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Elaboration, characterization and color stability of an isotonic beverage based on whey permeate with carotenoid powder from pequi

Elaboração, caracterização e estabilidade de cor de bebida isotônica à base de permeado de soro de leite com carotenoide em pó de pequi

Elaboracion, caracterización y estabilidad de color de bebida isotônica a base de permeado de suero com carotenoides em polvo de pequi

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Letícia Rocha Ferreira
ORCID: https://orcid.org/0000-0003-0983-5584
Federal University of Viçosa, Brazil
E-mail: leticia.r.ferreira@ufv.br
Edimar Aparecida Filomeno Fontes
ORCID: https://orcid.org/0000-0002-4370-8805
Federal University of Viçosa, Brazil
E-mail: eaffontes@ufv.br
Letícia Mafle Guimarães Marinho
ORCID: https://orcid.org/0000-0003-0724-1897
Federal University of Viçosa, Brazil
E-mail: leticia.mafle@gmail.com
Frederico Augusto Ribeiro de Barros
ORCID: https://orcid.org/0000-0001-7300-8773
Federal University of Viçosa, Brazil
E-mail: fredbarros@ufv.br
Paulo César Stringheta
ORCID: https://orcid.org/0000-0002-1229-7038
Federal University of Viçosa, Brazil
E-mail: paulocesar@ufv.br
Afonso Mota Ramos
ORCID: https://orcid.org/0000-0001-9435-7745
Federal University of Viçosa, Brazil
E-mail: amramos@ufv.br

Abstract
The aim of our research was to elaborate, characterize and evaluate the stability under light/darkness and different temperatures (4 and 25 ºC) of the color of an isotonic beverage based on whey permeate with carotenoid extract powder from pequi, and verify its microbiological safety and sensory acceptance. The 3% (w/v) concentration of powdered carotenoid from pequi was chosen because it has osmolality (314.89 mOsmol/L) in the range of hydroelectrolytic beverages and light-yellow tint. The beverage was evaluated for minerals Na (662 mg/L), K (1363.73 mg/L), total carotenoids (75.9 mg/L) and antioxidant capacity by the radicals ABTS (10.79 μmol equivalent Trolox/100 mL) and DPPH (73.38 μmol equivalent Trolox/100 mL), had good sensory acceptance by athletes and remained within microbiological criteria during stability study. The color coordinate L^* has undergone less change and C^*, greater change. The condition darkness at 4 ºC showed less change in yellow tint, for more than 30 days ($t_{1/2}$=121.6 day). Due to its characteristics, the beverage has potential benefits for consumption by athletes, because besides being isotonic, it has bioactive properties of pequi carotenoids and natural constituents of whey permeate. The use of permeate, often discarded as effluent, has benefits for the environment.
Keywords: Total carotenoid content; Bioactive secondary metabolites; Natural pigments; Stability study; Sensory acceptance; Microbiological safety.

Resumo
O objetivo de nossa pesquisa foi elaborar, caracterizar e avaliar a estabilidade sob condições de armazenamento em claro/escuro e diferentes temperaturas (4 e 25 ºC) da cor de uma bebida isotônica à base de permeado de soro com extrato de carotenoide em pó de pequi, e verificar sua segurança microbiológica. e aceitação sensorial. A concentração de 3% (m/v) de carotenoide em pó de pequi foi escolhida por apresentar osmolalidade (314,89 mOsmol/L) na faixa das bebidas hidroeletrolíticas e tonalidade amarelo-claro. A bebida foi avaliada quanto aos minerais Na (662 mg/L), K (1363,73 mg/L), carotenoides totais (75,9 mg/L) e capacidade antioxidante pelos radicais ABTS (10,79 μmol

equivalente Trolox/100 mL) e DPPH (73,38 μmol equivalente Trolox/100 mL), teve boa aceitação sensorial pelos atletas e manteve-se dentro dos critérios microbiológicos durante o estudo de estabilidade. A coordenada de cor L* sofreu menos alteração e C*, maior alteração. A condição escuridão a 4 ºC apresentou menor mudança na tonalidade amarela, por mais de 30 dias ($t_{1/2}$ = 121,6 dias). Pelas suas características, a bebida apresenta benefícios potenciais para o consumo de atletas, pois além de ser isotônica, possui propriedades bioativas dos carotenoides do pequi e constituintes naturais do permeado do soro de leite. O uso de permeado, muitas vezes descartado como efluente, traz benefícios ao meio ambiente.
Palavras-chave: Teor total de carotenoides; Metabólitos secundários bioativos; Pigmentos naturais; Estudo de estabilidade; Aceitação sensorial; Segurança microbiológica.

Resumen
El objetivo de nuestra investigación fue elaborar, caracterizar y evaluar la estabilidad bajo luz/oscuridad y diferentes temperaturas (4 y 25 ºC) del color de una bebida isotónica a base de permeado de suero con extracto de carotenoide en polvo de pequi, y verificar su seguridad microbiológica. y aceptación sensorial. Se eligió la concentración del 3% (m/v) de carotenoide en polvo de pequi porque tiene osmolalidad (314,89 mOsmol/L) en el rango de las bebidas hidroelectrolíticas y tinte amarillo claro. La bebida fue evaluada en cuanto a minerales Na (662 mg/L), K (1363,73 mg/L), carotenoides totales (75,9 mg/L) y capacidad antioxidante por los radicales ABTS (10,79 μmol equivalente Trolox/100 mL) y DPPH (73,38 μmol equivalente Trolox/100 mL), tuvo una buena aceptación sensorial por parte de los atletas y se mantuvo dentro de los criterios microbiológicos durante el estudio de estabilidad. La coordenada de color L* ha sufrido menos cambios y C*, un cambio mayor. La condición de oscuridad a 4 ºC mostró menos cambio en el tinte amarillo, por más de 30 días ($t_{1/2}$ = 121.6 día). Por sus características, la bebida tiene potenciales beneficios para el consumo de los deportistas, pues además de ser isotónica, posee propiedades bioactivas de los carotenoides de pequi y constituyentes naturales del permeado de suero. El uso de permeado, a menudo descartado como efluente, tiene beneficios para el medio ambiente.
Palabras clave: Contenido total de carotenoides; Metabolitos secundarios bioactivos; Pigmentos naturales; Estudio de estabilidad; Aceptación sensorial; Seguridad microbiológica.

1. Introduction

The growing appeal of a healthy life among the consumers has increased the demand for natural products with added value, such as beverages with health-promoting properties, which has led to the development of isotonic beverages enriched with fruits as a source of nutrients and bioactive compounds (Ferreira et al., 2020; Gironés-Vilaplana, Mena, Moreno, & García-Viguera, 2014; Porfírio et al., 2019; Raman, Ambalam, & Doble, 2019).

Whey permeate is a co-product of the production of concentrated and isolate protein from whey by ultrafiltration. It is obtained in large volume and has limited applications, being discarded, in most cases, as effluent. Its chemical composition includes mainly water, lactose, minerals, non-protein nitrogen and low molar mass compounds such as water-soluble vitamins (riboflavin, pantothenic and nicotinic acid) (Parashar, Jin, Mason, Chae, & Bressler, 2016), in addition to minerals such as calcium, sodium, magnesium and potassium that confer electrolytic characteristics (Fontes, Alves, Fontes, & Minim, 2015) and exercise activity in biological processes (Petrus, Assis, & Faria, 2005). Thus, whey permeate has osmolyte characteristics and natural nutrients, which shows its promising use as a basis in the formulation of isotonic beverages designed to assist hydration after physical activity (Fontes et al., 2015).

