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Development of a Ricotta Cheese Whey-based Sports Drink

Natali Knorr Valadão, Ivana Morais Geremias de Andrade, Jacqueline Costa Jory, Fabio Augusto Gallo, Rodrigo Rodrigues Petrus*

Department of Food Engineering, College of Animal Science and Food Engineering, University of São Paulo, São Paulo, Brazil

***Corresponding author:** Rodrigo Rodrigues Petrus, Department of Food Engineering, College of Animal Science and Food Engineering, University of São Paulo, Duque de Caxias Norte Avenue 225, Pirassununga, São Paulo, Brazil, Tel: +55 19 3656 4308; E-mail: rpetrus@usp.br

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Abstract

Ricotta cheese whey (RCW) is a by-product of the dairy industry; it is either destined to the feeding of swine or simply disposed of. This study focused on RCW to develop a sports drink. Sensory tests were performed for this purpose. Measurements of pH, soluble solids content, osmolality, and sodium and potassium levels were also performed. The sports drink was pasteurized at 85ºC/30 s, aseptically filled into plastic bottles and submitted to commercial sterility test. A nine-point hedonic scale test was carried out to assess the appearance, aroma and flavor. The results of the physicochemical tests were in compliance with Brazilian Food Regulation (pH 3.1, 6.3 °Brix, 306 mOsm/L, 500 mg/L sodium and 650 mg/L potassium). Commercial sterility was achieved. The mean ratings assigned to appearance, aroma and flavor were 6.9, 6.5 and 6.0, respectively. The findings indicate that RCW could be a technologically feasible alternative to produce a shelf stable sports drink.

Keywords: Dairy product; Isotonic drink; By-product; Sensory evaluation; New product development

Chemical Compounds Studied in this Article

Peracetic acid (PubChem CID: 6585); Sucrose (PubChem CID: 5988); Sodium chloride (PubChem CID: 5234); Citric acid (PubChem CID: 311).

Introduction

Whey is a raw material for making ricotta cheese. The principle of its production is based on the flocculation of whey proteins through heat associated with acidification. In the production of ricotta cheese, about 40 to 50 g/L of the yield is cheese [1] and the rest (950 to 960 g/L) is ricotta cheese whey. According to the most recent data on ricotta cheese production found in literature and on the Brazilian annual milk production in 2004 and 2014 (production estimates) [2,3], government sources estimate that in 2014 almost 230,000 tons of ricotta cheese whey were produced. Such a large amount of effluents may cause environmental problems connected with their disposal.

According to Sansonetti et al. [4], ricotta cheese whey is mostly formed by water (932 to 938 g/L), the remaining whey constituents are proteins from the flocculation process (1.5 to 2.2 g/L), lactose (48 to 50 g/L), minerals (10 to 13 g/L) and organic acids (2.0 to 2.5 g/L).

The mineral level in electrolyte drinks, popularly known as sports drinks, is similar to that found in the blood (approximately 300 mOsm/L). Such beverages are meant to restore the water and electrolytes lost during physical exercise when the body metabolizes huge amounts of water and electrolytes (sodium, potassium, chloride, etc.) as it loses heat to the environment. If those losses are not replenished, the body lingers in a state of low water level, which leads to increased risks of exhaustion and thermal shock, complications in kidney function and muscle cramps [5].

According to the Brazilian Association of Soda and Non-Alcoholic Beverages Industries [6], the consumption of sports drinks increased by 20% in 2011, a significant increase compared to the average (13.2%) between 2005 and 2009.

According to Resolution no. 18 (09/27/2010) [7], an electrolyte drink is a product designed to help hydration and must meet the following identity standards:

- The level of sodium in the beverage must range from 460 to 1150 mg/L; only inorganic salts generally added to food must be used as a source of sodium.
- Osmolality must range between 270 and 330 mOsm/kg of water.
- Carbohydrates may represent up to 80 g/L of the beverage.
- Potassium may be added to the product; up to 700 mg/L.
- Other nutrients and non-nutrients may not be added to the product.
- Dietary fibers, starches and polyols may not be added to the product.

Some studies seek alternatives to the use of ricotta cheese whey, such as the production of bio-ethanol [8-12].

