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Vitamin C content and amino acid identification in carbonated beverage with kiwifruit (*Actinidia deliciosa*), ACHOTILLO (*Nephelium lappaceum*) and MORINGA (*Moringa oleifera*).

Contenido de vitamina c e identificación de aminoácidos en bebida carbonatada con kiwi (*Actinidia deliciosa*) ACHOTILLO (*Nephelium lappaceum*) y MORINGA (*Moringa oleífera*)

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Abstract: The carbonated beverage industries are constantly changing due to the growing demand for the inclusion of natural products. Consumers are looking for beverages that are sources of nutrients with low caloric content. In this context, an experimental study was carried out with the main objective of determining the vitamin C and amino acid content in a carbonated beverage made from kiwi, achotillo and moringa. Sensory, physicochemical and microbiological analyses were also performed on the best treatment selected by a sensory panel of 30 untrained judges. The treatment that received the best sensory evaluation was the one formulated with 20% kiwifruit, 70% achotillo and 0.1% moringa. This treatment presented a vitamin C content of 212.24 mg/kg and total amino acids of 2.29 w/w. The physicochemical results obtained were a pH of 4.3, a citric acid content of 0.27% and total solids of 6.2 °Brix. The microbiological results showed an absence of mesophilic aerobes, less than 2 NPM/ml of total coliforms and less than 10 up/cm3 of molds and yeasts. These values are within the range established by INEN 1101:2008 for carbonated beverages. Analyses of the proposed beverage show that the product has a vitamin C content that exceeds 10% of the recommended daily intake, in addition to providing amino acids. This makes the beverage an interesting option compared to current carbonated beverages that do not include natural ingredients.

Keywords: achotillo, amino acids, kiwifruit, moringa, vitamin C

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Resumen: Las industrias dedicadas a la elaboración de bebidas carbonatadas cambian constantemente debido a la creciente demanda de inclusión de productos naturales. Los consumidores buscan bebidas que sean fuentes de nutrientes con un bajo contenido calórico. En este contexto, se llevó a cabo un estudio experimental con el objetivo principal de determinar el contenido de vitamina C y aminoácidos en una bebida carbonatada de kiwi, achotillo y moringa. Se realizaron también análisis sensoriales, fisicoquímico y microbiológicos al mejor tratamiento seleccionado por un panel sensorial conformado por 30 jueces no entrenados. El tratamiento que recibió la mejor evaluación sensorial fue el que se formuló con un 20% de kiwi, un 70% de achotillo y un 0.1% de moringa. Este tratamiento presentó un contenido de vitamina C de 212.24 mg/kg y aminoácidos totales de 2.29 p/p. Los resultados fisicoquímicos obtenidos fueron un pH de 4.3, un contenido de ácido cítrico del 0.27% y sólidos totales de 6.2 °Brix. En cuanto a los resultados microbiológicos, se encontró ausencia de aerobios mesófilos, menos de 2 NPM/ml de coliformes totales y menos de 10 up/cm3 de mohos y levaduras. Estos valores se mantienen dentro del rango establecido por la norma INEN 1101:2008 para bebidas carbonatadas. Los análisis realizados a la bebida propuesta demuestran que el producto tiene un contenido de vitamina C que supera el 10% de la ingesta diaria recomendada, además de proporcionar aminoácidos. Esto convierte a la bebida en una opción interesante en comparación con las bebidas carbonatadas actuales que no incluyen ingredientes naturales.

Palabras clave: achotillo, aminoácidos, kiwi, moringa, vitamina C

Introduction

In Ecuador, there are around 4,000 companies dedicated to the production of beverages, including carbonated beverages, juices, tea, energy drinks, water and dairy products. These beverage industries are in a constant process of innovation with the purpose of applying the appropriate techniques that guarantee the safety of the processes and that in turn will help contribute to the generation of nutritional beverages (Valverde, 2018).

