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FOOD ENGINEERING

Effect of the addition of stabilizers on physicochemical, antioxidant and sensory properties of a beverage made with hydrolyzed sweet whey permeate enriched with agraz (*Vaccinium meridionale Swartz*) and cape gooseberry (*Physalis peruviana* L) pulp

INGENIERIA DE ALIMENTOS

Efecto de la adición de estabilizantes sobre las propiedades fisicoquímicas, antioxidantes y sensoriales de una bebida elaborada con permeado de lactosuero dulce hidrolizado enriquecida con pulpa de agraz (*Vaccinium meridionale Swartz*) y uchuva (*Physalis peruviana* L)

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Abstract

The global beverage industry continues to grow with the particularity that the current market shows a trend for healthy, functional products that incorporate sustainable processes and raw materials that reduce negative impacts on the environment. The objective of this study was to develop a fruit drink with cape gooseberry (*Physalis peruviana L*) and agraz (*Vaccinium meridionale Swartz*) pulp, from sweet whey ultrafiltered and hydrolyzed permeate (80% degree of hydrolysis/lactase) , evaluating the effect of the addition of stabilizers on the physicochemical, antioxidant and sensory properties. The results indicated

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that the addition of a mixture incorporating pectin at 0.1% and Carboxymethylcellulose (CMC) at 0.1% with a participation of fruit pulp of 15% w/w achieves the best colloidal stability. Physicochemical analyzes for beverages with stabilizers showed the following values: pH (3.65±0.01), total soluble solids (11.9±0.05°Bx), titratable acidity (0.415±0.004%), viscosity (27.6±1.9 mPa.s), density (1.0387±0.0012 g/ml), humidity (87.34±0.124 % w/w), ashes (0.49 g/100g), protein <2.5 g/100g , total phenols (38.14±1.52 mg, gallic acid/100g), total carotenoids (292.99±6.72 µg β-carotene/100 g), antioxidant capacity by ABTS 452.42±12 µg trolox/ml, and DPPH 219.4±13.34 µg trolox/ml. Most of these attributes remained stable for 32 days both for the drink with stabilizers and for the one that did not incorporate them (control), however there were losses of 17% in total phenols and 4% in total carotenoids at the end of the storage period at 4 ºC. Sensory tests in the beverage with stabilizers indicated general acceptance of attributes above 80% by consumers, allowing to conclude that the developed beverage offers an important alternative to generate added value to whey permeate with commercial projection compared to other applications where only separated and concentrated whey proteins are used.

Keywords: *Whey, ultrafiltration, functional drink, cape gooseberry, andean blueberry.*

Resumen

La industria de bebidas a nivel mundial se mantiene en aumento con la particularidad que el mercado actual nos muestra una tendencia por productos saludables, funcionales y que incorporen procesos y materias primas sustentables que reduzcan los impactos negativos sobre el medio ambiente. El objetivo de este estudio fue desarrollar un refresco de frutas con pulpas de uchuva (*Physalis peruviana* L) y agraz (*Vaccinium meridionale Swartz*), a partir de un permeado de lactosuero hidrolizado al 80% con lactasa, evaluando el efecto de la adición de estabilizantes sobre las propiedades fisicoquímicas, antioxidantes y sensoriales del mismo. Los resultados indicaron que la adición de una mezcla con incorporación de pectina en 0,1% y carboximetilcelulosa (CMC) en 0,1%, con una participación de pulpa de frutas de 15% p/p logró alcanzar la mejor estabilidad coloidal. Los análisis fisicoquímicos para la bebida con estabilizantes presentaron los siguientes valores: pH 3,65±0,01, sólidos solubles 11,9±0,05°Bx, acidez titulable 0,415±0,004%, viscosidad 27,6±1,9 mPa.s, densidad 1,0387±0,0012 g/ml, humedad 87,34±0,124 %b.h, cenizas 0,49 g/100g, proteína <2,5 g/100g, fenoles totales 38,14±1,52 mg ácido gálico/100g, carotenos totales 292,99±6,72 µg β-caroteno/100 g, capacidad antioxidante por ABTS 452,42±12 µg trolox/ml y DPPH 219,4±13,34 µg trolox/ml; la mayoría de estos atributos se mantuvieron estables durante 32 días tanto para la bebida con estabilizantes como para la que no los incorporó, sin embargo se presentaron perdidas del 17% en fenoles totales y del 4% para carotenos totales al final del período de almacenamiento a 4°C. Las pruebas sensoriales por consumidores en la bebida con estabilizantes indicaron una aceptación general de atributos superiores al 80%, permitiendo concluir que la bebida desarrollada ofrece una alternativa importante para generar valor agregado al permeado de lactosuero con proyección comercial frente a otras aplicaciones en donde solo se aprovechan las proteínas séricas separadas y concentradas.

Palabras clave: *Lactosuero, ultrafiltración, bebida funcional, uchuva, agraz.*

1. Introduction

The production of whey —a co-product of the cheese industry— has been considered traditionally as a problem for the dairy sector, being a highly polluting component for the environment since it is traditionally discharged into water sources or soils without any treatment, even when it has important nutrients such as proteins, carbohydrates, and minerals. In order to minimize the environmental impact of this co-product (whey), the industry has fostered the use, development, innovation and optimization of technologies such as membrane separation systems (1).