As for the use of ricotta cheese whey in human food, Gerhardt et al. [13] developed a fermented milk-based beverage using ricotta cheese whey and hydrolysed collagen. Chávez [14] developed a sport drink and Fontes et al. [15] developed an electrolyte repository with permeated milk ultrafiltration, flavors strawberry and lemon, respectively. Notably, none of these studies mention the use of ricotta cheese whey in sports drinks production as described in the work presented in this paper.

An off-the-record survey of the four largest dairy farms in the country producing ricotta cheese revealed that ricotta cheese whey is, as of today, either destined to the feeding of swine or simply disposed of [Brazilian Association of Cheese Industries].

The aim of this study was therefore to investigate and propose a method which could be implemented to utilize the by-product whey that is mostly thrown away, for developing a sports drink.

Material and Methods

Defining the sports drink flavor

In order to identify the flavor that should be used in the development of the sports drink, Rank Preference tests were performed on sixty panelists with ages ranging from seventeen to fifty years old. The sensory analyses performed for this purpose followed the procedures described by Meilgaard et al. [16] on Chapter 12 (Affective Tests).

The panelists were served with seven samples of different flavors of a leading brand of sports drink, as follow: grape, passion fruit, strawberry with passion fruit, orange, lime, mandarin and citrus (composed of orange and grapefruit). A randomized complete block design was presented. The samples were served simultaneously in individual booths lighted by fluorescent white lamp. Approximately 50 mL of drink at a temperature of about 4°C was poured into plastic cups coded with random 3-digit numbers for identification.

Given the light flavoring of the drink, seven samples simultaneously served were not considered to be a major factor for test fatigue. According to Meilgaard et al. [16], Rank Preference tests are carried out with three or more samples.

The sensory analyses were approved by the Committee of Ethics in Research of the University of São Paulo (Protocol 359.879).

Processing the ricotta cheese whey

The ricotta cheese whey (RCW) was produced in the dairy plant at University of São Paulo, at Pirassununga campus, in accordance with the methodology described by Albuquerque [17]. The enzyme lactase (β-D-galactoside galactohydrolase/EC 3.2.1.23) Granolact M 7500 (Granolab, Brazil) was added to the RCW in the ratio of 1.05 g enzyme to each liter whey. The RCW powered with the enzyme was stored for 24 hours at 8°C in order for the lactose to hydrolyze.

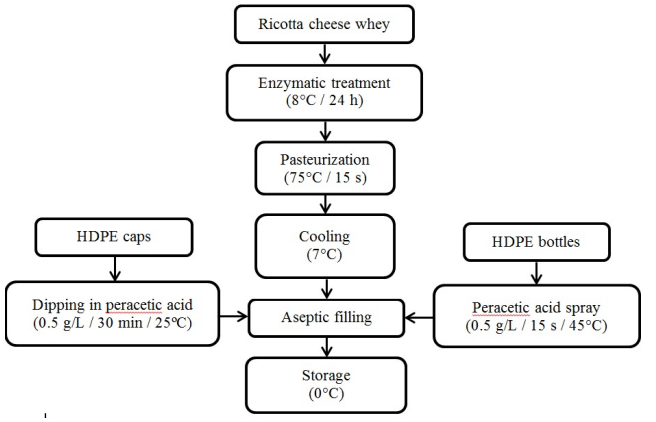

Figure 1: Flowchart of the processing of the ricotta cheese whey used in the development of the sports drink formulation.

After the enzymatic treatment, the RCW was pasteurized in an electric plate heat exchanger, with a nominal capacity of 300 L/h (Sumá Indústria e Comércio Ltda, Brazil) at 75°C/ 15 s, and aseptically filled in plastic bottles (HDPE) (Usicomp, Brazil) decontaminated beforehand. For decontamination, the bottles were sprinkled with a peracetic acid solution (Thech Desinfecção Ltda, Brazil) (0.05% (v/v)/15 s/45°C). The filling was made using a semi-automatic gravimetric filling machine (Polienva-Movitron, Brazil) installed on the inside of an ISO class 5 horizontal unidirectional airflow cabinet (Veco do Brasil, Brazil). The batch was stored in the dark at 0°C in order to preserve the quality of the whey during the time necessary for the development of the sports drink formulation. Figure 1 depicts the flowchart for experimental processing of ricotta whey.

Microbiological analysis of the ricotta whey

In order to evaluate the microbiological stability of the pasteurized whey kept at 0°C, standard mesophilic aerobic plate counts were carried out during storage period (eight months), according to the methodology described by Silva et al. [18].

