

Role of Thermal Process on the Physicochemical and Rheological Properties and Antioxidant Capacity of a New Functional Beverage Based on Coconut Water and Rice Flour

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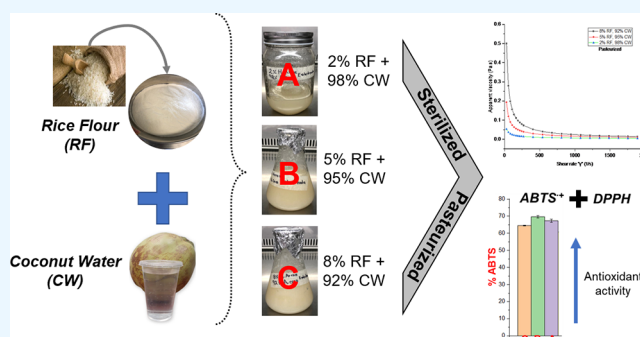
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ABSTRACT: Different substrates have been implemented for the production of functional beverages. To avoid the presence of pathogens, beverages have been subjected to thermal treatments, such as sterilization or pasteurization, which can interfere with the physicochemical, rheological, functional, and organoleptic properties of the final product. The objective of the present study was to evaluate the effects of heat treatment on the physicochemical properties, such as acidity, pH, total solids, density, total and reducing sugar, as well as the antioxidant activity of a beverage formulated from rice flour (RF) and coconut water (CW). Three beverage formulations were evaluated: A (2% RF; 98% CW), B (5% RF; 95% CW), and C (8% RF; 92% CW), each of which was subjected to two heat treatments: sterilized (121 °C/15 psi/15 min) or pasteurized (60 °C/60 min and subsequently 73 °C/15 s). The heat treatments increased the acidity and reducing sugars but decreased pH, total sugar, and antioxidant activity. As for the rheological properties, the mixtures were pseudoplastic fluid. The physicochemical properties from RF and CW mixtures were dependent on the heat treatment, but these can be introduced as new nondairy substrates for the elaboration of functional beverages to be consumed mainly by those lactose intolerant.



1. INTRODUCTION

Eating habits have changed for better or worse and, with it, people's way of life. These changes sometimes lead to metabolic syndrome diseases, such as obesity, diabetes, hypertension, and among others. Facing with these health problems, it is essential to implement interventions that help prevent these diseases, among which is the use of functional foods and products that have a positive effect on health beyond basic nutrition and help reduce the risk of disease.^{1,2}

The use of functional beverages with the incorporation of lactic acid bacteria has increased considerably in recent years, benefiting a large percentage of the population.^{1–3} These bacteria have been widely incorporated in dairy foods, which cannot be ingested by the lactose-intolerant population. Therefore, the need has arisen to evaluate new functional nondairy beverages based on fruits and cereals. Coconut water and rice flour can be considered innovative matrices, which can be used as substrates for the maintenance of lactic acid bacteria.^{4–6}

Several countries are rich in tropical fruits, which can provide the beneficial potential for human health and which could be added to the creation of new functional products on the market.^{7–9} In this context, coconut is a tropical fruit that

has various health benefits thanks to its bioactive properties.¹⁰ Coconut water is a widely used by-product of coconut. Coconut water is a clear liquid obtained from the endosperm of coconuts (*Cocos nucifera* L.), with low in calories and fat.¹⁰ Coconut water is rehydrating, used as a sports drink, and rich in vitamins, minerals, and amino acids.¹¹ Also, it has antimicrobial,¹² anti-inflammatory,^{13,14} cardioprotective,¹⁵ and antioxidant activity.¹⁶ Globally, coconut water remains a traditional and underutilized resource, which is extracted in few tropical and subtropical areas.¹⁷ Currently, the use of coconut water has increased due to its diverse health benefits, highlighting its importance as a probiotic encapsulant.¹⁸

Fruits and vegetables are not the only ones that provide a high nutritional value and therapeutic effects on health but also cereals such as rice, which is a hypoallergenic and easily

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digestible food and rich in fiber, vitamins, minerals, and carbohydrates.^{2–19} Thus, it is also used in various formulations of food products to provide texture, consistency, smell, flavor, and nutritional value; hence, it is also one of the most preferred grains by consumers for its sensory qualities, appearance, and texture.²⁰ Rice flour is a valuable ingredient in baby foods and in gluten-free products.^{21,22}

At present, heat treatment is the most widely used method to commercialize fermented beverages obtained from these matrices;^{23,24} however, alterations have been observed in the sensory quality of the product, modifying its nutritional content. Thermal pasteurization and sterilization are important thermal treatments that ensure the safety of beverages. Thermal pasteurization inactivates vegetative microorganisms and enzymes, while sterilization is a more drastic treatment that destroys all microorganisms, including spores.²⁵

Although both matrices, coconut water and rice flour, have been of great importance, there are still challenges faced by the industrial sector to preserve their functional properties and guarantee the survival of lactic acid bacteria.^{1,6} Therefore, it is essential to evaluate and study processing methods that guarantee good product quality. The objective of the present study was to evaluate the effect of heat treatment on the rheological properties and physicochemical properties, such as acidity, pH, total solids, density, total and reducing sugar, as well as the antioxidant activity of a functional beverage formulated from rice flour (RF) and coconut water (CW). The hypothesis of the present work was to develop an innovative matrix in order to incorporate bacteria with probiotic potential, which generates a nondairy functional product, to be consumed mainly by vegans and/or vegetarians, and those who are lactose intolerant.