The use of cape gooseberry (*Physalis peruviana* L) in functional beverages has been increasing, given its nutritional properties and the phytochemical components, mainly antioxidants such as carotenoids, polyphenols, and vitamin C, properties required in the prevention of some diseases such as scurvy, cardiovascular diseases, cataracts, macular degeneration, as well as blood sugar regulation, prevention of cellular oxidative damage, preventive effect on the development of tumors and some types of cancer, modulation of immune response and inflammatory processes (2, 3).

Interesting properties have been found for agraz (*Vaccinum meridionale Swartz*) and its incorporation in functional beverages thanks to the health benefits, favored by the amount of phenols and anthocyanins present, which includes enhancing defense mechanisms of the body against toxicity, diabetes, prevents cell damage, inhibits or delays the oxidative damages generated by some chronic and/or inflammatory diseases, and protection against cardiovascular diseases (4, 5).

Several studies have been reported on the development of beverages (soft drinks) using whey, including isotonic beverages. Torres (6) used ultrafiltered whey permeate to develop an isotonic beverage with a tangerine flavor, which presented characteristics of pH, color, and opacity similar to the commercial product Gatorade® with a good physicochemical and microbiological stability in a period of 20 days under refrigeration conditions (4°C) and a percentage of sensory acceptability of 68%. Beucler et al. (7), designed a blue raspberry (*Rubus leucodermis*) flavored beverage using hydrolyzed whey permeate in which the formulation was replaced with the permeate at concentrations of 0, 25, 75 and 100% of the water used; the results of this study showed high values in the content of electrolytes (Na, Ca, Mg, Zn, P) and sensory acceptance similar to commercial beverages for permeate participation between 25 and 50%. Cuellas & Wagner (8) conducted a feasibility study to produce products from whey by mixing it with orange, grapefruit, and strawberry flavors and hydrolyzing it in percentages of 80%, 50% and 20% lactose conversion; the results indicated that the isotonic drink formulated with 80% hydrolyzed whey, sucrose, and orange flavoring presented high sensory acceptability with wide possibilities of insertion in the market because of its low production cost compared to other whey-based products from cheese factories. Morales & Vivas (5) evaluated the antioxidant activity of a refreshing drink based on whey with banana passion fruit (*Passiflora tarminiana*) pulp during storage; the results indicated that the analyzed beverages showed differences in the pH and acidity tests, this is due to the variation of the pulp content, where the antioxidant activity varied from 2824.8125 µM trolox for the drink with 10% pulp to 20230.1375 µM trolox for the drink with 20% pulp throughout the storage time, determining that the drink with 20% pulp is the one that shows the best antioxidant activity. Véliz (9) developed a milk drink with sweet whey, adding orange juice and polydextrose; the results indicated that the most accepted formulation was the mixture of 70% (whey), 30% (orange juice) with 12% polydextrose in terms of taste, color, smell, and viscosity. Montesdeoca & Intriago (10) evaluated the effect of adding lactase (β-galactosidase) and sucrose (as sweetener) to an isotonic drink from sweet whey; the combination of 0.5% lactase and 8% sucrose was determined as the best treatment based on an affective test where the participation of sucrose increased carbohydrates and calories above the value defined by Ecuatorian regulations.

Most of the scientific studies on the use of whey for the development of beverages have focused on the development of isotonic drinks, therefore, the objective of this work was to develop a fruit soft drink using sweet whey ultrafiltered and hydrolyzed permeate with the addition of cape gooseberry (*Physalis peruviana* L) and agraz (*Vaccinium meridionale* Swartz) pulp, evaluating their quality characteristics for 32 days under constant storage conditions at 4°C.

2. Materials and methods

Formulation and elaboration of the drink

The formulation of the drink was set up considering the parameters established in NTC 3549 (11) for fruit soft drinks and resolution 3929 of 2013 of Ministry of Health and Social Protection (Colombia) for processed fruits (12). Initially, 4 treatments were evaluated with different percentages of incorporation of carboxymethylcellulose (CMC) and pectin as stabilizers to evaluate their effect in mixtures with agraz (*Vaccinium meridionale Swartz*) and cape gooseberry pulp (*Physalis peruviana* L).

The fruits were selected according with the Colombian quality standards NTC 6391 (13) for fresh agraz and NTC 4580 (14) for fresh cape gooseberry. Subsequently, a washing procedure with water and acetic acid (5%) was carried out to remove impurities and reduce their microbial load, and both fruits were scalded by immersion in water at 90°C for 90 seconds. The fruits were subjected to pulping (SPH 7.0- pulp machine, Andalucía, Sevilla, Spain) and sieving processes (0.8 mm diameter mesh).

To obtain the permeate, the whey was filtered, heated to 45°C, and skimmed by centrifugation at 7700 rpm (Westfalia SIH10007). Subsequently, it was pasteurized at 72°C for 30 minutes before being taken to the ultrafiltration equipment. This ultrafiltration process was carried out in the PERINOX S.A (Spain) brand filtration pilot equipment (membrane tangential technology): membrane filtration system equipped with a semi-permeable polyethersulfone spiral membrane with a 10 kDa cut-off size and the following operating conditions: pressures of 1 and 3 bars at the outlet and inlet, respectively; a temperature of 48°C, and a concentration factor of 18.