Natural dyes, extracted from fruits and vegetables, can be used in the formulation of beverages with isotonic characteristics, providing color and bioactive properties to the final product. Pequi (*Caryocar brasiliense* Camb.) is a typical Brazilian Cerrado fruit that has bioactive compounds with antioxidant capacity such as phenolic and carotenoid compounds, molecules associated with reducing the risk of developing chronic diseases such as cancer, cataracts, age-related macular degeneration and cardiovascular diseases. However, carotenoids are sensitive to heat and insoluble in water (de Mendonça et al., 2017; Machado, Mello, & Hubinger, 2013; Nascimento-Silva & Naves, 2019). According to Schweikert (2017), the major forms of solubility of carotenoids nowadays are oily suspensions, oil-in-water emulsions and water-dispersible powders. There are some studies in the literature on ways to increase the solubility and stability of colored beverages with natural carotenoids (Mesnier, Gregory, Fança-Berthon, Boukobza, & Bily 2014, Bovi, Petrus, & Pinho 2017 and Ligia Focsan, Polyakov, &

Kispert 2019). Another study by Pinto et al. (2018) reports on extracts rich in pequi carotenoids encapsulated by drying in a foam layer to protect against the deleterious effects of exposure to light and emulsified to be solubilized in an aqueous base.

In this context, the use of natural fruit extracts such as pequi, as dye and the whey permeate, ingredients with bioactive properties for the production of isotonic beverages, beverages associated with sports activities, becomes advantageous for the food industries due to the growing demand for health-promoting foods for athletes. However, there are many limitations to the commercial use of natural dyes due to the low stability, making the application of natural extracts dependent on factors such as: pH, temperature, presence of oxygen, light, ascorbic acid, cofactors and chemical structure. Thus, the aim of this study was to elaborate an isotonic beverage based on permeate from the ultrafiltration of whey with carotenoid extract powder from pequi, and to study the color stability of this product under light and different temperatures (4 and 25 ºC), in addition to conducting microbiological and sensory analyzes on the product to verify its safety and acceptance.

2. Methodology

2.1 Permeate of whey ultrafiltration

The permeate used was obtained by the ultrafiltration of whey using the membrane system of the Innovative Laboratory of the Federal University of Viçosa (UFV, Viçosa, Minas Gerais, Brazil). The WGM Systems plant was used in ultrafiltration pilot standpoint equipped with a polysulfone/polyamide spiral membrane and with a molecular weight cut-off at 10 kDa. We used the temperature between 45 and 50 ºC to maximize the permeate flow during the concentration of the milk phase. It is necessary because milk has lower viscosity in this interval, which minimizes the precipitation of calcium salts. The pressure variation applied to the milk was 0.99 atm, with inlet pressure of 2.96 atm and outlet pressure of 1.97 atm. The membrane filtration area was 6 m^2 (Ferreira et al., 2020).

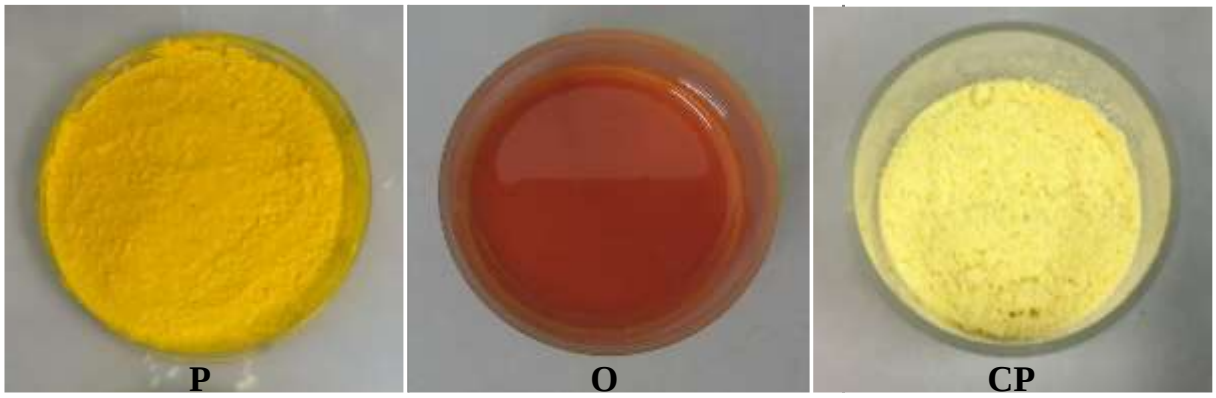
2.2 Pequi

Pequi (*Caryocar brasiliense* Camb.), from 2015 harvest at Santana do Pirapama-MG, located at 19º 00 21 S (latitude) and 44º 02 35 W (longitude), were used. These were manually peeled and pulped in a mechanical pulping machine (Itametal®, model Bonina 0.25 df). The pulps were stored in polyethylene packages in portions of approximately 250 g at 8 ± 2 ºC until used.

2.3 Acquisition of carotenoid powder from pequi: emulsification (O/W) followed by foam mat drying

The obtaining of carotenoid powder from pequi (Figure 1) from the oily carotenoids extract followed the methodology described by Pinto et al. (2018) with modifications. Fresh pequi pulp (Figure 1) was lyophilized (Lyophilizer - Terroni, model Fauvel LH 0400/4L) for 48 h. From the freeze-dried pequi pulp, carotenoids were extracted using the same procedure described by Pinto et al. (2018) to obtain the oily carotenoids extract (Figure 1). The extracts were stored in polypropylene bottles, wrapped in foil and stored in a freezer (−22 °C). For emulsification (O/W) powdered soy lecithin (Êxodo Científica) 1% (w/w) homogenized on a mechanical stirrer (AGI 103, Nova Ética) at 504 rpm for 10 min was added before Emustab® (Duas Rodas Industrial Ltda) 5% (w/w).

Figure 1. Fresh pequi pulp (PP); Oily carotenoids extract from pequi pulp (OC); and carotenoid powder from pequi (CP).



Source: Authors.

2.4 Elaboration of isotonic beverage

To define the formulation of the isotonic beverage, preliminary tests were carried out with different concentrations of carotenoid extract in pequi powder (1%, 2%, 3% and 4% (m/v)) with the purpose of presenting the final beverage osmolality between 270 and 330 mOmol/L and yellow color characteristic of powdered carotenoid that was added to the formulations. The beverage made with 3% of carotenoids powder from pequi was the one that met the requirements described previously (data not shown).

To elaborate the isotonic beverage according to Fontes, Alves, Fontes, & Minim (2015), 0.0075% (w/v) sucralose was added to the permeate of whey ultrafiltration. Then, it was acidified to pH 3.5 using a 2% (w/v) citric acid solution. The mixture underwent a pasteurization process (62.8-65 °C/30 min) and was then cooled to 40 °C for the addition of the preservatives (0.01% (w/v) potassium sorbate and 0.05% (w/v) sodium benzoate). We then cooled it to a temperature of 20 °C again to add the carotenoids in pequi powder and aroma identical to natural (0.01% (m/v)). The formulated beverages were distributed (50 mL) in previously sterilized transparent and amber bottles of 60 mL (121 ºC/15 min). Having defined the formulation of the beverage that met the requirements of osmolality and with a greater intensity of yellow color, the beverage was prepared for its physical, chemical and sensory characterization (addition of a pineapple or passion fruit flavor), in addition to microbiological analyzes during the color stability study.