Among the types of carbonated beverages are energy drinks, the energy provided by these beverages is due to the high fructose content. According to Maersk et al. (2012), fructose plays a very important role in weight gain and consequently in the development of obesity. In Ecuador, the per capita consumption of carbonated beverages is 50 l/year, due to the great demand for these beverages, there has been interest in creating research to develop products of this type, but with the purpose of containing and providing nutritional value, generating

more alternatives when consuming nutrients from a refreshing source (Gaona-Gonzeaga et al., 2019)..

Nowadays, people try to get food products, which contain a higher nutritional contribution and can also generate health benefits, therefore, there has been an increased interest in the study of beverages with these characteristics, which has contributed to the development of products made from the mixture of vegetable species and fruit (Llanes, 2015).

Nutrients are essential for the body because they enable the development, maintenance and functioning of our body, so by consuming carbonated beverages we are not giving any nutritional contribution that can benefit human health (FAO, 2019). However, this situation can be addressed with proposals for beverages in this category that incorporate ingredients with bioactive compounds of nutritional interest in their formulation.

The kiwi is a fruit that has a high nutritional value, it contains vitamin C and vitamin E, with a higher contribution of nutrients compared to other commonly consumed fruits, vitamin C has beneficial effects on the immune system and antioxidant power, helps in the gastrointestinal function and the respiratory system. (Carr and Frei, 1999).

Achotillo is an exotic tropical fruit that grows in tropical or subtropical climates. Its main component is fiber, which is resistant to digestion and absorption in the small intestine. Among the vitamins it possesses is vitamin C, which groups dehydroascorbic, isoascorbic and L-ascorbic acids, which make up the active biological part that has protective effects against various diseases such as cardiovascular diseases, it also has phenolic compounds that help reduce the risk of diseases such as diabetes (McEvily et al., 1992)..

Moringa is one of the fast growing plant species known as the "tree of life" and this is due to its great nutritional contribution, moringa leaves have a high protein content with all the essential amino acids, also, it has a variety of vitamins such as A, B, C and E and a large number of nutrients and micronutrients such as calcium, zinc, magnesium among others... (Rubio-Sanz, 2020). (Rubio-Sanz, 2020)Amino acids are essential for our organism, since they are the compounds in charge of combining and forming the proteins that provide energy to our body. (Solutions., 2021). Likewise, because the body does not produce vitamin C, it needs to obtain it from the diet (Carr and Maggini, 2017).

Based on the above, the importance of generating alternatives to the consumer with regard to innovative products that provide nutrients is of

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greater interest every day. As well as in the proposal to generate a positive change in the choice of beverages that denote a benefit.
Rodriguez (2020) determined the presence of antioxidants in a beverage made with soy milk (*Glycine max*) and kiwi (*Actinida deliciosa*), flavored with passion fruit. From this study it is highlighted that the best sensorially qualified treatment of the soy beverage was the one elaborated with 40 % soy, while, of the kiwi concentrations studied, the treatment with the highest sensorial acceptance was the one containing 40 % kiwi.
Sanchinelli (2004) conducted an investigation to determine the protein content, essential amino acids in leaves, flowers and stems of Moringa oleifera, which is cultivated in Guatemala. The protein content in *Moringa oleifera* was found to range from 1.30 to 4.62 %, with the highest content in the leaves. The essential amino acid content was found to range from 98 to 1384 mg/100 g and from 90 to 270 mg/100 g in dehydrated and fresh leaves, respectively. In flowers the range was from 23 to 122 mg/100 g and in stems from 11 to 37 mg/100 g. The limiting amino acid in leaves was lysine; in flowers the limiting amino acids were valine and methionine; and in stems the limiting amino acid was valine.
Cedaro et al. (2015) presented a paper on the analysis of vitamin C in soft drinks, the results of a survey carried out on 52 products that are part of the usual offer of these beverages Obtained ascorbic acid levels in a range from 0 to 82.36±4.84 mg/200mL, corresponding the highest values to orange flavored products. This study determined the presence of vitamin C and the amino acid profile in a carbonated beverage with kiwi (*Actinidia deliciosa*), achotillo (*Nephelium lappaceum*) and moringa (*Moringa oleifera*) that will provide a beverage with nutritional appeal. The antioxidant properties of kiwi and achotillo, together with the nutritional benefits such as the amino acids provided by moringa, will be used to generate a new carbonated beverage that not only provides the sensation of freshness, but is also potentially beneficial to health.