The permeate was cold hydrolyzed by adding lactase in a ratio of 0.5 ml/L for 24 hours keeping the solution at 4 °C, until to reach a level of lactose hydrolysis of 80%. The hydrolysis level was quantified by the cryoscopic method (15, 16), seeking to significantly reduce the amount of lactose to prevent the unpleasant effects caused by intolerance associated with the same.

Colloidal stability tests

Preliminary tests indicated the need to add stabilizers in order to avoid the sedimentation. An experimental design of two independent variables (% pectin and % CMC incorporated in the beverage) was established: (T1: Without hydrocolloids, T2: 0.1% pectin, T3: 0.1% CMC, and T4: 0.1% pectin + 0.1% CMC, respectively). The response variables were viscosity, Z potential according to the methodology described by Genovese & Lozano (17), and particle size according by Keshtkaran et al. (18). These colloidal stability tests were performed looking for a suitable mixture to subsequently carry out the characterization of the final beverage in comparison with the T1 control without hydrocolloids.

Determination of physicochemical parameters in the final beverage

Physicochemical parameters were determined for the beverage obtained without stabilizers (T1) and for the selected final formulation (T4) given in Table 1.

Table 1. Formulation of the drink for treatments without and with the addition of stabilizers.

Treatments	Hydrolyzed permeate (% w/w)	Agraz pulp (%w/w)	Cape gooseberry pulp (%w/w)	Sugar (%w/w)	Pectin (%w/w)	CMC (%w/w)
T1	80	7.5	7.5	5	-	-
T4	79.8	7.5	7.5	5	0.1	0.1

The pH was determined using the potentiometric method (NTC 4592/1999). The titratable acidity was carried out by a standard acid-base titration (NTC 4623/1999). Total soluble solids (measured as degree Brix) by digital refractometer (NTC 4624/1999). The viscosity of the suspension at 4ºC was obtained by means of flow curves using a concentric cylinder system N°SC4-27 coupled to a Brookfield DV-III ultra rheometer (19). Density was determined by 10 ml pycnometer (AOAC 945.06-1990). The color was evaluated by the CIE L*a*b* method with a Konica Minolta CR-400 series colorimeter. Moisture content by gravimetric method (AOAC 977.21-1990). Protein content by Kjedahl volumetric method (AOAC 2001.11), and total ash by gravimetric method (AOAC 972.15 -1974).

Evaluation of analytes and antioxidant capacity

For the extraction of antioxidant components, 10 g of each sample of the drink were taken in

Falcon tubes and 15 ml of a methanol/water solution (70/30) were added, then they were stirred in a vortex for 30 seconds, and then subjected to centrifugation for 15 minutes, 8000 rpm and 20°C in a Universal 320 R Hettich Zentrifugen centrifuge. The supernatant was filtered into 25 ml flasks, then the filtered extract was calibrated to the remaining volume with a methanol/water solution (70/30) and left refrigerated for 24 hours in dark conditions. Total phenols were determined using Folin-Ciocalteu spectrophotometric method and expressed as mg gallic acid/100 g (20). **Total carotenoids** were expressed as β-carotene, quantified spectrophotometrically at 450 nm and expressed in μg β-carotene/100g of sample according to the methodology described by Ordoñez et al. (21).

Antioxidant Capacity (DPPH): 2.2-diphenyl-1-picrylhydrazyl (DPPH) free radical decolorization technique was used according to the adjusted method of Gelareh et al. (22). **Antioxidant Capacity (ABTS)**: evaluation of the antioxidant capacity by the ABTS cationic radical method and the results were expressed as values (μM Trolox/g) (23).

Physicochemical stability studies

The physicochemical parameters of the beverages were determined for those without stabilizers (T1) and with stabilizers (T4: 0.1% CMC and 0.1% pectin), which was the treatment that presented the best behavior and stability compared to the others initially tested, evaluating their stability at 0, 8, 16 and 32 days under constant refrigeration temperature (4 ºC). For both treatments, pH, titratable acidity, soluble solids and viscosity were determined. In addition, total polyphenols, total carotenoids and antioxidant capacity (ABTS and DPPH) were also evaluated. Three repetitions were performed for all the defined variables.

Sensory analysis

The respective microbiological analysis was carried out to guarantee the safety of the product before preparing the sensory test. For the beverages defined in the control treatment (T1) and the chosen treatment (T4), for day 1, analyzes of the microbiological parameters established in resolution 3929 of 2013 for fruit soft drinks and NTC 3549/2012 were performed: count of mesophilic microorganisms in CFU/ml (AOAC 988.18/1990), count of fecal coliforms for Escherichia coli in CFU/ml (AOAC 966.24/1990) and count of molds and yeasts in CFU/ml (AOAC 995.21/1990). Participants were chosen as frequent consumers of juices and soft drinks, men and women between 18 and 60 years old, who were previously informed of the ingredients of the product and the content of milk derivatives, guaranteeing hygiene and safety measures for the consumption of the samples, according to the ethical guidelines established for this type of tests. The samples used had 3 days of storage at 4°C. A sensory panel was carried out with a group of consumers

(72) to evaluate the acceptability of the product (T4) in parameters of taste, smell, appearance and texture using a hedonic scale from 1 to 5: "like strongly" (5), "like moderately" (4), "neither like nor dislike" (3), "dislike moderately" (2), and "dislike strongly" (1), according to GTC 293/2018 ICONTEC for conducting hedonic tests with consumers.

