality. This beverage is also an excellent source of antioxidants including vitamin C, total phenolics, α-tocopherol and carotenoids and exhibits an excellent scavenging ability for different forms of free radicals. The high contents of phenolic compounds and significant linear correlation between the values of the concentration of phenolic compounds and antioxidant activity indicated that these compounds contribute to the strong antioxidant activity. Natural bioactive compounds in this beverage may be responsible for its high antiradical activity and play an important role to meet the health-conscious consumes' demanded. Moreover, this beverage is light, refreshing, healthful and nutritious. The successful development of whey-based mango beverages will open a new avenue for the utilization of whey.

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