Materials and methods

The present work proposed the study of the vitamin C content and amino acid profile in a carbonated beverage composed of three ingredients: kiwi, achotillo and moringa. The details of the proposed formulation are presented in Table 1.

Table 1. *Percentages of ingredients used for the different treatments in the formulation of the carbonated beverage.*
Description of the percentages of ingredients for the different treatments in the formulation of the carbonated beverage.

Ingredients	T1	T2	T3
Actinidia deliciosa (kiwi)	25%	20%	15%
Nephelium lappaceum (Achotillo)	45%	50%	55%
Moringa oleifera	0.1 %	0.1%	0.1%
Monk fruit	0.25 %	0.25%	0.25%
Water	29.04%	29.04%	29.04%
CO_2	0.5%	0.5%	0.5%
Sodium benzoate		0.05%	0.05%
Citric acid	0.05%	0.06%	0.06%
	0.06%		

Armijos, 2022

From the results obtained, the beverage with the best score was selected. The formulated beverage with the best sensory score had its vitamin C content, amino acid profile, physical-chemical and microbiological analysis determined.

Material resources

The raw materials (kiwi, achotillo, moringa, monkfruit, lemon, sodium benzoate, carbon dioxide) used were obtained from local businesses.

Methods and techniques

Figure 1 shows the flow diagrams of the processes used to obtain the beverage under study.

Sensory evaluation method

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For the sensory evaluation of the organoleptic characteristics (color, odor and flavor), a panel composed of 30 untrained persons between 18 and 30 years of age was selected; each member of the panel received 3 containers, each containing a treatment to be evaluated. Subsequently, they received an evaluation form where they recorded the data using a 5-point hedonic scale, where 5 is the highest score and 1 is the lowest score.

The score of 5 had a category of "I like it very much", the score of 4 had a category of "I like it", the score of 3 had a category of "I neither like nor dislike it", the score of 2 had a category of "I dislike it" and the score of 1 had a category of "I dislike it very much". To evaluate the vitamin C content, amino acid profile, physicochemical and microbiological parameters of the best evaluated treatment, analyses were performed in an accredited laboratory.

Physicochemical parameters

pH determination

For pH titration, the procedure detailed in INEN 1087 was followed.

Determination of carbon dioxide gas

For the determination of carbon dioxide gas, the procedure detailed in INEN 1082 was followed.

Determination of Brix degrees

For the determination of Brix degrees, the procedure detailed in INEN 1083 was followed.

Acidity determination

For the determination of acidity, the procedure detailed in NTE INEN 1091 was followed.

Determination of vitamin C by HPLC

The sample chosen with the best score by the sensory panel had its vitamin C content determined by HPLC.

Liquid chromatography (HPLC) is a technique used to separate the components of a mixture. It consists of a non-polar stationary phase (column) and a mobile phase. The stationary phase is silica that has been treated with RMe_2 SiCl. The mobile phase acts as a sample carrier. The sample in solution is injected into the mobile phase. The components of the solution migrate according to the non-covalent interactions of the compounds with the column. These chemical interactions determine the separation of the contents of the sample. The use of the different detectors will depend on the nature of the compounds to be determined (Miranda and Martín, 2013).

C18 column and an RP-18 precolumn were used for the stationary phase, and the mobile phase, sulfuric acid was used to reach pH 2.2.

The flow rate was 0.4 mL/min, and the UVVIS detector at 225 nm. (Fang, 2017).

Determination of amino acid profile by the Burbach Institute Prederivatized (HPLC-FLD) method.

The sample chosen with the best score by the sensory panel had its amino acid profile determined by the Burbach Institute Prederivatized (HPLC-FLD) method (Castillo-Portela et al., 2011). (Castillo-Portela et al., 2011)..