3. Results and Discussion

Colloidal stability

In Figure 1, a statistically significant difference is observed mainly between the formulation without stabilizers and the treatment with the addition of CMC and pectin ($p < 0.05$), even with higher values for viscosity and Z potential compared to the treatments where only one of the stabilizers was incorporated, except the Z potential between T1 and T2 where there were no statistically significant differences ($p>0.05$). This result suggests a synergy between both hydrocolloids providing better colloidal stability to the beverage and avoiding the agglomeration of suspended particles, thereby contributing to the appearance and texture of the beverage (24). With the increase of the Z potential, the repulsion between the suspended particles is favored, granting greater turbidity and viscosity, and preventing the separation of phases during storage, results that coincide with Ciro et al. (25) in tree tomato-based beverages.

Both the use of pectin (T2) and CMC (T3) individually show a significant difference compared to the beverage without stabilizers (T1), but a better behavior in the stability attributes is found when combining their effect (T4) (Figure 1).

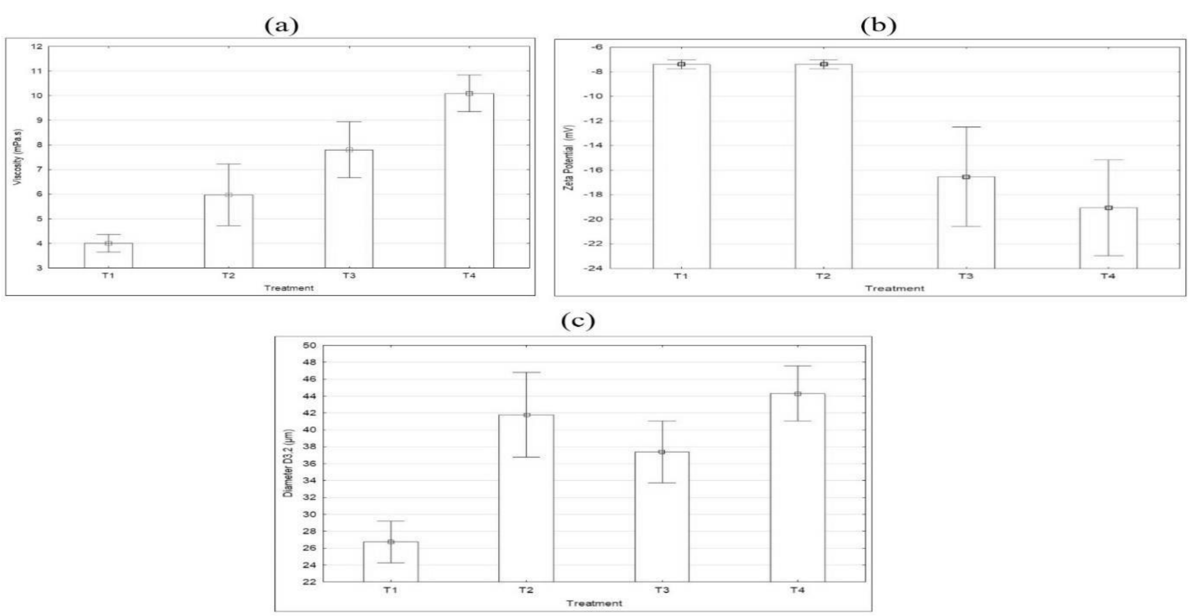

Figure 1 Effect of treatments with different stabilizers used in beverage formulation on colloidal stability parameters: (a) Viscosity (mPa.s). (b) Z-potential (mv). (c) Average Particle Size. T1: without added stabilizers; T2: 0.1% pectin; T3: 0.1% CMC; T4: 0.1% pectin + 0.1% CMC. The mean values are presented with their confidence intervals at 5% by Fisher's test.

The particle size presented a statistically significant differences (p <0.05) between the beverage without hydrocolloids (T1) compared to the other treatments (T2, T3 and T4), where an increase in the average diameter was evidenced, probably due to the encapsulating effect and the formation of an external film in the polysaccharide and cellulosic components as described by Ruihuan et al. (26). For effects of the stability, texture and presentation of the beverage, the formulation that included CMC at 0.1% and pectin at 0.1% (T4) was selected. A similar study by Vizcarra (27) on stabilization of acidified dairy beverages with the addition of CMC and pectin, concluded that the inclusion of both stabilizers in a double homogenization process decreases the sediment in the final product, since there is a synergistic effect between both hydrocolloids.

Characterization of the soft drink type.

Table 2 shows the initial characterization of the physicochemical parameters of the control treatment (T1) without stabilizers and the defined formulation with hydrocolloids after evaluating the colloidal stability results (T4).