2.5 Physical and chemical analyzes of isotonic beverage

2.5.1 Centesimal composition

The determinations of moisture, total nitrogen and fixed mineral residue were performed as described by AOAC (2006). The total carbohydrate content was obtained from the difference in percentage of the sum of the other nutrients (protein, fixed mineral residue, and water) and lactose by titrimetric analytical procedure (ISO/IDF, 2007).

2.5.2 Titratable acidity, pH and total soluble solids

The beverages were characterized in relation to pH, titratable acidity, total soluble solids according to the methods described by AOAC (2006).

2.5.3 Determination of osmolarity

The osmolarity was determined by cryoscopy, according to Fontes, Alves, Fontes, & Minin (2015). For quantification, Eq. (1) was used.

$$\text{Osmolality}\left(\text{mOsmol}/\text{L}\right) = \frac{T_c}{K_c} \cdot 1000 \quad (1)$$

Where K_c is cryoscopic water constant (1.86 °C mol/kg) and T_c is freezing point temperature of the beverage samples, in degrees Celsius.

2.5.4 Minerals

To determine sodium, potassium, calcium, magnesium, and phosphorus, the isotonic beverage was digested according to the methodology described by Gomes & Oliveira (2011). Sodium and potassium concentrations was determined by flame photometry (Celm FC-280), while atomic absorption (Spectraa 220FS) was used for calcium and magnesium concentrations. The atomic absorption spectrophotometry (Femtro 600S) was used for phosphorus using analytical curves for the quantification of each mineral.

2.5.5 Color

Parameter lightness (L^*), redness (a^*), yellowness (b^*) of isotonic beverage were carried out using the Colorquest XE (HunterLab, Reston, VA) colorimeter. The equipment, connected to a computer provided with the universal software system, was duly calibrated for included reflectance, 10º observer angle and D65 illuminant. From the parameters, the cylindrical coordinate H^* (hue angle) was calculated according to Pinto et al. (2018), and C^* (chroma) and total color difference (E) according to Mutsokoti et al. (2017).

2.5.6 Total Carotenoids Content

Total carotenoids content was determined according to the method described by Rodriguez-Amaya, (2001). The carotenoids were extracted with acetone, separated in petroleum ether, diluted in a volumetric flask and subsequently read in a spectrophotometer (UV BEL Photonics SP 1105) at the wavelength of 450 nm to determine the total carotenoid content, expressed in β-carotenoids.

2.5.7 Antioxidant capacity

The *in vitro* antioxidant capacity was analyzed using spectrophotometric methods (spectrophotometer-BEL Photonics UV-M51) in low light environment. The antioxidant capacity was determined by the capture of the free radical ABTS according to the methodology described by Re et al. (1999) with modifications. In an amber bottle, the same volume of ABTS solution (7.0 mmol/L) was mixed with potassium persulfate solution (2.45 mmol/L), which remained in the dark for 12-16 h for the generation of the ABTS•+ chromophore cation. After this period, the solution of the radical was diluted in 80% ethanol until reached an absorbance of 0.700 ± 0.005, at wavelength of 734 nm, in a spectrophotometer previously calibrated with 80% ethanol (white). Then, we added 0.5 mL aqueous beverage dilutions and 3.5 mL of the ABTS•+ radical solution to test tubes, followed by homogenization in a tube shaker. After 6 min of reaction, the absorbance was read of the samples at same wavelength. As control, we used 80% ethanol instead of the addition in the samples.

The antioxidant capacity was also determined by the DPPH assay following the methodology described by Brand-Williams, Cuvelier, e Berset (1995), with modifications. Aliquots of 0.1 mL of aqueous beverage dilutions were transferred to test tubes, followed by the addition of 2.9 mL of 60 µmol/L methanolic solution of the DPPH•+ radical previously prepared. After homogenization in a tube shaker, they were allowed to rest, in the dark, for 25 min. The absorbance at 515 nm was used for reading the samples in a spectrophotometer, previously calibrated with methanol (white). As control, we used methanol instead of the addition in the samples. Analytical Trolox curves were constructed for each method, and the results were

expressed in μmol equivalent of Trolox/100 mL of the isotonic beverage.

2.6 Microbiological analysis

Microbiological analyses were carried out according to a methodology standardized by the American Public Health Association (APHA, 2001), evaluating Most Probable Number (MPN) for coliforms at 35 ºC, total counts of aerobic mesophilic microorganisms, filamentous fungi and yeasts and psychotropic and identification of the presence or absence of *Salmonella* spp.

2.7 Color stability study of isotonic beverage

To assess the effect of light on color stability, beverage samples were packaged in transparent flasks, then stored in a light cabinet, at 25 ºC containing lamps in the color temperature range corresponding to daylight, which more approaches sunlight (~ 6500 Kelvin), keeping a distance of about 4 cm from each other, protected from any other light source, at 25 ºC, and the other flasks, in a display refrigerator at 4 ºC, adapted with fluorescent lamps. The beverages in amber bottles were distributed and placed in closed cardboard boxes and stored in a display refrigerator (4 ºC) and at room temperature (25 ºC) in the darkness for 30 days.

The kinetics of changing the color coordinates a^*, b^*, C^* and h^* were evaluated to determine the speed of change (k) and the half-life ($t_{1/2}$ - time required to change a given color coordinate in 50%).

For the calculation of the rate of change of the color coordinates, the first order kinetics was admitted according to Eq. (2).

$$\ln\frac{C}{C_0} = \frac{k}{t} \quad (2)$$

Where, C is the value of the color coordinate after the storage time of the beverage samples under conditions of light and controlled temperature and C_0 is the value of the color coordinate at time zero; *k* the speed of change and t the time. By linear regression analysis, the rates of change were estimated.

To calculate the half-life ($t_{1/2}$), from the values obtained for k, the values of $t_{1/2}$, were calculated from the first order relations according to Eq. (3).

$$t_{1/2} = \frac{\ln 2}{k} \quad (3)$$

2.8 Sensory acceptance test

After approval by the Ethics Committee (CAAE: 56368416.7.0000.5153) the acceptance of isotonic beverages formulated with pineapple and passion fruit aroma were assessed by 100 athletes, untrained tasters, from the Athletic Association-LUVE / UFV in the Sensory Analysis laboratory at UFV.

Acceptance was assessed according to the attributes color, flavor, aroma and global impression, using the nine-point hedonic scale according to Lawless & Heymann (2010). The samples were presented refrigerated in transparent 50 mL disposable cups identified with three-digit codes, monadically, sequentially and randomly to the evaluators. At each evaluation, the evaluators were instructed to rinse the mouth with water to remove possible residues inside the mouth.

2.9 Statistical analysis

The experiment to study the color stability of the isotonic beverage formulated with carotenoid powder from pequi based on the permeate was conducted in a completely randomized design in a split-plot scheme. The controlled light and temperature conditions in the plot, totaling 4 storage conditions: light at 25 ºC, light at 4 ºC; dark at 25 ºC and dark at 4 ºC. In

the subplot, storage time (zero to 30 days) was a factor.