Microbiological analysis for mesophiles by BAM-FDA method CAP#3

This method is based on the certainty that a vital microorganism present in a food sample, when inoculated in a solid nutrient medium, will reproduce forming a visible individual colony. To make colony counting possible, decimal dilutions of the initial sample suspension are made and the nutrient culture medium is inoculated. The inoculum is incubated at 30°C for 72 hours and then the number of colonies formed is counted. The count is used to calculate the amount of microorganisms per gram or per cubic centimeter of food (INEN, 2006). (INEN, 2006).

The sample chosen with the best score by the sensory panel underwent the respective microbiological analysis by the most probable number method for *the* detection of mesophiles.

Multiple fermentation tube method for coliform analysis

The method is based on the detection of the most probable number (MPN) by the tube dilution technique using the selective liquid medium bile-lactose brilliant green broth or similar for the presumptive assay and the tubes that present gas are plated on Eosin methylene blue agar (E M B). The incubation temperature for the presumptive and confirmatory assay is 30 +-1°C for refrigerated products and 35+-1°C for products kept at *room* temperature. (INEN, 1990).

Analysis of molds and yeasts by the plate count method by deep sowing

This method is based on the cultivation between 22ºC and 25ºC of the propagating units of molds and yeasts, using the plate count technique by deep sowing and a medium containing yeast extract, glucose and mineral salts. (INEN, 2013).

Sensory evaluation method

A sensory panel of 30 untrained judges was selected for the analysis of odor, color and flavor of the samples of each of the treatments under

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study. A 5-point hedonic scale was used to measure the sensory characteristics.

Statistical analysis

The information obtained at the end of the carbonated beverage treatments for each of the sensory variables, using the 5-point scale planned to be used, was statistically evaluated by analysis of variance (ANOVA). In the case of obtaining significant differences, the Tukey test was used as a test for comparison of means. These two statistical tools were applied at 5% significance.

The analysis of variance model is detailed in Table 2.

Table 2. *Analysis of variance for sensory variables*

Sources of variation	Degrees of freedom
Total (n-1)	89
Treatments (t-1)	2
Repetitions (judges)(R-1)	29
Experimental error (t-1) (R-1)	58

Presentation of analysis of variance for sensory variables, Armijos, 2021

3. Result

Results of formulation selection of carbonated beverage formulation made with kiwi achotillo and moringa from a sensory panel.

Sensory tests were conducted to evaluate a carbonated kiwi, achotillo and moringa beverage. Three treatments were established with different proportions of kiwifruit and achotillo, while moringa, monkfruit, citric acid and sodium benzoate were held constant in the three formulations. The preparation of the beverage followed the process previously detailed in Figure 1. 20 kg of kiwifruit and achotillo in good condition and of acceptable quality were selected, based on their physiological maturity index and sugar content (12ºBrix for kiwifruit and 17ºBrix for achotillo). The fruits were washed with water and disinfected with 0.5% lactic acid for 3 minutes. They were then peeled and the pulp was weighed according to the proportions required for each formulation. The pulps were liquefied to obtain the juice, which was then filtered to separate solid residues.

Subsequently, the pulps were mixed and citric acid was added as a stabilizer and sodium benzoate as a preservative. Pasteurization was carried out at 70ºC for 30 minutes, followed by thermal shock in water

and ice to rapidly reduce the temperature to 20ºC and ensure the elimination of microorganisms. Water, non-caloric sweetener (monkfruit) and moringa powder were added. The juice was cooled to 4ºC for one day and then carbonated by mixing with carbon dioxide. Finally, it was packaged in amber glass bottles and stored at 4ºC.

Analysis of variance and tukey's test to the treatments evaluated by the panelists.

The results obtained from the sensory tests of the treatments performed on the panelists with their replicates were recorded in a Microsoft Excel spreadsheet, and then exported to Infostat (2020), followed by an analysis of variance using the Tukey test at 5% probability, which made it possible to determine the significant difference between treatments by checking the significance level (p-value).