Table 2. Physicochemical parameters of beverages formulated with and without stabilizers.

Variable	**Treatment (T1)**	**Treatment (T4)**
pH	3.64 ± 0.01 [a]	3.65 ± 0.01 [a]
Titratable acidity (% eq citric acid)	0.417 ± 0.0006 [a]	0.415 ± 0.004 [a]
Total solids (°Bx)	11.9 ± 0.11 [a]	11.9 ± 0.05 [a]
Viscosity (mPa.s)	4.0 ± 0.14 [a]	27.60±1.76[b]
Supernatant viscosity (mPa.s)	1.69 ± 0.06 [a]	27.1 ± 1.90 [b]
Sediment viscosity (mPa.s)	6.33 ± 0.37 [a]	27.75 ± 1.86 [b]
Z-potential (mv)	-6.79 ± 2.22 [a]	-19.20 ± 1.21 [b]
Density (g/mL)	1.0427 ± 0.0005 [a]	1.0387 ± 0.0012 [b]
Color		
L*	18.96 ± 0.07 [a]	21.04 ± 0.76 [b]
a*	6.21 ± 0.51 [a]	6.35 ± 0.45 [a]
b*	3.96 ± 0.32 [a]	4.29 ± 0.26 [b]
Humidity (% w/w)	87.18 ± 0.153 [a]	87.34 ± 0.124 [a]
Particle Size D (3;2) (µm)	26.73 ± 0.99 [a]	43.95 ± 1.87 [b]
Total Phenols (mg gallic acid/100g)	35.6 ± 1.13 [a]	38.14 ± 1.52 [a]
ABTS (µg trolox/ml)	411.7 ± 29.77 [a]	452.42 ± 12 [a]
DPPH (µg trolox/ml)	217.47 ± 8.17 [a]	219.4 ± 13.34 [a]
Carotenoids (µg β-carotene/100 g	294.25 ± 3.4 [a]	292.99 ± 6.72 [a]

sample)		
Crude protein (g/ 100g)	< 2.5 [a]	< 2.5 [a]
Ashes (g/ 100g)	0.45 [a]	0.49[a]

*The averaged results are presented with their standard deviation. The values marked with different letters per row represent significant differences between the formulations ($p < 0.05$).

The results obtained for both formulations show that this beverage is an acid product with a pH below 4.0, soluble solids by refractometric reading between 11 and 12 ºBx, and percentages of acidity between 0.4 and 0.5% equivalent to citric acid, which conforms to the parameters defined by the Colombian regulations for fruit soft drinks according to NTC 3549 (11) and resolution 3929 of 2013 of Ministry of Health and Social Protection (Colombia) (12); for these variables there was no statistically significant difference between both formulations ($p > 0.05$).

Viscosity values showed statistically significant differences ($p < 0.05$) between the formulation without the addition of hydrocolloids and the one with the addition of CMC and pectin as stabilizers, improving the colloidal stability of the beverage and its appearance to the consumer by reducing the tendency to phase separation due to agglomeration of particles during storage, a result that coincides with the findings by Contreras, Ciro & Arango (28). The increase in viscosity can occur due to the water retention capacity of hydrocolloids, forming a three-dimensional network with water and reducing its mobility by forming hydrogen bonds (29, 30).

Additionally, the value of the Z potential remained in absolute values slightly below the light stability range (-21 to -30) (31); however, it is superior to the drink formulated with hydrocolloids because both the CMC and pectin increases the electrostatic repulsion between particles, improving the steric and electrostatic stability of suspensions (17).

For the value of luminosity (L) an increase is observed with the addition of hydrocolloids, which can be explained by the white or yellowish color characteristic of hydrocolloids, giving a clearer and brighter appearance. The increase in luminosity can also be related to the variation in particle size due to the formation of larger aggregates, affecting the diffraction pattern (18). The value of the coordinate a* in both samples shows a tendency towards red, probably due to the content of anthocyanins provided by the agraz pulp, while the value of b* shows a tendency towards yellow, which may be due to the presence of carotenes provided by the cape gooseberry pulp in the formulation.

In the particle size, a statistically significant difference ($p < 0.05$) is observed between the formulation without stabilizers and the one added with CMC and pectin, the latter presenting higher values. Corredig, Kerr & Wicker (32) comment that this behavior is due to the fact that hydrocolloids can increase the diameter of the particles in suspension due to their encapsulating capacity of insoluble polysaccharide fractions.

Regarding the content of total phenols, carotenoids, DPPH and ABTS, no statistically significant differences were found between the formulations (p> 0.05).

Physicochemical stability of formulations

The physicochemical parameters of pH, titratable acidity, total soluble solids and viscosity evaluated for both treatments with (T4) and without hydrocolloids (T1) during storage under refrigeration conditions are presented in Figure 2.