The data were interpreted by analysis of variance (ANOVA), using t test (sensory analysis), Tukey test for comparison of means and regression analysis, adopting a level of 5% probability. Statistical Analyzes System (SAS), version 9.4, licensed for use by UFV, was used to carry out the statistical analysis.

3. Results and Discussion

3.1 Beverage characterization

Table 1 shows the results of the analysis of the characterization of the isotonic beverage made with ultrafiltration permeate of whey with 3% carotenoid powder from pequi added.

Table 1. Mean values (± standard deviation) of the chemical characteristics of the isotonic beverage (IB) formulated with whey permeate with carotenoid powder from pequi (CP).

Characteristics	Mean (n=3)
Water (% w/v)	91.63 ± 0.28
Total nitrogen (% w/v)	0.02 ± 0.00
Ash (% w/v)	0.47 ± 0.02
Lactose (% w/v)	4.67 ± 0.03
Total Carbohydrates (% w/v)	7.88 ± 0.20
pH	3.66 ± 0.04
Total titratable acidity (% w/v) expressed in citric acid	0.51 ± 0.06
Total soluble solids (ºBrix)	7.9 ± 0.0
Sodium (mg/L)	662 ± 19.14
Potassium (mg/L)	1363.73 ± 33.23
Phosphorus (mg/L)	295.27 ± 8.26
Calcium (mg/L)	294.40 ± 65.87
Magnesium (mg/L)	62.25 ± 1.06
Osmotic concentration (mOsm/L)	314.89 ± 2.56
Total Carotenoids content (µg/100 g)	77.95 ± 6.22
Antioxidant capacity ABTS (µmol equivalent of Trolox/100 mL)	10.79 ± 1.50
DPPH (µmol equivalent of Trolox/100 mL)	73.38 ± 3.49

Source: Authors.

The total carbohydrate content obtained (7.88% (w/v) is within the range that defines isotonic beverages (6% to 8%) and is also adequate according to Resolution RDC 18 (ANVISA, 2010), which is up to 8% in the ready-to-beverage beverage.

The total soluble solids content of the isotonic beverage analyzed in the present study showed a value equal to 7.9 ºBrix, a value higher than that found by Ferreira et al. (2020) (5.83 ºBrix), in an isotonic beverage with anthocyanin extract from jabuticaba. By drying in a foam mat, the carotenoids, natural dyes of the present work, had the addition of wall materials (maltodextrin) and emulsifier (soy lecithin), which increased the soluble solids content of the isotonic beverage.

Isotonic beverages must have high acidity (pH<4.6) to limit the growth of microorganisms and guarantee their microbiological stability and safety (Petrus et al., 2005; Valadão, Shimoda, Jory, Fratassi, & Petrus, 2019), thus, in the

elaboration of the beverage citric acid was added to the permeate. The pH value of the isotonic beverage ready for consumption was 3.66 and for total titratable acidity 0.51% (w/v) (expressed as citric acid) (Table 1). Fontes et al. (2015), when studying electrolyte beverage based on skimmed milk permeate, found a pH value of 3.42 and an acidity of 0.66% (expressed as citric acid). These results demonstrate small variations between the same characteristics for the same type of beverage.

Regarding the mineral content of the elaborated beverage, the Na, K, Ca concentrations are within the values required by the legislation (ANVISA, 2010). The concentrations of these minerals and P, were similar to those obtained in the characterization of an isotonic beverage made with ultrafiltration permeate added with anthocyanins extract from jabuticaba in the work of Ferreira et al. (2020). When compared to commercial isotonics of different brands, isotonics with standardized mineral concentration, the elaborated beverage presented a concentration of minerals higher than those found by Coombes (2005) and Leśniewicz, Grzesiak, Żyrnicki, & Borkowska-Burnecka, (2016). Ferreira et al. (2020) state that these minerals are important in replacing the electrolytes of athletes and because it is an isotonic beverage (314.89 mOsmoL/L), the electrolytes are absorbed more quickly during training without causing gastrointestinal problems.

The color of the beverage is provided by carotenoids, with the total carotenoid content equal to 77.95 µg/100 g (Table 1). Carotenoids, in addition to providing color to the beverage, also confer antioxidant characteristics, as they are able to eliminate reactive oxygen species (ROS) and inhibit the formation of antioxidant species (Mariutti, Chisté, & Mercadante, 2018).

The in vitro antioxidant capacity of the elaborated beverage was evaluated by ABTS and DPPH analysis, due to the carotenoids' ability to eliminate radicals, respectively ABTS•+ and DPPH•+. The beverage showed values of antioxidant capacity equal to 10.79 µmol equivalent of Trolox/100 mL (ABTS) and 73.38 µmol equivalent of Trolox 100 mL (DPPH), indicating that consumption of the beverage may increase the antioxidant potential (Bovi et al., 2017; Cerezal Mezquita et al., 2020; Schweikert, 2017).

The permeate, the base of the elaborated isotonic beverage, is, in itself, a practically sterile product. Even so, a slow pasteurization was performed on the permeate, with the addition of chemical preservatives and the beverages were poured into previously sterilized glass bottles. The beverage had a relatively low microbiological count for filamentous fungi and yeasts, aerobic and psychotropic mesophiles below 10^1 CFU/mL, Most Probable Number (MNP) of coliforms at 35 ºC below 3 NMP/mL and absence of *Salmonella* spp. throughout the color stability study, under different storage conditions, indicating the microbiological safety of the beverage.

3.2 Isotonic beverage stability study

3.2.1 Effect of storage conditions

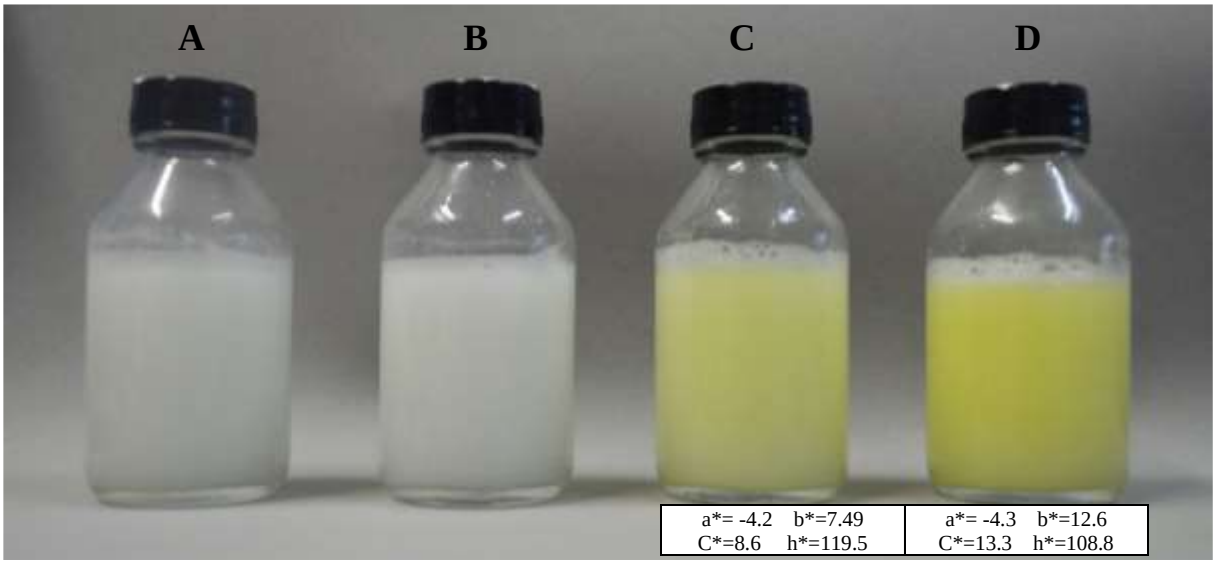
Effects of exposure or not of light and temperature, refrigeration (4 ºC) and environment (25 ºC) were observed in the color coordinates in samples of isotonic beverages during 30 days of storage (Table 2). Visually, beverages stored in the presence of light became whitish after the storage period (Figure 2), due to the presence of ingredients that were added to the formulation, such as maltodextrin and emulsifiers, that influenced the final color of the samples stored under the presence of light. It should be noted that with 40 days and 60 days of storage, the color of the beverages was still preserved in the conditions of darkness at 25 ºC and 4 ºC, respectively.