As can be seen in Table 4, treatment 2 did not present a significant difference with treatment 1 for the attributes of color and flavor, so treatment 2 was chosen for having the highest mean, which was 3.73 for the attribute of color, while the mean for this same treatment in the attribute of flavor was 3.60 and for the attribute of odor, treatment 2 had a mean of 3.50, presenting a significant difference between treatments 1 and 3, which did not present a significant difference between them with respect to the aforementioned attribute.

Table 3 shows the results of the sensory evaluation of the treatments.

Table 3. *Sensory evaluation of the kiwi, achotillo and moringa carbonated beverage treatments.*

Treatments	Color	Odor	Flavor
Treatment 1	3.57^{b}	2.93^{a}	3.39 ^{b}
Treatment 2	3.73^{b}	3.50^{b}	3.60 ^{b}
Treatment 3	3.06^{a}	2.86^{a}	2.86 ^{a}
CV	27.91	37.89	35.92

Means of sensory attributes in the proposed treatments of the carbonated beverage.

Means with a letter in common are not significantly different (p>0.05).

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Determination of the Vitamin C content and amino acid profile of the carbonated beverage made with kiwi, achotillo and moringa selected as the best scored from the sensory panel.

According to the analysis of the vitamin C content of the kiwi, achotillo and moringa carbonated beverage corresponding to treatment 2, selected as the one with the highest sensory acceptance, it registered a vitamin C content of 212.39 mg/kg (21.24 mg/100g), highlighting that the raw materials used for its preparation are a good source of this vitamin.

Amino acid profile results of the best scoring treatment of the kiwi, achotillo and moringa carbonated beverage.

Table 4 shows the values corresponding to the amino acid profile of treatment 2 of the carbonated beverage selected as the best scored by the sensory panel.

Table 4. *Amino acid profile of carbonated beverages*

Amino acids gAA/100g
Aspartic Acid 0.18
Glutamic Acid 0.35
Serine 0.12
Histidine 0.00
Threonine 0.12
Glycine 0.16
Arginine 0.12
Alanine 0.00
Tyrosine 0.72
Valine 0.09
Methionia 0.04
Phenyl alanine 0.12
Isoleucine 0.09
Leucine 0.13
Lysine 0.05
Total amino acids 2.29 w/w

Results of the amino acid profile of the carbonated beverage.

Evaluation of the physicochemical (acidity, ºbrix, pH, CO Volume$_2$) and microbiological (total coliforms, mesophilic molds and yeasts) parameters of the kiwi (*Actinidia deliciosa*) achotillo (*Nephelium lappaceum*) and moringa (*Moringa oleifera)* carbonated

beverage according to the NTE INEN 1101:2008 Standard for carbonated beverages for the treatment with the highest score in the sensory panel.

Physicochemical results of the kiwi, achotillo and moringa carbonated beverage.

The results of the physicochemical parameters for treatment 2 indicate that they are within the ranges established by NTE INEN 1101:2008 for carbonated beverages.

Table 5 presents the results obtained from the physicochemical analysis:

Table 5. *Physicochemical parameters of the treatment with the highest acceptance by the sensory panel.*

Parameters	Results
Acidity	0.27%
Brix	6.2
pH	4.3
Volume CO2	2.6

Physicochemical analysis of the best-scoring carbonated beverage treatment
Armijos, 2022

Results of the microbiological analysis of the best-scoring treatment of the kiwi, achotillo and moringa carbonated beverage.

The results of the microbiological analyses performed on treatment 2 of the kiwi, achotillo and moringa carbonated beverage indicate that they are within the microbiological parameters established by NTE INEN 1101:2008 for carbonated beverages. Table 6 shows the results of the microbiological quality analysis of the carbonated beverage:

Table 6. *Results of microbiological analysis of the treatment with the highest acceptance by the sensory panel.*

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Requirements	Unit	Results	Test method
Total coliforms	NMP/ml	<3	PEE.LASA.MB.01b;BAM
Mesophilic			CAP.
	4,ED.2017		
	CFU/g	$3.0x10^4$	BAM-FDA CAP.#3
Molds and yeasts	2001		
			(Plate count)
	CFU/g	<10	INEN 1529-10 1998
			(Plate count)

