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Antioxidant activity and sensory acceptability of whey protein-based smoothie beverages made from mango (*Mangifera indica* L.) cv Haden and strawberry (*Fragaria* × *ananassa* Duch.) cv Festival

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ABSTRACT
Objective: To assess the effect of different whey proteins levels on the physicochemical, antioxidant activity, and sensory acceptability of smoothie beverages made from mango and strawberry.
Design/methodology/approach: Twenty-four formulations were evaluated (type of fruit and concentration level of whey protein).
Results: Adding of whey protein to smoothies composed of mango and strawberry increased the protein content and antioxidant activity by 2-2.5-fold compared to control smoothies' samples without whey protein. Sensory analysis showed that in terms of overall acceptance, all produced smoothies were considered very acceptable by the panelists.
Limitations on study/implications: Smoothies enriched with whey proteins can be a good new food product that incorporated nutritional and functional compounds into the human diet.
Findings/conclusions: Smoothies produced from mango and strawberry fruits enriched with whey proteins can be considered valuable products as source of bioactive compounds and from sensory points of view.

Keywords: smoothie, antioxidant activity, whey protein, acceptability

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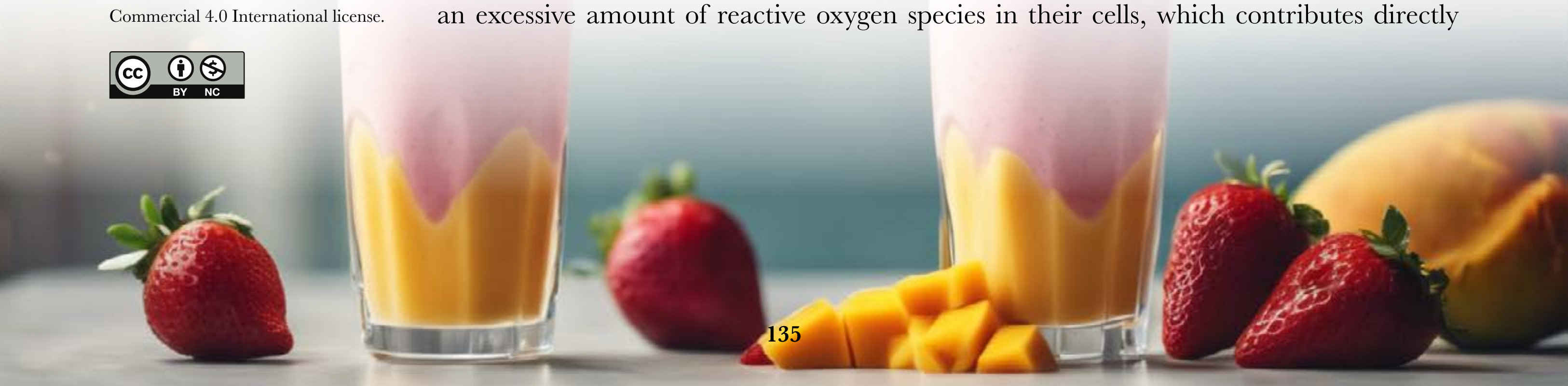
INTRODUCTION

Currently, consumers are concerned about topics related with their health, correct nutrition, and well-being because the modern life is characterized by a stressful life-style, vegetable and fruit-poor diet, and lack of physical activity (Ali & Ali, 2020; Bailey *et al.*, 2020). The above triggered oxidative stress in the body of consumers because there is an excessive amount of reactive oxygen species in their cells, which contributes directly



to developing inflammatory diseases, ischemic diseases, certain cancers, and accelerated aging (Checa & Aran, 2020).

In this context, functional foods have emerged as a complementary alternative in order to improve the lifestyle of consumers. Besides, in recent years consumer trends are orientated to ready-to-eat and/or reformulation of typical food products to increase their nutritional value (Kidoń & Uwineza, 2022). One of foods than can fulfill these requirements and demands are the smoothies, which can be enriched with some bioactive compound with health-promoting properties.

Typically, smoothies are semi-liquid beverages resulting from the blend of fruit- and/or vegetable-based products (*e.g.*, juice and/or puree) with the possible incorporation of milk or yogurt (Kidoń & Uwineza, 2022; Uzodinma *et al.*, 2020). Besides, other ingredients can be added such as species, ice, water, sugar or sweeteners (Uzodinma *et al.*, 2020). In the functional food field, smoothies have been generated by the addition of fruit seeds and peels or by using raw materials rich in phytochemicals (Baiano, Mastromatteo, & Del Nobile, 2012; Fernandez *et al.*, 2020; Picouet *et al.*, 2016; Saini & Sharma, 2020; Tkacz *et al.*, 2021).