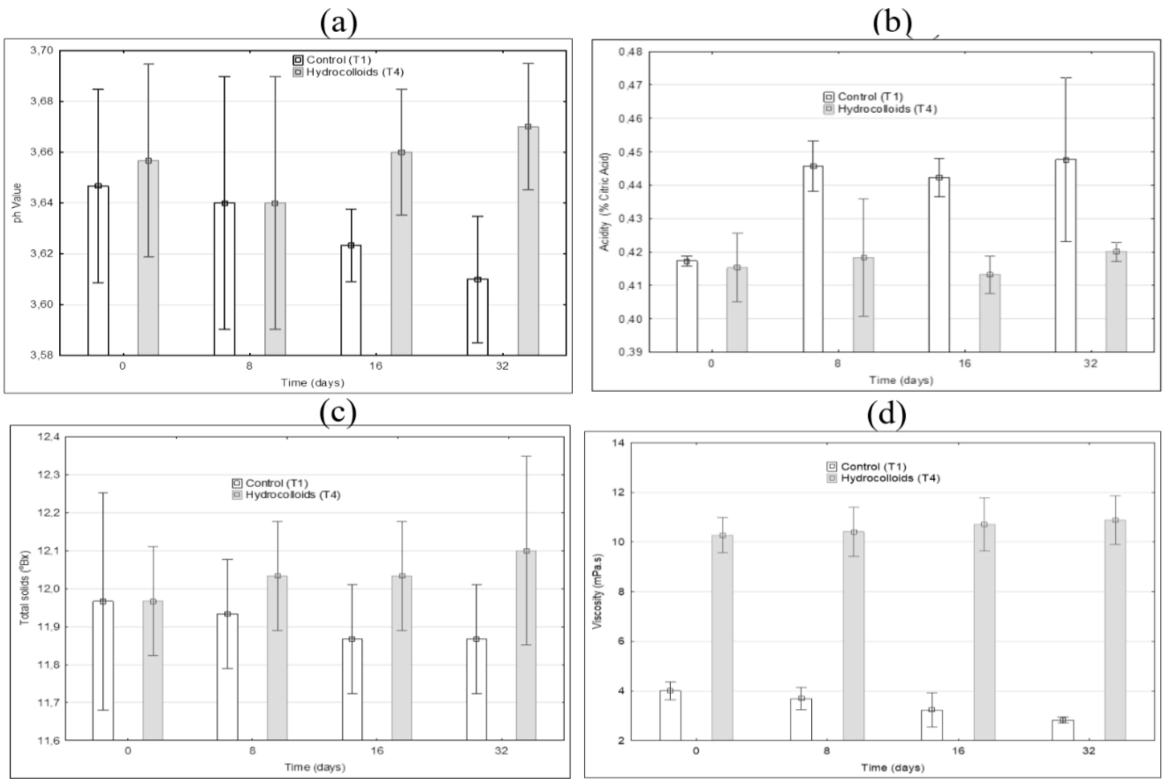

Figure 2. Stability of physicochemical parameters: (a) pH. (b) Titratable acidity (% citric acid w/w). (c) soluble solids (°Bx). (d) Viscosity (mPa.s). The values represent the means and confidence intervals of the data by Fisher's test at 5%.

For most of the physicochemical attributes evaluated, the values remained constant during the 32 days of follow-up and within the parameters established in NTC 3549 (11) and Resolution 3929 of 2013 of Ministry of Health and Social Protection (Colombia) (12). Both in the determination of the pH (Figure 2a) and in the total soluble solids (Figure 2c) no statistically significant differences were observed between the treatments over time (p >0.05), a behavior reported by Franco et al. (33) for agraz nectar, the contents of total phenols and antioxidant capacity determined for both treatments were slightly lower than those found by in a similar study in storage for 40 days. The titratable acidity (Figure 2b) remained constant during the storage time, although an initial increase was observed between day 0 and day 8 for the drink without stabilizers from 0.417 to 0.445 (% citric acid) to then remain constant, a situation not observed in the formulation with hydrocolloids. This result may be due to the release of organic acids present in the peel and fragmented cellular structures of the fruits, which in the case of the beverage with stabilizers may have been encapsulated and would reduce their detection by the titration method (26).

Figure 2d shows statistically significant differences in the viscosity values when comparing the beverage without hydrocolloids (T1) to the mixture with CMC and pectin (T4), where the values were higher due to the thickening effect provided using hydrocolloids that give greater consistency to suspensions of this type and form a gel-like structure that limits the precipitation of suspended particles (25). To the mixture with CMC and pectin (T4), no statistically significant differences were observed for the same treatment over time ($p > 0.05$), showing a high stability of this attribute during the 32 days of observation.

Total polyphenols, ABTS, DPPH and carotenes.

Figure 3 shows the content of total phenols, total carotenoids, and antioxidant capacity (ABTS and DPPH) for the beverage as a function of time.