Microbiological analysis results
Armijos, 2022

For the present study, the vitamin C content and amino acid profile of a carbonated beverage with kiwi, achotillo and moringa were determined, for which three different treatments were formulated with different percentages as indicated in previous sections (T1: 25% kiwi, 45% achotillo, 0.1% moringa, 0.25% monkfruit and 0.25% sodium benzoate).1% moringa, 0.25% monkfruit and 0.25% sodium benzoate) T2: 20% kiwifruit, 50% achotillo, 0.1% moringa, 0.25% monkfruit and 0.25% sodium benzoate) T3: 15% kiwifruit, 55% achotillo, 0.1% moringa, 0.25% monkfruit and 0.25% sodium benzoate). According to the results obtained from the sensory panel, the most acceptable treatment in terms of color, odor and flavor attributes was treatment 2, which was analyzed for vitamin C content, amino acid profile, physicochemical analysis (acidity, pH, °brix and volume of CO_2) and microbiological analysis (total coliforms, mesophiles, molds and yeasts). In a study conducted by Gonzaga (2017), where he conducted sensory analysis of a carbonated beverage made from naranjilla pulp, the formulation of the beverage made up of 25% naranjilla pulp and 10% sweetener obtained greater acceptance; this formulation had a greater amount of pulp extract, which allowed it to maintain the characteristic flavor, color and smell of the fruit; however, the panelists recommended reducing the intensity of the sweet flavor. Based on the above, it can be indicated that in the carbonated beverage proposed with kiwi, achotillo and moringa, a value of 2% was considered for the sweetener based on the juice of the fruits that were added, so that it would not be affected in terms of the sweet flavor and thus could highlight a characteristic flavor of the fruits used in the beverage.

Rodriguez (2020) evaluated the organoleptic characteristics of a beverage made from soy milk and kiwi, flavored with passion fruit. The

best qualified treatment of this beverage was treatment 4 made with 40% soy and 40% kiwi, while the remaining treatments showed no significant difference between them, with measurements well below the winning treatment. Similar results were shown in the sensory evaluation of the kiwi, achotillo and moringa carbonated beverage, where it was shown that the treatment with the highest sensory mean was treatment 2, but in this case it did not show a significant difference with treatment 1, for which the highest mean corresponding to T2 was chosen.

The analysis to determine the vitamin C content of treatment 2, chosen by the sensory panel for being the highest scoring, resulted in a value of 212.39 mg/kg (21.24 mg/100g), a value higher than the vitamin C content obtained by Maldonado and Moncayo (2012), in their carbonated passion fruit drink where they recorded a vitamin C value of 10.02 mg/100g of vitamin C, this result is surpassed by the proposed carbonated drink of kiwi, achotillo and moringa because the matrices used have a high vitamin C content, highlighting for example 70 mg/100 g is the vitamin C content of achotillo, 93 mg/100 g is that of kiwi and 70 mg/100 g in moringa, all these matrices exceed the fruit used by Maldonado and Moncayo (2012), which contains 30 mg/100 g of this vitamin. On the other hand, in a study conducted by Cantillo (2020), where he elaborated nectar based on achotillo and apple enriched with alfalfa as a nutritional contribution, he evaluated the vitamin C content by the method of volumetric titration to several treatments in which the control treatment consisting of 50% achotillo and 50% apple obtained a higher vitamin C content which was 26.25mg/100 ml, followed by the treatment consisting of alfalfa 2.5%, achotillo 40% and apple 57.5% with a vitamin C content of 24.13 mg/100 ml and the lowest value was the treatment made up of 5% alfalfa, 50% achotillo and 45% apple which was 18.25/100 ml, resembling these vitamin C contents with that of the present project under study which includes one of the fruits used (achotillo) by the authors mentioned above, this being a carbonated beverage. It is important to highlight that pasteurization is a treatment that affects the loss of vitamin C as confirmed by Villareal, Mejía, Osorio, and Ceron (2013), who analyzed the effect of pasteurization on sensory characteristics and vitamin C content in fruit juice, which were tree tomato juice, costeño mango juice, valencia orange and castilla blackberry, initially these juices had a vitamin C content of 6.4mg/100g, 7.2mg/100g, 55mg/100g and 2.4mg/100g, after being subjected to