Whey proteins, once considered waste from cheese manufacture, have proven to be a source of bioactive components, and when used in functional foods can potentially lead to prevention of lifestyle diseases (Sharma, 2019). Besides, those proteins can provide to beverages some tecnofunctional properties such as foaming, emulsifying, and solubility, which contributes to the increase in the viscosity, texture, and taste of smoothie that influences the organoleptic characteristics of the beverage (Kristensen *et al.*, 2021). Therefore, the aim of this study was the development of new smoothie formulations based on fruits and whey protein, as well as to investigate their sensory characteristics and antioxidant activity of the obtained products.

MATERIALS Y METHODS

Low-fat milk (LALA®), natural yogurt (LALA®), mangoes, and strawberries were obtained from a local supermarket in Hermosillo, Sonora, Mexico. The fruits used in the present study were mango (*Mangifera indica* L.) cv Haden and strawberry (*Fragaria* × *ananassa* Duch.) cv Festival. The fruits were selected with an optimum degree of maturity (intense color, characteristic aroma, and firmness). A commercial unflavored whey protein isolate (Isopure, 86.2% protein content) was obtained from General Nutrition Center (GNC, Mexico). All chemicals used were analytical or gradient grade (for HPLC) purity and provided by Sigma Aldrich (St. Louis, MO, USA) and Thermo Fisher Scientific (Waltham,MA, USA).

Preparation of smoothies-type mango and strawberry beverages

Four different formulations were prepared in triplicate for each fruit (mango and strawberry), obtaining a total of 24 experimental units. The smoothie formulation was prepared by mixing milk (250 mL), natural yogurt (250 mL), fruit (20 g), chipped ice (250 g), and different concentrations of whey protein (0, 10, 20, 30% w/v). The ingredients were homogenized using an Osterizer blender model 450–10 (Sunbeam

Mexicana, S.A. de C.V., Mexico). Smoothie's samples were stored under refrigerated conditions at 4 °C in sterile bottles until analysis in the same day.

Physicochemical analysis

Titratable acidity (AOAC 947.05, 1997), pH (AOAC 981.12, 1997), total protein (Kjeldahl, AOAC 920.123,1997) and total fat (Babcock, AOAC 989.04,1997) contents were determined according to the methods described in the Association of Official Analytical Chemist.

Determination of antioxidant activity

Samples were centrifugated at 12,000 g (Bechman J2-21, Fullerton, USA) during 40 min at 4 °C. The supernatant of each sample was collected and was used to determine the antioxidant activity through 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) assay according to the methodology reported by Re *et al.* (1999). Briefly, the ABTS radical was prepared by mixing a solution of ABTS reagent (7 mM) with potassium persulfate (2.45 mM). The mixture was kept in the dark during 16 h at 30 °C. The ABTS working solution was prepared by dilution of the ABTS radical stock solution with PBS (5 mM, pH 7.2) until reached an optical density on 0.7 ± 0.02 (734 nm). Next, 10 μL of sample was mixed with 1 mL of ABTS working solution and the optical density was recorded at 734 nm after 7 min. Trolox (6-hydroxy-2,3,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich) was used as a standard to prepare a reference curve (0 to 3.5 mM in PBS) to compare those readings obtained by the samples. The results were expressed as millimoles of Trolox equivalents.

Sensory evaluation

Samples were evaluated using a 5-point hedonic scale (5-like very much; 4-like slightly; 3-neither like nor dislike; 2-dislike slightly; 1-dislike very much). The assessment included the following quality attributes: aroma, appearance, acidity, taste, and overall acceptance. The sensory evaluation was conducted by a group of 27 semi-trained panelists (11 women and 16 men, aged 20-45) with formal sessions training in sensory evaluation. To qualify, panelists had to be non-smokers, and had to have no allergy to any of the smoothie ingredients. The panelists were selected based on their interest and skill in sensory assessment and were instructed in the evaluation of the parameters before described. The training was adapted to achieve understanding of the sensory parameter measurement scope and sensory borderlines, and this was achieved via individual testing, followed by group discussions. The sensory tests of each sample were conducted properly in a sensory assessment room equipped with separated cabinets and individual lightning at 20 ± 2.0 °C. The samples were coded with three-digit numbers, served randomly to the panelists, and the data was analyzed.

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA). Differences among means were determined at 5% level of significance by the Tukey's test, for physicochemical parameters and antioxidant activity, or Fisher's test, for sensory evaluation data. All analyses were performed using the NCSS software version 2007 (NCSS Statistical software, Kaysville, UT, USA).