Table 2. Mean values of the color coordinates of the isotonic beverage (IB) formulated with whey permeate with carotenoid powder from pequi (CP) stored under different light and temperature conditions for 30 days.

Condition	L*	a*	b*	C*	h*	ΔE
Initial	58.96	-3.76	15.75	16.20	103.42	-
Light at 25 ºC	59.60[a]	-2.57[a]	3.85[a]	5.36[a]	147.40[a]	12.20[a]
Light at 4 ºC	62.63[a]	-2.51[a]	5.64[b]	7.11[b]	141.23[a]	10.89[a]
Dark at 25 ºC	60.48[a]	-4.00[b]	13.23[c]	13.86[c]	107.70[b]	3.09[b]
Dark at 4 ºC	59.84[a]	-3.85[b]	14.91[d]	15.40[d]	104.56[b]	1.40[c]

Means (n = 3) followed by at least the same letter in the column do not differ by Tukey's test (p> 0.05). Source: Authors.

Figure 2. Isotonic beverages (IB) formulated with whey permeate with carotenoid powder from pequi (CP) stored under different light and temperature conditions. Presence of light at 25 ºC after 30 days (A); dark at 4 ºC after 30 days (B); darkness at 25 ºC after 40 days (C) and darkness at 4 ºC after 60 days (D).



Source: Authors.

It is observed that the luminosity (L^* coordinate) of the beverages was not affected by the storage conditions ($p>0.05$), since all samples initially presented a light color, therefore, this coordinate did not change significantly during the 30 days of storage.

The change in the coordinate a*, which represents the green chromaticity parameter, was observed in the storage conditions in the presence of light that differed from the conditions of darkness ($p<0.05$). On the other hand, changes in the b* coordinate that represents the chromaticity parameter of yellow and in the saturation (C^*) of yellow color, differed between all conditions ($p<0.05$), being more preserved in the darkness at 4 ºC. Changes with an increase in the shade angle (h^*) were observed in the conditions of presence of light ($p<0.05$) at 25 ºC and 4 ºC, indicating loss of yellow tint. Isotonic beverages stored in the darkness under refrigeration, on the other hand, maintained their tonality close to that of the day they were made during the entire stability study.

The loss of yellow tint is directly related to the degradation of carotenoids. Because they are molecules with a highly unsaturated structure, a characteristic that gives them the property of being antioxidants by sequestering singlet oxygen, interacting with free radicals, they are more susceptible to isomerization and oxidation, being unstable at high temperatures and light (Maiani et al., 2009; Rodriguez-Amaya, 2001).

The color of the beverages stored in the dark condition was preserved when compared to the beverages stored in the presence of light. The condition of storage under refrigeration temperature also preserved the color more when compared to storage under room temperature, even in the darkness. According to Boon et al. (2010) exposure to light degrades carotenoids by producing radical cations leading to rapid degradation of carotenoids and consequently loss of yellow color.

In the work of Chen, Peng, & Chen (1996) who evaluated the stability of different carotenoids and vitamin A in carrot juice under different storage conditions (light and dark storage at 4; 25 and 35 ºC for 3 months), as result that the concentration of all carotenoids decreased with increasing storage temperature and that the carotenoids from samples stored in the dark were less destroyed than in the presence of light. Under light storage, β-carotene concentration decreased from 59.7 to 48.7, 47.3 and 46.2 µg mL after storage for 3 months at 4; 25 and 35 ºC, respectively and under dark storage, β-carotene concentration decreased from 54.7 to 50.9, 49.0 and 46.4 µg mL, in the same conditions of time and temperature of storage.

The stability of carotenoids during storage is affected due to oxidation and isomerization reactions. These reactions result in the loss of sensory attributes related to product quality and the purchase intention based on color (Castro-López et al., 2016).

Regarding the variable ΔE (global color difference), the higher its value, the greater the total color difference of the product over time in relation to the product at time zero, on the day it was produced. The values found for ΔE showed that isotonic beverages stored in the condition of presence of light reached the limit of detection of the global color difference more quickly and would be easily perceived by the consumer. According to Schubring (2009) differences of the order of 3 can be described as "very pronounced", since limits below 1 (SCHUBRING, 2009) and 2 (MESNIER et al., 2014) are not perceptible by human eyes.

3.2.2 Effect of time on storage conditions

The storage time of 30 days affected the color coordinates of the beverages under the studied conditions ($p>0.05$).

Figure 3 shows the variations in color coordinates during the 30 days of storage, under different conditions.

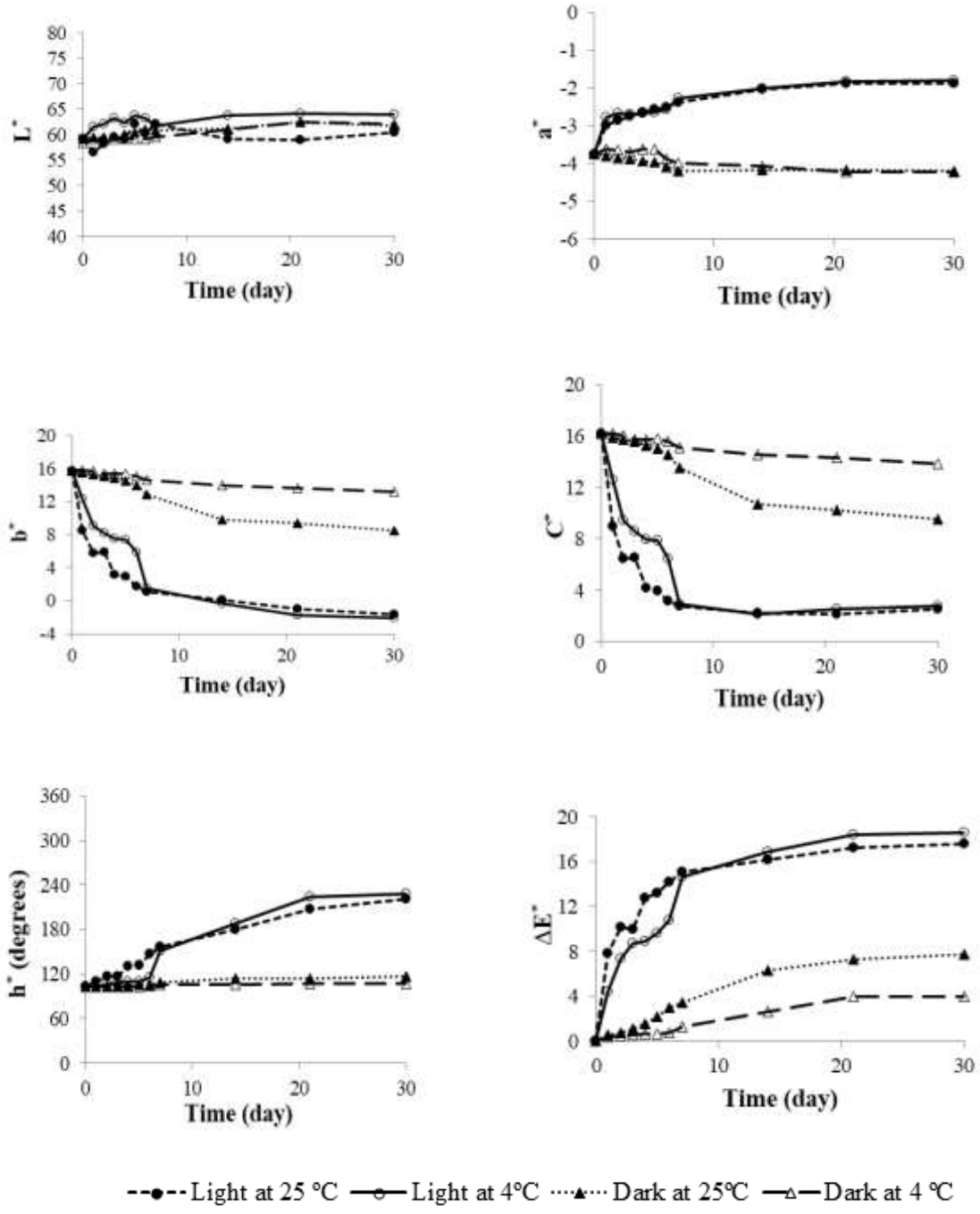
It can be seen in Figure 3 that the luminosity (L*), suffered little influence from the storage time, regardless of the storage conditions, due to the fact that the beverage samples initially presented a light color.