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different temperatures and pasteurization times for each juice, corresponding as follows, blackberry juice T=80°C and t=120 s, mango juice T=85°C and t=120 s, tree tomato juice T=80°C and t=90 s and orange juice T=75°C and t=90 s, evidenced a loss of vitamin C in each fruit juice, for tree tomato juice the loss was 87.72%, mango juice 89.82%, orange juice 99.43% and blackberry juice 84.67%. On the other hand, Rodriguez (2007), elaborated and determined the stability of an isotonic drink based on Kiwi and Guava added with conjugated linoleic acid (CLA), where the fruits with peel were subjected to a steam scalding at 80°C for 30 minutes, the analysis of vitamin C was carried out by the spectrophotometric method, obtaining as a result a vitamin C content of 41.This value is almost double that found in the carbonated kiwi achotillo and moringa drink with a vitamin C content of 21.24 mg/100 g. It is important to note that guava has 3 times more vitamin C than kiwifruit and almost 4 times more than achotillo, and the heat treatment applied to the isotonic beverage influences the vitamin C value to be higher than that found in the carbonated kiwifruit achotillo and moringa beverage, which was pasteurized at 75°C for 25 minutes.

The results for the amino acid profile of the kiwi achotillo and moringa carbonated beverage of treatment 2 chosen as the most acceptable by the sensory panel, recorded the following results; Aspartic Acid 0.18 g/100 g (1.8mg/g sample), Glutamic Acid 0.35 g/100 g (3.5 mg/g sample), Serine 0.12 g/100g (1.2 mg/g sample), Threonine 0.12 g/100g (1.2 mg/g sample), Glycine 0.16 g/100 g (1.6 mg/g sample), Arginine 0.12 g/100 g (1.2 mg/g sample), Tyrosine 0.75g/100 g (7.5 mg/g sample), Valine 0.09 g/100 g (0.9 mg/g sample), Methionine 0.04 g/100 g (0.4 mg/g sample), Phenyl alanine 0.12 g/100 g(1.2 mg/g sample), Isoleucine 0.09 g/100 g (0.9 mg/g sample), Leucine 0.13 g/100 g (1.3 mg/g sample), Lysine 0.05 g/100 g (0.5 mg/g sample), with total essential amino acids (lysine, leucine, threonine, isoleucine, methionine, valine, phenylalanine) of 0.64 g/100 g except for Histidine and Alanine, results that are well below that evidenced by Santos (2019), who performed a characterization of a Tea by using moringa leaves which determined the amino acid profile in moringa leaves dehydrated at different temperatures 35, 45 and 55°C characterizing the amino acid profile by the HPLC method where the highest content of essential amino acids was identified in the treatment at 55°C obtaining as a result 16.It is important to highlight that the author mentions that the heat applied to the fresh moringa leaves to dehydrate them influenced the concentration of amino acids, because there was a greater loss of water in the leaves when the temperature of 55°C was applied,

so that at this temperature the concentration of amino acids was not high enough.