RESULTS Y DISSCUSION

Physicochemical parameters

Titratable acidity, pH, total protein, and total fat contents are presented in Table 1. The results showed that the titratable acidity of both type of smoothies (mango and strawberry) ranged between 3.6 and 4.8, and that pH ranged between 4.7 and 5.03. The pH and titratable acidity were different due to different ingredient ratios of whey protein used in the formulation of smoothies. In this context, it was observed a progressive increase of pH and titratable acidity according to the concentration of whey protein in the produced smoothies regardless of if was made with mango or strawberry. This behavior could be explained by the fact that whey protein can increase the pH due to their buffering capacity (Sodini, Mattas, & Tong, 2006). Some physicochemical parameters are important features in foods, such is the case of pH and acidity because are important for sensory characteristic and for the prevention of microbial spoilage. It has been described that some fruit juices could successfully replace of current artificial acidity regulators, particularly in functional food development (Koss-Mikołajczyk *et al.*, 2015). The values found in the present study were in accordance with those reported in the literature. For example, a smoothie made with pineapple, watermelon, banana, and coconut milk had a pH about 4.5 (Uzodinma *et al.*, 2020), whereas other similar smoothie made with banana, pumpkin, and purple carrot had a pH of 4.45, while a smoothie made with mango showed a pH of 4.57 (Balaswamy *et al.*, 2013).

On the other hand, the incorporation of whey protein in the smoothie had a clearly direct effect on their protein content, namely, as the amounts of whey protein increased, the content of the proteins on the smoothies also increased. In both type of smoothies (mango and strawberry), the samples with 30% of whey protein added, showed the highest protein content (4.92-4.94%), while control samples showed lowest protein content (1.75-1.90%). The increase of protein in the sample with 30% of protein whey added was of 2.6-fold compared to control sample. Our results are according to similar studies reported in the literature (Camargo *et al.*, 2018; Maravić *et al.*, 2022; Zavareze, Morales, & Salas-

Table 1. Physicochemical characteristics of smoothies made from mango and strawberry with different concentration of whey proteins.

Parameters	Mango				Strawberry			
	0%	10%	20%	30%	0%	10%	20%	30%
Protein (%)	1.75±0.28[a]	2.38±0.06[b]	4.14±0.09[c]	4.92±0.45[d]	1.90±0.12[a]	2.41±0.05[b]	4.17±0.32[c]	4.94±0.16[d]
pH	4.70±0.09[a]	4.94±0.14[b]	4.97±0.12[c]	5.03±0.25[d]	4.82±0.23[a]	4.91±0.11[b]	5.03±0.29[c]	5.01±0.08[d]
Titratable acidity (g/L of lactic acid)	3.62±0.34[a]	3.94±0.37[b]	4.60±0.28[c]	4.81±0.11[d]	3.60±0.37[a]	4.00±0.24[b]	4.09±0.18[c]	4.70±0.13[d]

* Values are mean of triplicate determinations. Different letters indicate statistical difference among formulations with different level of whey proteins for each parameter and type of smoothie. Fat was evaluated but was not included in the table due to did not show changes for all samples (ca. 3% of fat).

Mellado, 2010), where more whey protein is added to a food product formulation, the higher is the progressive protein increase. This is important because whey protein is a nutritious product that contain biologically active superior proteins (Musina *et al.*, 2018). In a study realized by Corgneau *et al.* (2019) found that among various commercial protein supplements, the whey protein showed highest in vitro digestibility (ca. 90%), while egg white powder showed less digestibility (ca. 81%). In addition to their excellent digestibility, whey proteins showed outstanding bioavailability and essential amino acid contents that meeting the human needs (McGregor & Poppitt, 2013). Then, the addition of whey protein increases the nutritional quality of smoothie's samples. In contrast, the content of fat did not show changes at different levels of the incorporation of whey protein in both type of mango and strawberry smoothie's samples (ca. 3%).

Antioxidant activity

It is known that the antioxidant activity of bioactive compounds can help deal with the effects of the pathological condition called oxidative stress, which is a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses. Oxidative stress contributes directly to developing inflammatory diseases, ischemic diseases, certain cancers, and accelerated aging (Checa & Aran, 2020). Antioxidant activity data showed that all smoothies formulations made from mango and strawberry with different levels of why proteins had antioxidant proprieties in different extents (Table 2).

It has been observed that as the amounts of whey protein increased, the antioxidant activity on both type of smoothies also increased, being statically different ($P > 0.05$) the formulations with highest content of whey protein (20 and 30%). The antioxidant activity of the smoothies with 30% of whey protein added were ca. 2-2.5-fold higher than the control smoothie without whey protein. In related works, it has been reported the antioxidant activity of different formulations of smoothies. The results of our study was higher than those reported by Yassin *et al.* (2020), who evaluated the antioxidant activity of 17 commercial smoothies marketed in Brazil. Their results showed that the antioxidant activity ranged from 1.487 to 7.333 mM of Trolox equivalents for DPPH method and from 1.819 to 9.801 mM of Trolox equivalents for ABTS method. Similarly, Nowicka, Wojdyło, and Samoticha

Table 2. Antioxidant activity (mM of Trolox equivalents) of smoothies made from mango and strawberry with different concentration of whey proteins.