The change in coordinate a^* occurs quickly until 12h, in light conditions at 25 ºC and 4 ºC and then begins to slow down the change up to 30 days of storage (Figure 3). For the condition without light at 25 ºC, the change in the value a^* remains increasing until the 7th day and from that point, it decelerates until the 30th day. However, in the condition without light at 4 ºC, there was no change until the 6th day (Figure 3) and from then on, a slight decrease was observed up to 30 days of storage.

The b^* coordinate, which represents the yellow chromaticity parameter, and the cylindrical coordinates C^* and h^* also underwent similar changes in light conditions at 25 ºC and 4 ºC, with increasing changes up to the 7^{th} day, presenting deceleration curves up to 30º storage day (Figure 3). In the darkness conditions at 25 ºC and at 4 ºC, a decrease in the values b*, C* and h* is observed during the 30 days of storage, however with less change in the condition without light at 4 ºC.

Therefore, the first order kinetic model was applied and adjustment to experimental data ($p<0.05$) was observed for color coordinates (a^*, b^*, C^* and h^*) in samples of isotonic beverages stored under storage in the presence and darkness at 4 ºC and 25 ºC to represent changes in a given period of time.

Figure 3. Variation of color coordinates in the isotonic beverage (IB) formulated with whey permeate with carotenoid powder from pequi (CP) in different storage conditions (light/darkness and 4/25 ºC) for 30 days. The study was carried out in three experimental replications, with duplicate analysis.



Source: Authors.

Table 3 shows the estimates of kinetic coefficients to change the color coordinates of isotonic beverages under different storage conditions, in a given period of time. For the results obtained, the values of the speed constants are higher in the presence of light at room temperature (25 ºC).

Table 3. Estimation of kinetic coefficients to change color coordinates in samples of isotonic beverage (IB) formulated with whey permeate with carotenoid powder from pequi (CP), stored under different light and temperature conditions for 30 days.

Coordinate	Condition	Period of time (day)	*k* (day)	$t_{1/2}$ (day)	R^2
a*	Light at 25 ºC	0 at 0.5	0.4076	1.7	0.9729
	Light at 4 ºC	0 at 0.5	0.4127	1.7	0.9280
	Dark at 25 ºC	0 at 7	0.0152	45.6	0.9350
	Dark at 4 ºC	6 at 30	0.0035	198.0	0.8659
b*	Light at 25 ºC	0 at 7	0.3424	2.0	0.9786
	Light at 4 ºC	0 at 7	0.2807	2.5	0.9277
	Dark at 25 ºC	0 at 30	0.0232	29.9	0.9422
	Dark at 4 ºC	0 at 30	0.0066	105.0	0.9323
C*	Light at 25 ºC	0 at 7	0.2445	2.8	0.9536
	Light at 4 ºC	0 at 7	0.1979	3.5	0.9251
	Dark at 25 ºC	0 at 30	0.0202	34.3	0.9304
	Dark at 4 ºC	0 at 30	0.0057	121.6	0.9315
h*	Light at 25 ºC	0 at 7	0.0571	12.1	0.9784
	Light at 4 ºC	0 at 7	0.0402	17.2	0.9423
	Dark at 25 ºC	0 at 30	0.0044	157.5	0.9223
	Dark at 5 ºC	0 at 30	0.0017	407.7	0.8911

Source: Authors.

It is observed that the coordinate a^* (green chromaticity), presented a rate of change very close in the light conditions at 25 ºC and 4 ºC and the most affected in these conditions, for a short period of time. Probably, the rate of change in this coordinate is due to the degradation of riboflavin (vitamin B2), which is greenish yellow, is responsible for the color of whey or for ultrafiltration permeate and is bleached by oxidation, being degraded under these conditions of the study (Goulding, Fox, & O'Mahony, 2020).

The change in the b^* coordinate (yellow chromaticity) is related to the degradation of the carotenoid powder from pequi that was added in the formulation of the beverage and that presents a characteristic yellow color. It can be seen in Table 3 that the rates of change for the b^* coordinate were higher under conditions of light, indicating intense oxidation of the carotenoids. On the other hand, the rate of change in the b^* coordinate in the darkness was lower with a lower rate of change for refrigeration temperature (0.0066 days).

As expected, the saturation (C^*) and the hue (h^*) of the yellow color of isotonic beverages stored under the light at 25 ºC and 4 ºC underwent significant changes up to 7 days, according to the rates of changes found (Table 3). The C * values practically did not change until 30 days of storage. Regarding samples stored in the darkness at 25 ºC, the C^* value changed up to 30 days at a rate equal to 0.0202 days, whereas beverages stored at 4 ºC showed lower values of degradation rate (0.0057 days), requiring approximately 122 days to reduce the C* value by 50%, while for beverages stored at room temperature (25 ºC), the same reduction would occur in approximately 34 days. The samples of isotonic beverages stored in the presence of light showed a degradation rate value for samples stored at 25 ºC equal to 0.2445 days and 4 ºC equal to 0.1979 days.

The h^* coordinate degradation rate values for samples of isotonic beverages stored in the darkness were higher for those stored at 25 ºC (0.0044 days), requiring about 160 days for a 50% reduction in the value of this coordinate, while for those stored at 4 ºC (0.0017 days) about 408 days.