Developing the sports drink formulation

After processing the ricotta whey, the levels of sodium and potassium were analyzed on a B462 flame photometer (Micronal, Brazil), the level of water-soluble solids on an AR 200 portable digital refractometer (Reichert, USA), the pH on a 300M Analyzer pHmeter (Hanna Instruments, Romania) and the osmolality on a PLZ 1000 osmometer (PLZ Tecnologia, Brazil). From the results, numerous sports drink formulations were developed with variations in the levels of ingredients and additives (ricotta cheese whey, deionized water, sodium chloride, citric acid, sucrose, mandarin flavor and twilight-yellow coloring). Both physicochemical and sensory analyses were also performed to guide the development of the formulation.

Sensory analyses: During the development of the sports drink formulation Just About Right Scales and 9-point Hedonic Scale tests were performed, according to the procedures described by Meilgaard et al. [16] and Ferreira et al. [19]. Sixty panelists with ages ranging from seventeen to fifty years old were recruited. Each panelist was served 40 mL of a drink formulation at approximately 4°C.

Physicochemical analyses: Physicochemical analyses were performed on the ricotta whey and on the developed beverage according to the methodology of the Association of Official Analytical Chemists [20]. To determine the level of water-soluble solids an AR 200 portable digital refractometer (Reichert, USA) was used. The pH was determined on a 300M Analyzer pHmeter (Hanna Instruments, Romania). The titratable acidity was determined by using NaOH 0.1 M; and the end point, the one at which the pH was 8.3. The osmolality was determined on a PLZ 1000 osmometer (PLZ Tecnologia, Brazil) and the levels of sodium and potassium on a B462 flame photometer (Micronal, Brazil).

Processing the new product

The sports drink developed from ricotta whey was pasteurized at 85°C for 30 s, aseptically filled into white pigmented (TiO_2) polyethylene terephthalate (PET) bottles with a volume capacity of 330 mL and hermetically sealed with polypropylene screw lids (PP) with aluminum seals by electromagnetic induction. A batch of 180 packages was obtained.

Commercial sterility test of the end product

At the end of the processing, the packages were incubated at 25°C for 10 days in the dark. The pre-incubation technique was developed in an attempt to increase the number of viable microorganisms if present. Under these conditions, if the product spoils, the presence of contaminants is determined. The methods described by Deibel et al. [21] and Denny et al. [22] were followed in order to evaluate the commercial sterility of the sports drink (pH 3.0).

Inspection of the samples: After incubation, the packages were examined for abnormal conditions such as leakage, swells, flippers etc., and prior to opening. After the bottles were opened, changes in appearance, odor and flavor were investigated prior to pH determination. If the package is defective and/or spoilage is evident the commercial sterility is not achieved. Otherwise, the samples are submitted to microbiological tests.

pH determination: After the inspection of the product, the pH values of samples from ten packages were determined. A variation of less than 0.2 pH units in relation to the determination made immediately after processing was tolerated.

Microbiological analysis: Five samples of the product (pH 3.0) that presented no evidence of spoilage or pH variation were submitted to microbiological tests. After the samples were homogenized, 1 mL-aliquot were aseptically transferred to sterilized plates where Thermoacidurans Agar (TAA), acidified Potato Dextrose Agar (PDA) and All Purpose Tween Agar (APT) were separately poured. TAA plates were incubated at 30°C for 5 days under aerobic and anaerobic conditions for the purpose of identifying facultative mesophilic aerobes. PDA plates were incubated at 25°C for 5 days under aerobic condition, for yeasts and molds detection. APT plates were incubated at 30°C for 5 days under aerobic condition, for lactic acid bacteria.

Assessment of end product´s sensory acceptability

In order to evaluate the sensory acceptability of the pasteurized sports drink, an affective test was applied to a panel consisting of sixty habitual consumers (age of twenty two years on average) of sports drinks. The panelists were asked to evaluate the sensory attributes of appearance, aroma and flavor by assigning a liking score on a 9-point hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely) [16]. Tests were accomplished in individual booths lighted with a white fluorescent lamp, and the samples were monadically presented in 50 mL plastic cups labeled with a 3-digit code and presented at a temperature of about 4°C.