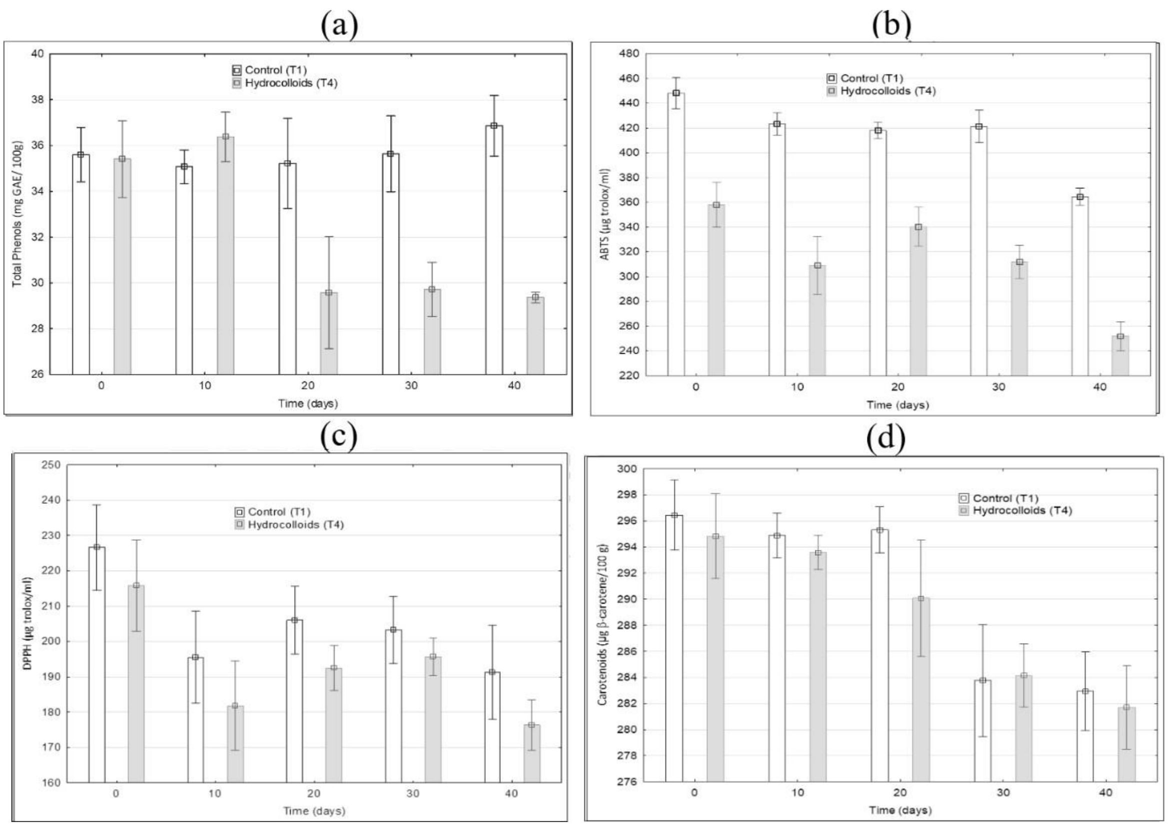

Figure 3. Stability of total phenols, total carotenoids and antioxidant capacity over time: (a) Total Phenols. (b) ABTS. (c) DPPH. (d) β-carotene. The mean values with their corresponding confidence intervals for control treatment (T1) and treatment with hydrocolloids (T4) are presented.

Initially (time = 0 days), no statistically significant differences ($p<0.05$) were found between the treatments for total phenols, DPPH and total carotenoids, probably due to the fact that the addition of hydrocolloids in the amounts used had no effect on the determined concentration.

However, for the antioxidant capacity by ABTS, as observed in Figure 3b, there were differences with a tendency to decrease over time, a situation that could be due to the fact that the presence of hydrocolloids influenced the extraction of compounds of a hydrophilic nature detected by ABTS but not by DPPH that only detects compounds extracted by organic solvents. These hydrophilic compounds may be linked by hydrogen bonds to the hydrocolloids used, making their extraction difficult (34).

As shown in Figure 3a, for the content of total phenols, there were only significant differences between the treatments ($p < 0.05$) from day 20 on, being lower for the treatment with stabilizers, this decrease could be explained as for the current moment electrophilic aromatic substitution reactions favored by the presence of hydrocolloids are happening, thereby reducing the presence of phenolic compounds (35). For the antioxidant capacity by ABTS (Figure 3b), no statistically significant differences were observed between the treatments for the same evaluation times ($p> 0.05$) but it is possible to identify a decrease in the average antioxidant capacity by DPPH with the storage time, probably due to the degradation of these components by factors such as light, aromatic electrophilic substitution reactions and oxidation reactions (36). Higher values were found in the analytes determined for the formulation without stabilizers compared to the one that included CMC and pectin; this result may be due to the fact that the incorporation of hydrocolloids in the beverage generates encapsulation of the phenolic components, antioxidants and carotenoids, making their extraction difficult and, therefore, indicating lower concentrations by the spectrophotometric method used in their quantification.

Although there were statistically significant differences in the contents of all the components when comparing day 0 with day 40 (Figure 3), these losses are not as marked as in other stability studies of phenols and carotenoids in functional beverages under similar refrigerated storage conditions. Santander, Osorio & Mejía (37), for a mixed drink with tree tomato and milk, determined losses of up to 33% in total phenols and 37% in carotenoids by day 28. Compared to the drink developed with cape gooseberry and agraz, with very similar initial values, it only presented a decrease of 17% towards day 40 in total phenols, and only 4% for the carotenoids of the drink with hydrocolloids. This suggests that the antioxidant and functional components of agraz and cape gooseberry may be more stable over time than those obtained from other fruits and would have significant potential as preservatives in other food matrices

(38). Similar works with agraz nectar have shown a stable behavior in its phenolic components and antioxidant capacity (ABTS and DPPH), finding statistically significant differences from day 60 of storage (33).