Whey protein (%)	Smoothies	
	Mango	Strawberry
0	17.54 ± 1.89^{a}	14.15 ± 1.24^{a}
10	19.12 ± 1.02^{a}	14.88 ± 1.00^{a}
20	23.93 ± 2.03^{b}	27.30 ± 2.32^{b}
30	33.36 ± 0.79^{c}	33.92 ± 0.93^{c}

*Values are mean of triplicate determinations. Different letters indicate statistical difference among formulations with different level of whey proteins for each type of smoothie.

(2016) reported the antioxidant activity of 13 smoothies based on different combinations of *Prunus* fruits (*e.g.*, cherry (*Prunus cerasus* L.) cv Lutowka, peach (*Prunus persica* L.) cv Hardow beauty, apricot (*Prunus americana* L.) cv Miodowa, and plum (*Prunus* L.) cv Promis). The authors found that the antioxidant activity ranged from 2.35 to 6.81 mM of Trolox/100 g sample for ABTS method and from 1.33 to 4.23 mM of Trolox/100 g sample for ORAC method. For the above studies, it is possible that the differences are due to the proportion and constituents of each beverage not being uniform, justifying the variation. Likewise, a vegetable smoothie made from Beet plants (*Beta vulgaris* L. cv Conditiva) showed an antioxidant capacity of 219.2 mM of Trolox/kg sample for FRAP and 28.9 mM of Trolox/kg sample for DPPH (Fernandez *et al.*, 2020). In a study realized by Keenan *et al.* (2010) reported values of antioxidant activity of 253.6 mg of Trolox equivalents/100 g dry sample of a smoothie made from a blend of strawberry, apple, banana, and orange. Besides, the authors treated the smoothie sample thermally way, which increased their antioxidant activity by 15%.

Because methodologies for evaluating antioxidant activity may vary in different aspects, such as the assay used and the way to express the results, comparison of results from different studies is as difficult as it is to distinguish only one mechanism or compound involved in the antioxidant activity (Alam, Bristi, & Rafiquzzaman, 2013).

Sensory evaluation

In the sensory evaluation of smoothies made from mango and strawberry (Table 3), it was not found significant differences ($P>0.05$) in the most formulations for the follow attributes: aroma, appearance, taste, and overall acceptance; however, the acidity was the only one which showed significant difference in the smoothies from mango formulated with high content of whey proteins (20% and 30%) compared with those formulated with lowest content of protein whey. Besides, highest sensory score in all attributes was found in smoothies made from strawberry compared with those showed in the smoothies made from mango.

Furthermore, most formulations scored above 3.5 for overall acceptance, which represent the equivalent to “like slightly”. Thus, the also showed that in terms of overall acceptance, all produced smoothies were considered very acceptable by the panelists.

Table 3. Sensory analysis of smoothies made from mango and strawberry with different concentration of whey proteins.

Attributes	Mango				Strawberry			
	0%	10%	20%	30%	0%	10%	20%	30%
Aroma	3.77^{a}	3.85^{a}	3.63^{a}	3.60^{a}	3.74^{a}	3.96^{ab}	3.48^{b}	3.88^{ab}
Appearance	3.66^{a}	3.70^{a}	3.59^{a}	3.61^{a}	3.51^{a}	3.85^{a}	3.55^{a}	3.81^{a}
Acidity	3.96^{a}	3.96^{a}	3.25^{b}	3.25^{b}	3.70^{a}	3.81^{a}	3.51^{a}	3.70^{a}
Taste	3.77^{a}	3.48^{ab}	3.00^{b}	3.25^{ab}	4.00^{a}	4.14^{ab}	3.51^{b}	3.85^{ab}
Overall acceptance	4.00^{a}	3.74^{ab}	3.37^{b}	3.54^{ab}	3.96^{a}	3.9^{a}	3.55^{a}	3.88^{a}

Values are mean of evaluators. Different letters indicate statistical difference among formulations with different level of whey proteins for each attribute and type of smoothie.

CONCLUSIONS

Smoothies produced from mango and strawberry fruits enriched with whey proteins can be considered valuable products as source of bioactive compounds and from sensory points of view. Incorporation of whey proteins into mango and strawberry smoothies significantly enriched protein content of the product with the concomitant increase in their antioxidant activity. Besides, the sensory panelists approved of all the smoothies formulated. However, more research is needed to check changes of bioactive compound content during the storage of smoothies.

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