Data analysis

The data were subjected to the variance analysis with a 5% confidence level and Tukey's test for comparison between the averages of the results obtained from the microbiological and physicochemical tests. The data were analyzed on SAS (Statistical Analysis System, SAS Institute Inc., USA) version 9.2. For the Rank Preference tests, the 10% significance chart was used [23].

Results and Discussion

Defining the sports drink flavor

For the analysis of the results of the Rank Preference test with seven flavors and sixty panelists, the statistical analysis chart for the aforementioned test with 10% significance was applied [23], whose significant minimal difference among the samples is 64 points. The flavors mandarin, passion fruit, strawberry with passion fruit and grape were considered the most preferred statistically; therefore, lime and citrus were rejected.

With the five remaining flavors a new Rank Preference test was performed. The 10% significance level chart [23] indicated that the significant minimal difference among the samples is 42 points. The passion fruit, the strawberry flavored with passion fruit and the mandarin were the preferred flavors. Mandarin was chosen as the sports drink flavor in developing the formulation, since according to ABIR [24] mandarin is one of the two best-liked flavors among those made available by the national market brand leader and, according to a research done by Marins et al. [25] and Brito et al. [26], mandarin was the sports drink flavor preferred among 420 athletes and 200 judokas respectively.

Microbiological analysis of the ricotta whey

Table 1 shows the results of the microbiological analyses performed in triplicate on the samples of the ricotta whey used in the development of the sports drink formulation.

Storage time at 0°C (weeks)	Average count ± standard deviation (CFU/mL)
0	<1est
18	10.7 ± 1.5
25	13.3 ± 2.1
36	15.0 ± 1.0

Table 1: Mean counts of mesophilic aerobic microorganisms in pasteurized ricotta whey during the period in which the sports drink formulation was developed. The averages (three samples) were not different ($P > 0.05$); est – estimated value.

According to Table 1 the ricotta whey remained microbiologically stable throughout the development of the sports drink, since the count averages were inferior to 16 UFC/mL.

Developing the formulation

According to the sports drinks identity standards established by Agência Nacional de Vigilância Sanitária [National Health Surveillance Agency Brazil] (ANVISA) [7], the level of potassium found in the whey (1700 mg/L) exceeded the limit allowed (700 mg/L). Analogously the osmolality of the whey (436.9 mOsm/L) also exceeded the limit allowed by ANVISA (330 mOsm/L), which called for the dilution of the whey in deionized water. After dilution with sodium chloride, the minimum levels required by law (460 mg/L) were restored.

An initial formulation was developed by applying the Just About Right Scale test along with the Hedonic Scale test to identify how close to "just about right" that the orange color, the mandarin aroma and flavor, the acidity, the sweetness and the savory taste were, according to each panelist's opinion. Physicochemical analyses were performed in parallel.

Based on the results obtained in the first test, a new formulation was developed in which the quantity of ingredients and additives were altered based on the panelists' responses.