Sensory analysis

The results of sensory acceptance of the color, smell, taste, texture, and general acceptance attributes are given in Figure 4 (a, b).

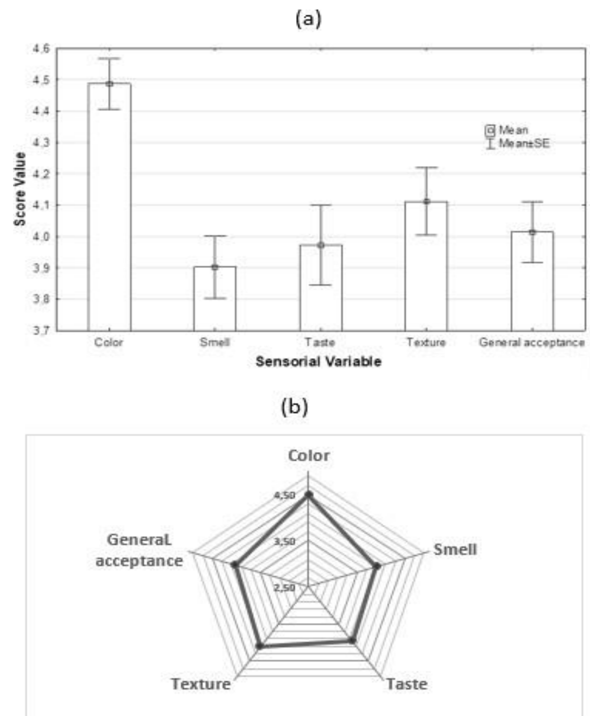

Figure 4. Sensory acceptance test by consumers: (a) acceptance for each attribute from 1 to 5 (color 4.49 ±0.69; smell 3.90 ±0.84; taste 3.97 ±1.07; texture 4.11 ±0.91; general acceptance 4.01 ±0.83. (b) Sensory acceptance hedonic profile (from 1 to 5).

The panelists evaluated the samples of treatment 4, which presented the best behavior and stability according to the physicochemical analyzes previously carried out and the microbiological results, where the counts of mesophiles, fecal coliforms, fungi and yeasts were below 10 CFU/ml, complying with the parameters of the NTC 3549/2012 for fruit drinks. The results indicated acceptance values in all the characteristics or attributes above 78%, with a general acceptance rating between " like moderately" and " like strongly " (Figure 4a).

The results of Figure 4b allow us to establish that the best evaluated attribute was that of color with 89.7%, probably due to the intensity of the tones provided by the agraz and the cape gooseberry pulps, where colored compounds such as anthocyanins and carotenes predominate, favoring the appearance and presentation of the product to the consumer. Texture (perceived viscosity) was also an attribute positively evaluated with 82.2%, suggesting that the effect of CMC and pectin was favorable for the mixture. This agrees with Abedi et al. (24) who, in an affective sensory evaluation of a dairy drink with raspberry juice added with CMC and pectin, found that texture and appearance attributes were better valued in the formulation with hydrocolloids compared to the control without said addition, attributing the effect to the increase in viscosity and consistency. Such assessments were described by Soukoulis et al., and Azarikia & Abbasi (39, 40) who suggest that, although hydrocolloids stabilize fruit drinks, they can also influence the sensory properties where the increase and addition of hydrocolloids decrease the intensities of aroma and flavor, and this is reached when the concentration is higher than a critical value.

It is observed that the smell was the least valued attribute compared to the others, although it was above 78% with some responses of "neither like nor dislike" this was interpreted as some respondents not perceiving the smell of the evaluated beverage intensely. This may be due to the fact that low temperatures (the beverage was served at 4 ˚C) reduce the release of volatile components that give food odor (41) since they are not perceived with the same intensity by people depending on the environment (42).

4. Conclusions

The addition of hydrocolloid stabilizers such as 0.1% CMC and 0.1% pectin to the whey-based formulation made it possible to obtain a product with a better appearance, consistency, and colloidal stability thus favoring its sensory presentation. During the storage time under refrigeration conditions, the product maintains its physicochemical stability for up to 32 days and does not present significant changes for most of its quality attributes, which suggests that it can be commercialized and distributed over longer time frames without presenting alteration. The mixture of whey hydrolyzed permeate with cape gooseberry and agraz pulps allowed to obtain a product with outstanding sensory characteristics, despite minor losses in terms of content of phenols, carotenes and antioxidant capacity during the storage, which would allow this product to be offered as a functional beverage in the food industry. The commercial potential of the beverage was observed by having established compliance with the requirements of Colombian regulations for soft drinks defined in NTC 3549/2012 and resolution 3929/2013; Likewise, according to the regulation on labeling defined in resolution 810/2021, it would be important to carry out future analyzes of sugars provided by pulps, whey permeate and added sugar to establish whether the product should bear the front warning stamps on sugars added, or if, on the contrary, it is possible to add a positive seal and declare, according to its antioxidant content and health properties.

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