In another study conducted by Vendrell (2021), on the protein and antioxidant enrichment of cookies with moringa dried leaves powder, where he added moringa powder to wheat flour in different proportions called G1 and G10, G1 consisted of 1% moringa powder and 53% wheat flour while G10 consisted of 10% moringa powder and 44% wheat flour in this study determined the amino acid profile of the cookie made with these formulations using the gas chromatography method, the highest results of amino acids were given in the formulation G10 which were; for Isoleucine 0.02 g/100 g sample, Aspartic Acid 0.33 g/100 g sample, Glutamic Acid 0.45 g/100 g sample, Serine 0.18 g/100g sample, Threonine 0.1 g/100g sample, Proline 0.015g/100 g sample, Asparagine 0.03 g/100 g sample, Hydroxyproline 0.01 mg/100 g sample, Tyrosine 0.02 g/100 g sample, Methionine 0.09 g/100 g sample, Phenylalanine 0.02 g/100 g sample, α-aminoadipic acid 0.6 g/100 g sample, as can be evidenced the results only contain 3 of the essential amino acids (Isoleucine 0.02 g/100 g sample , Methionine 0.09 g/100 g sample and Phenylalanine 0.02 g/100 g of sample), these quantities are lower than the essential amino acids found in the carbonated kiwi, achotillo and moringa beverage, of which Isoleucine with 0.09 g/100 g and Phenylalanine with 0.12 g/100 g are found in greater proportion. This loss of essential amino acids in the case of the cookie is due to the high baking temperature (180 ºC) applied for the elaboration of the cookie, as stated by the author Vendrell (2021).

The results obtained for the physicochemical parameters of the kiwi achotillo and moringa beverage of treatment T2 selected as the most acceptable by the sensory panel were 6.2 °brix, 4.3 pH, titratable acidity 0.27% and CO2 volume of 2.6 /L, which are within the range established by the INEN (2008) standard for low-calorie carbonated beverages, however, in the physicochemical results obtained by Gonzaga (2017), where he elaborated a carbonated beverage with naranjilla pulp presented 10°brix, pH 3.5, titratable acidity 0.55% and CO2 volume of 2.5 /L, this beverage reflects higher values in terms of acidity and pH, this is due to the fact that naranjilla has an acidity of 2.56%. Gonzaga (2017)being more acidic than kiwi with 1.18% Godoy and Dome (2013), and achotillo with 0.30% acidity Avendaño-Arrazate et al., (2017), fruits that were occupied for the elaboration of the carbonated beverage of the present research.

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Finally, microbiological parameters were evaluated for treatment 2, based on the INEN (2008) standard for carbonated beverages, showing results for total coliforms < 3 (Absence), Mesophiles < 1/d (Absence), molds and yeasts < 10, showing that all these values are in accordance with the specifications established by the INEN 1101 standard:2008 for carbonated beverages, it should be mentioned the importance of performing microbiological analyses since they determine the safety of the process and can affect the shelf life and sensory parameters of the beverage.

4. Conclusions

According to the sensory analysis carried out on the carbonated beverage made with kiwi (15%), achotillo (25%) and moringa (0.1%), treatment 2 was chosen as it presented the greatest acceptance by the sensory panel with respect to the attributes of color, odor and flavor, evaluated with the help of the infostad program, where it was determined through the analysis of variance that treatment 2, although it did not present a significant difference with treatment 1, was chosen as it showed the highest mean among the three treatments carried out.

From the results obtained by the laboratory with respect to vitamin C and the amino acid profile of treatment 2 of the carbonated beverage, 212.39 mg/kg (21.24 mg/100 g) of vitamin C content was obtained, this amount would represent approximately 20% of the recommended daily value of vitamin C indicated as a voluntary declaration for nutrients in INEN 1334-2 Labeling of Food Products for Human Consumption Part 2: Nutritional Labeling. Requirements (NTE INEN 1334-2, 2011).

In relation to the amino acid profile, it could be evidenced that the beverage presents a total of 2.29 AAg/100 gram of sample, being able to highlight this presence of nutrients when compared with other carbonated beverages in the market. It should be emphasized that for this research only 0.1% of moringa was used, which is a good source of amino acids, but for formulation purposes the amount added was low due to its intense flavor and aroma, which is not accustomed to the palate of consumers, which altered the sensory quality of the proposed beverage.

The results of the physicochemical parameters for the kiwi, achotillo and moringa carbonated beverage of treatment 2 were 6.2 ° Brix, 4.3 pH, titratable acidity 0.27% and CO2 volume of 2.6 /L. These results show that they are within the ranges established by the INEN 2008 standard for carbonated beverages. With respect to the microbiological results for mesophilic aerobes, total coliforms, molds and yeasts, the

carbonated beverage complies with the parameters required by the NTE INEN 2008 standard.

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