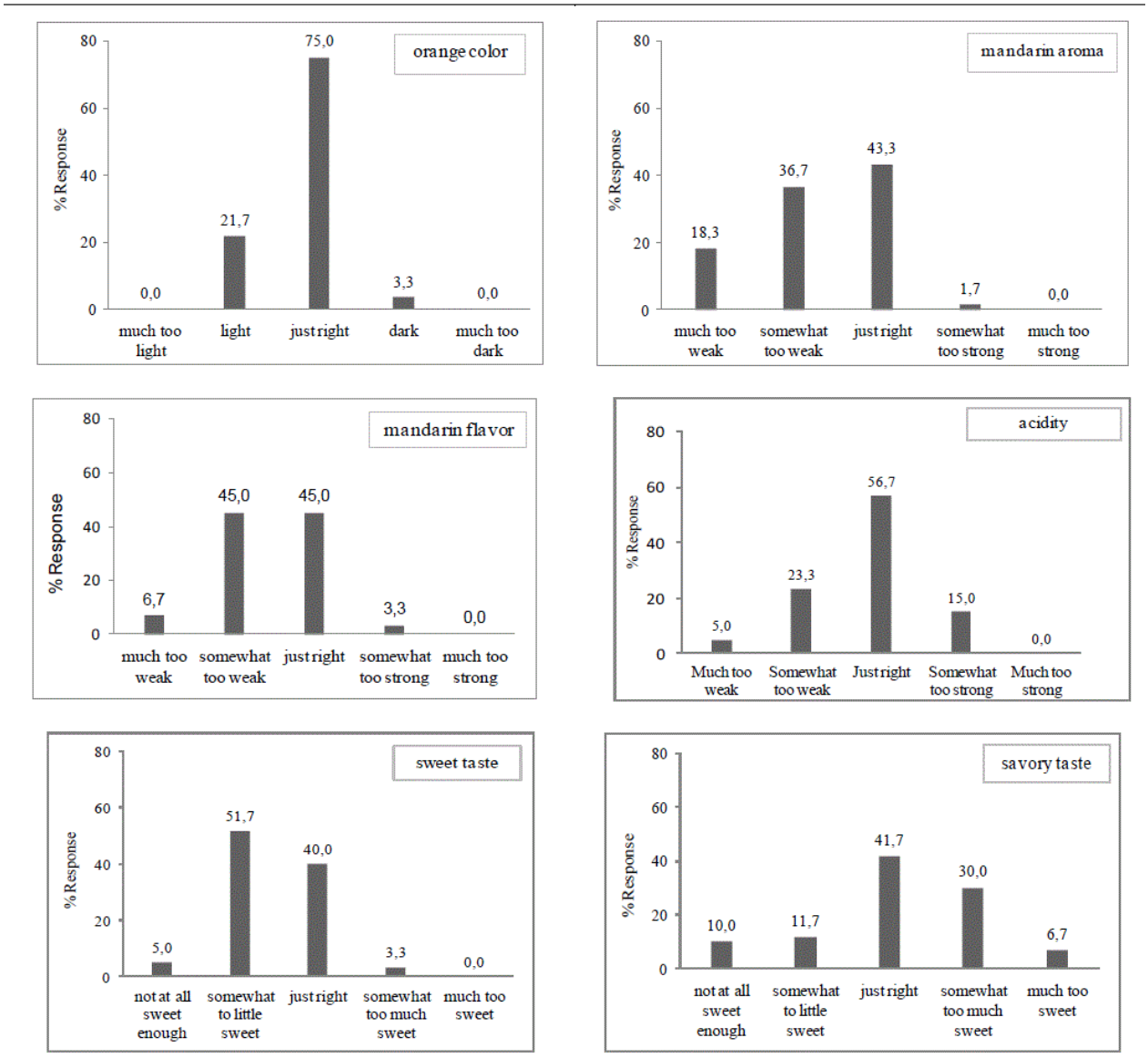

Figure 2: Responses obtained in the Just About Right (JAR) scale tests.

During the development of the formulation, which lasted six months, ten sensory analysis sessions (Just About Right and Hedonic Scales) were held. The responses obtained in the sensory and physicochemical analyses led to the development of a palatable supplement (Table 2) that scored an average of 6.3 (classified between "liked slightly" and "liked moderately") in the 9-point hedonic scale and in conformity with the identity standards established by current food legislation.

Fontes et al. [15] developed an electrolyte repository (lemon flavoured) with permeated of milk ultrafiltration. The formulated drink reached values (pH of 3.4, soluble solids content of 5.9 °Brix and osmolality of 311.6 mOsm/L) which is close to those found in this study.

Adjustments to the deionized water/ricotta cheese whey ratio and the concentrations of citric acid, sodium chloride, mandarin flavor and twilight-yellow coloring were made to every formulation. Monteiro et al. [5] state that sports drinks having 60 to 80 g/L of carbohydrates in their composition enter the bloodstream faster than water. As presented in Table 2, the developed formulation met the physicochemical requisites required by Brazilian Regulation.

Figure 2 shows the results obtained for the orange color, mandarin aroma, mandarin flavor, acidity, sweet and savory tastes attributes, respectively, in the Just About Right Scales tests for the sports drink developed.As can be observed in Figure 2, only the color attribute achieved the recommended 70% of responses in the Just About Right scale [19]. One could notice a tendency among the panelists to prefer a beverage having a sweeter taste. However, for the beverage to be able to meet the required physicochemical standards, no more sucrose was

added to the formulation. There was an opposite response to the savory taste; a percentage of about 35% of the panelists answered they still felt a strong or extremely strong savory taste in the formulation, but the added sodium chloride achieved the minimum concentration necessary for the beverage to meet the legislation standards. The beverage acidity was considered strong by some panelists and weak by others; the majority (57%) considered it to be just about right. The results suggest the necessity of increasing the concentration of mandarin flavour. Nevertheless, for several of the created formulations - those with substantial increments of flavor concentration - the responses obtained in the Just About Right Scales test remained constant; therefore, the flavor concentration was maintained.