Castro-López et al. (2016) guaranted the stability of total carotenoids of eight fruit juices under refrigeration (4, 8 and 11 ºC) for 20 days was determined by determining the content of β-carotene and find that the content of total carotenoids decreased during storage. According to the authors, the loss of yellow tint is directly related to the oxidation of carotenoids, which undergo isomerization and consequent loss of color. Carotenoids are photo and thermosensitive, as they showed higher degradation speeds and consequent shorter half-life in conditions with the presence of light and room temperature. The color loss of the carotenoid also occurred in the work of Cerezal Mezquita et al. (2020), who, as in this work, aimed to prepare an isotonic beverage with natural dye (emulsion of astaxanthin oleorin) and evaluate the stability under the presence of light.
As the samples of isotonic beverages stored in the conditions of darkness at 25 ºC and 4 ºC, obtained the best results for maintaining the color (Figure 2), the studies extended to 40 and 60 days, respectively.

3.3 Sensory acceptance test

The averages and standard deviations of the scores attributed by the tasters to the attributes in samples of isotonic beverages formulated with whey ultrafiltration permeate added to pequi powder carotenoid extract and natural pineapple or passion fruit aroma are shown in Table 4.

Table 4. Average score values for the sensory attributes evaluated for isotonic beverage (IB) formulated with whey permeate with carotenoid powder from pequi (CP), added for the natural aromas of pineapple and passion fruit**.**

	Aroma		
Attribute	Pineapple	Passion fruit	p > F
Color	6.52 ± 1.68[a]	6.35 ± 1.65[a]	0.4555
Flavor	5.32 ± 2.13[a]	5.39 ± 2.25[a]	0.8257
Aroma	5.69 ± 1.86[a]	5.96 ± 1.82[a]	0.2774
Overall impression	5.78 ± 1.83[a]	5.87 ± 1.85[a]	0.7364

Values presented represent an average of 100 judges ± standard deviation. Averages followed by the same letters, on the same line, do not differ from each other by t test (p>0.05). Source: Authors.

The analysis of variance (ANOVA) showed that the averages of all evaluated attributes did not differ significantly (p>0.05) between the formulated beverages. Both beverages were equally accepted by the tasters with respect to all attributes. The color attribute was the one with the highest average, 6, which indicates that consumers liked it slightly more. The other attributes obtained an average above 5, indicating that consumers were indifferent in terms of taste, aroma and overall impression.

The ricotta cheese whey-based sport-beverage prepared by Valadao e Geremias de Andrade (2015) had an acceptance equal to 6.3, a value close to that found in the whey permeate base beverage of this work. In the work of Martins, Chiapetta, Paula, & Gonçalves (2011) who made isotonic beverages with concentrated fruit and vegetable juice with different sanitization processes, the result was that the analyzed samples had a minimum score of around 4.92 (general aspect) and maximum 6.70 (odor) for all analyzed attributes, not differing statistically (p>0.05).

4. Conclusion

The storage condition in the presence of light at 25 ºC showed a greater and faster change in color coordinates and consequently degradation of carotenoids in the isotonic beverage. The condition of storage in the darkness, under refrigeration (4 ºC) proved to be the condition with the least degradation (the yellow color remained more stable) for the storage of the beverage. The isotonic beverage was accepted by athletes, consumers for whom the beverage is intended, being considered a promising supplement for obtaining carotenoids from pequi, which give the product a natural yellow tint and bioactive properties with antioxidant and pro-vitamin A activity, in addition to vitamin B2 and natural minerals from whey ultrafiltration permeate, which assist in the replacement of electrolytes lost during physical activity. The beverage can also present benefits to the environment, as it makes possible the use of permeate, which is often discarded as effluent.

New isotonic beverages can be elaborated with natural sources of carotenoids and new stability studies can be carried out in order to study the stability of the color and these carotenoids under different storage conditions.

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References

ANVISA (Brazilian Sanitary Surveillance Agency), (2010). Resolução - RDC nº 18, de 27 abril de 2010 – *Provides for food for athletes*

AOAC, A. O. A. C. (2006). *Official Methods of Analysis of AOAC INTERNATIONAL* (J. Dr. William Horwitz; Dr. George W. Latimer (ed.); (18th ed.). AOAC INTERNATIONAL.

APHA. (2001). (American Public Health Association). In *Compedium of methods for the microbiological examination of foods* (4th ed.). Compedium of methods for the microbiological examination of foods.

Boon, C. S., McClements, D. J., Weiss, J., & Decker, E. A. (2010). Factors influencing the chemical stability of carotenoids in foods. *Crit Rev Food Sci Nutr*, *50*(6), 515–532. https://doi.org/10.1080/10408390802565889

Bovi, G. G., Petrus, R. R., & Pinho, S. C. (2017). Feasibility of incorporating buriti (Mauritia flexuosa L.) oil nanoemulsions in isotonic sports drink. *International Journal of Food Science and Technology*, *52*(10), 2201–2209. https://doi.org/10.1111/ijfs.13499

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Castro-López, C., Sánchez-Alejo, E. J., Saucedo-Pompa, S., Rojas, R., Aranda-Ruiz, J., & Martínez-Avila, G. C. G. (2016). Fluctuations in phenolic content, ascorbic acid and total carotenoids and antioxidant activity of fruit beverages during storage. *Heliyon*, *2*(9). https://doi.org/10.1016/j.heliyon.2016.e00152

Cerezal Mezquita, P., Espinosa Álvarez, C., Palma Ramírez, J., Bugueño Muñoz, W., Salinas Fuentes, F., & Ruiz-Domínguez, M. del C. (2020). Isotonic Beverage Pigmented with Water-Dispersible Emulsion from Astaxanthin Oleoresin. *Molecules*, *25*(4), 841. https://doi.org/10.3390/molecules25040841

Chen, H. E., Peng, H. Y., & Chen, B. H. (1996). *Stability of carotenoids and vitamin A during storage of carrot juice*. *57*(4), 497–503.

Coombes, J. S. (2005). Sports drinks and dental. *American Journal of Dentistry*, *18*(2), 101–104.

de Mendonça, K. S., Corrêa, J. L. G., Junqueira, J. R. de J., Cirillo, M. A., Figueira, F. V., & Carvalho, E. E. N. (2017). Influences of convective and vacuum drying on the quality attributes of osmo-dried pequi (Caryocar brasiliense Camb.) slices. *Food Chemistry*, *224*, 212–218. https://doi.org/10.1016/j.foodchem.2016.12.051

Ferreira, P. R., Marins, J. C. B., de Oliveira, L. L., Bastos, D. S. S., Soares Júnior, D. T., da Silva, C. D., & Fontes, E. A. F. (2020). Beverage based on whey permeate with phenolic extract of jabuticaba peel: A pilot study on effects on muscle and oxidative stress in trained individuals. *Journal of Functional Foods*, *65*(December 2019), 103749. https://doi.org/10.1016/j.jff.2019.103749

Fontes, E. A. F., Alves, Y. P. C., Fontes, P. R., & Minim, V. P. R. (2015). Bebida eletrolítica a base de permeado da ultrafiltração de leite: Avaliação física, química e microbiológica durante o armazenamento. *Ciencia Rural*, *45*(2), 342–348. https://doi.org/10.1590/0103-8478cr20131432

Gironés-Vilaplana, A., Mena, P., Moreno, D. A., & García-Viguera, C. (2014). Evaluation of sensorial, phytochemical and biological properties of new isotonic beverages enriched with lemon and berries during shelf life. *Journal of the Science of Food and Agriculture*, *94*(6), 1090–1100. https://doi.org/10.1002/jsfa.6370

Gomes, J. C., & Oliveira, G. F. (2011). *Análises físico-químicas de alimentos* Ed. UFV.

Goulding, D. A., Fox, P. F., & O'Mahony, J. A. (2020). Milk proteins: An overview. In *Milk Proteins* (pp. 21–98). Elsevier. https://doi.org/10.1016/B978-0-12-815251-5.00002-5

Lawless, H. T., & Heymann, H. (2010). Acceptance Testing. In *Sensory Evaluation Practices* (pp. 325–347). https://doi.org/10.1007/978-1-4419-6488-5_14

Leśniewicz, A., Grzesiak, M., Żyrnicki, W., & Borkowska-Burnecka, J. (2016). Mineral Composition and Nutritive Value of Isotonic and Energy Drinks. *Biological Trace Element Research*, *170*(2), 485–495. https://doi.org/10.1007/s12011-015-0471-8

Ligia Focsan, A., Polyakov, N. E., & Kispert, L. D. (2019). Supramolecular carotenoid complexes of enhanced solubility and stability — The way of bioavailability improvement. *Molecules*, *24*(21). https://doi.org/10.3390/molecules24213947

Machado, M. T. C., Mello, B. C. B. S., & Hubinger, M. D. (2013). Study of alcoholic and aqueous extraction of pequi (Caryocar brasiliense Camb.) natural antioxidants and extracts concentration by nanofiltration. *Journal of Food Engineering*, *117*(4), 450–457. https://doi.org/10.1016/j.jfoodeng.2012.12.007

Maiani, G., Castón, M. J. P., Catasta, G., Toti, E., Cambrodón, I. G., Bysted, A., Granado-Lorencio, F., Olmedilla-Alonso, B., Knuthsen, P., Valoti, M., Böhm, V., Mayer-Miebach, E., Behsnilian, D., & Schlemmer, U. (2009). Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Molecular Nutrition and Food Research*, *53*(SUPPL. 2), 194–218. https://doi.org/10.1002/mnfr.200800053

Mariutti, L. R. B., Chisté, R. C., & Mercadante, A. Z. (2018). Carotenoids: Concept, Dietary Sources and Structure–Activity Relationships for Antioxidant and Health-related Properties. In F. Saura-Calixto & J. Pérez-Jiménez (Eds.), *Non-extractable Polyphenols and Carotenoids: Importance in Human Nutrition and Health* (1 st, pp. 134–164). Royal Scociety of Chemistry. https://doi.org/10.1039/9781788013208-00134

Martins, R. C., Chiapetta, S. C., Paula, F. D. De, & Gonçalves, É. C. B. a. (2011). Avaliação da vida de prateleira de bebida isotônica elaborada com suco concentrado de frutas e hortaliças congeladas por 30 dias. *Alimentos e Nutrição*, *22*(44), 623–629.

Mesnier, X., Gregory, C., Fança-Berthon, P., Boukobza, F., & Bily, A. (2014). Heat and light colour stability of beverages coloured with a natural carotene emulsion: Effect of synthetic versus natural water soluble antioxidants. *Food Research International*, *65*(PB), 149–155. https://doi.org/10.1016/j.foodres.2014.06.025

Mutsokoti, L., Panozzo, A., Tongonya, J., Kebede, B. T., Van Loey, A., & Hendrickx, M. (2017). Carotenoid stability and lipid oxidation during storage of low-fat carrot and tomato based systems. *LWT - Food Science and Technology*, *80*, 470–478. https://doi.org/10.1016/j.lwt.2017.03.021

Nascimento-Silva, N. R. R. Do, & Naves, M. M. V. (2019). Potential of Whole Pequi (Caryocar spp.) Fruit-Pulp, Almond, Oil, and Shell-as a Medicinal Food. *Journal of Medicinal Food*, *22*(9), 952–962. https://doi.org/10.1089/jmf.2018.0149

Parashar, A., Jin, Y., Mason, B., Chae, M., & Bressler, D. C. (2016). Incorporation of whey permeate, a dairy effluent, in ethanol fermentation to provide a zero waste solution for the dairy industry. *Journal of Dairy Science*, *99*(3), 1859–1867. https://doi.org/10.3168/jds.2015-10059

Petrus, R. R., Assis, J. De, & Faria, F. (2005). Processamento e avaliação de estabilidade de bebida isotônica em garrafa plástica. *Ciência e Tecnologia de Alimentos*, *25*(3), 518–524.

Pinto, M. R. M. R., Paula, D. de A., Alves, A. I., Rodrigues, M. Z., Vieira, É. N. R., Fontes, E. A. F., & Ramos, A. M. (2018). Encapsulation of carotenoid extracts from pequi (Caryocar brasiliense Camb) by emulsification (O/W) and foam-mat drying. *Powder Technology*, *339*, 939–946. https://doi.org/10.1016/j.powtec.2018.08.076

Porfírio, M. C. P., Gonçalves, M. S., Borges, M. V., Leite, C. X. dos S., Santos, M. R. C., Silva, A. G. da, Fontan, G. C. R., Leão, D. J., Jesus, R. M. de, Gualberto, S. A., Lannes, S. C. da S., & Silva, M. V. da. (2019). Development of isotonic beverage with functional attributes based on extract of Myrciaria jabuticaba (Vell) Berg. *Food Science and Technology*, *2061*, 1–7. https://doi.org/10.1590/fst.14319

Raman, M., Ambalam, P., & Doble, M. (2019). Probiotics, Prebiotics, and Fibers in Nutritive and Functional Beverages. In *Nutrients in Beverages*. Elsevier Inc. https://doi.org/10.1016/b978-0-12-816842-4.00009-5

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Resolução RDC nº18 de abril de 2010 - Regulamento técnico sobre alimentos para atletas, (2010). http://portal.anvisa.gov.br/wps/wcm/connect/65f5 b80047457f258ad 7de3fbc4c6735/RDC+dos+Atletas+-+Dicol.pdf?MOD=AJPERES

Rodriguez-Amaya, D. B. (2001). A Guide to Carotenoid Analysis in Foods. In *Life Sciences.*

Rodriguez, D. (2001). A Guide to Carotenoid Analysis in Foods. In *Life Sciences*.

Schubring, R. (2009). Colour Measurement. In H. Rehbein & J. Oehlenscläger (Eds.), *Fishery Products* (pp. 127–172). Wiley-Blackwell. https://doi.org/10.1002/9781444322668.ch7

Schweikert, L. (2017). The Bioavailability of Carotenoid Forms. In H. K. Biesalski, A. Drewnowski, J. T. Dwyer, J. Strain, P. Weber, & M. Eggersdorfer (Eds.), *Sustainable Nutrition in a Changing World* (pp. 289–294). Springer International Publishing. https://doi.org/10.1007/978-3-319-55942-1_22

Valadao, N. K., & Geremias de Andrade, I. M. (2015). Development of a Ricotta Cheese Whey-based Sports Drink. *Advances in Dairy Research*, *4*(3). https://doi.org/10.4172/2329-888x.1000156

Valadão, N. K., Shimoda, S. Y., Jory, J. C., Fratassi, G. C., & Petrus, R. R. (2019). Stability of a dairy-based electrolyte replenishment beverage. *Food Science and Technology*, *39*(4), 824–829. https://doi.org/10.1590/fst.09018