Ingredients and additives	
Deionized water (mL)	617.2
Ricotta cheese whey (mL)	382.8
Sucrose (g)	36.2
Citric acid (g)	3.2
Tangerine flavor (mL)	2.6
Twilight-yellow coloring (mg)	13
Physicochemical parameters (average ± standard deviation)	
Osmolality (mOsm/kgH2O)	306 ± 0.6
pH	3.15 ± 0.01
Soluble solids (°Brix)	6.40 ± 0.00
Sensory acceptance (9-point hedonic scale)	
Average score for overall impression	6.3 ± 1.8
Percentage of acceptance (%)*	81.7

Table 2: Composition of 1L-electrolyte supplement developed from ricotta whey. *Percentage of panelists that assigned scores above 5.

Commercial sterility test

Packages examination: After incubation at 25ºC for ten days, no defective packages were found (such as leakage or swelling); there was no evidence of spoilage.

Measurement of pH: The pH values of freshly pasteurized (time 0) sports drink and stored during ten days at 25ºC (incubation test) were 3.03 and 2.98, respectively. Thus, the variation ($|\Delta pH| = 0.05$) was less than 0.2 units, showing the physicochemical stability of the product.

Microbiological tests: All five tested samples showed the absence of microorganisms under the specific recovery media and incubation conditions, previously mentioned. In this way, the sports drink developed from ricotta whey was considered commercially sterilized (Figure 3).

It´s noteworthy that the combination of intrinsic (acid pH) and extrinsic factors (pasteurization and aseptic filling) employed in this study was effective in attaining a shelf stable sports drink. This is a very attractive achievement with respect to energy saving, since there is no need of cold chain for product´s storage, distribution and commercialization.

Assessment of end product´s acceptability: Figure 4 depicts the ratings histogram assigned to the attributes of appearance, aroma and flavor of the commercially sterilized sports drink.

Figure 4 shows a good acceptability of the drink, since most ratings assigned to appearance, aroma and flavor were greater than five in the nine-point hedonic scale. The percentages of responses equal or greater than 5 were 88.4, 91.6 and 76.6% for appearance, aroma and flavor, respectively.

Finally, some positive and appealing aspects regarding the present study are apparent:

- A by-product of dairy industry, which is usually disposed of, may be utilized to produce a high added-value drink.
- The developed sports drink may be consumed by lactose-intolerant individuals, since ricotta cheese whey was pre-treated with lactase.
- The hydrolysis of lactose into galactose and glucose enhances the sweetness of the drink with no caloric increase.

Figure 3: Shows the sports drink developed from ricotta cheese whey. By-products (a, b) of the dairy industry and the new product developed (c).

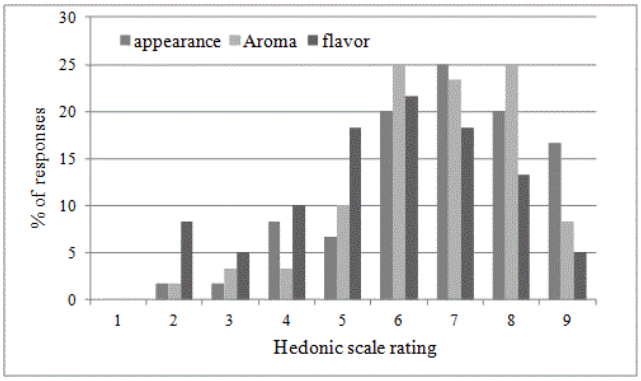

Figure 4: Ratings histogram assigned to the developed sports drink.

Conclusions

The findings demonstrated that the sports drink developed met the identity standards established by Brazilian current Food Regulation. The use of ricotta cheese whey in the production of a shelf stable sports drink is a technically feasible alternative, which adds value to a residue of the dairy industry that is usually disposed of or served as animal feed. Still, eventual adjustments to the sports drink formulation

developed in this study may be necessary in order to improve the palatability of the beverage.

Acknowledgements

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