2. RESULTS AND DISCUSSION

2.1. Preparation of the Mixtures. A calculation base was built with the minimum and maximum values corresponding to the amount of carbohydrates, fat, protein, and fiber in formulations of functional beverages in relation with CW or RF. These amounts of carbohydrates, fat, protein, and fiber were used as restrictions in an objective function to obtain the coordinates in *X* and *Y* plane for each of the nutritional components (Figure 1). For this case, 8%, 5%, and 2% of RF

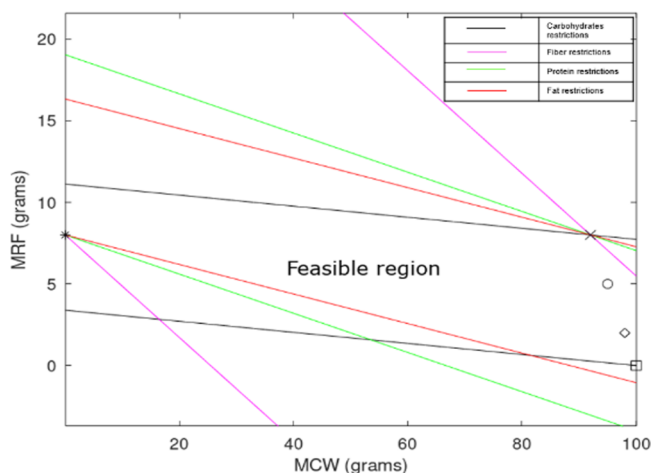


Figure 1. Feasible region showed the minimum and maximum limits. MRF (mass rice flour) and MCW (mass coconut water).

were used, making a total of three mixes that were closest to the maximum, intermediate, and minimum limit of the feasible region and the same for coconut water in a percentage of 92, 95, and 98% respectively on a calculation basis of 100 g. The mixtures were identified as A (2% RF; 98% CW), B (5% RF; 95% CW), and C (8% RF; 92% CW).

2.2. Physicochemical Characterization of the Mixtures. In this study, we determined the changes in the physicochemical properties of three beverage formulations: A (2% RF; 98% CW), B (5% RF; 95% CW), and C (8% RF; 92% CW). Each mixture undergoes two heat treatments, sterilization and pasteurization (Table 1). The results showed that the titratable acidity without heat treatment in B was higher compared to A ($p < 0.05$); however, A and C were similar. With heat treatments, the acidity of the mixtures increased in A greater than 100% ($p < 0.05$), B by 68% ($p < 0.05$), and C by 95% ($p < 0.05$). The pH without heat treatment was no significant difference between the mixtures. However, with heat treatments, the pH of the mixtures decreased in A and C by 9% ($p < 0.05$) and in B by 13% ($p < 0.05$). With sterilization, the mixtures were similar to each other, but with pasteurization, A was greater than B and equal to C and B was equal to C. The results obtained indicate that the heat treatment leads to the evaporation of water within the mixtures, concentrating the free amino acids present in the coconut water^{24,26} and gelatinizing the rice flour.^{19,27} The free amino acids that most abundant in coconut water are the alanine, γ -aminobutyric acid, glutamic acid, glutamine, serine, and the sum of arginine + cysteine, and heat treatments induce a slight increase of these amino acids due to protein hydrolysis or the hydrolysis of bonded free amino acids.²⁴ On the other hand, at temperatures higher than 50 °C, the gelation temperature of the starch present in the rice flour is reached, which leads to a loss of molecular organization or the breaking of hydrogen bonds within the granule.^{27,28} As a result, the acidity of the medium increases and the pH decreases due to the effect of the heat treatment.^{2,19,24}

Sample C presented a higher concentration of total soluble solids compared to A and B ($p < 0.05$). With heat treatment in mixture C, they decreased, which was not observed in A and B. The total soluble solids present in the mixtures of coconut water and rice flour were sugars such as fructose, glucose, and sucrose, which are mainly found in coconut water;^{29,30} however, the starch present in rice is a form of carbon that can intervene in the measurement of total solids, which is why the amount of total solids in mixture C increased. These total soluble solids are responsible for the sweet taste of the mixtures. As for the density of the mixtures, B and C were higher compared to mixture A ($p < 0.05$). Heat treatment did not affect the physicochemical property. This increase in density in mixture B and C is mainly due to the amount of rice flour present in the mixture.

The concentration of total sugars in A and C was higher compared to B ($p < 0.05$) and C higher compared to A ($p < 0.05$). This is mainly due to the fact that mixture A has the highest amount of coconut water and mixture C has the highest amount of rice flour, and both coconut water and rice flour influence the amount of total sugars present in the mixtures. When mixture C was subjected to heat treatment, the concentration of total sugars decreased ($p < 0.05$). This is because at high temperatures, starch tends to form dextrins, which, when hydrolyzed, form reducing sugars that are not detected by the type of technique used in this study to measure

Table 1. Physicochemical Properties of Functional Beverages Formulated from Rice Flour and Coconut Water^a

physicochemical parameters	A			B			C		
	NHT	S	P	NHT	S	P	NHT	S	P
titratable acidity (g/100 mL lactic acid)	0.102 ± 0.01 ^{aA}	0.205 ± 0.00 ^{bB}	0.205 ± 0.00 ^{bB}	0.126 ± 0.00 ^{bA}	0.212 ± 0.00 ^{bB}	0.212 ± 0.00 ^{bB}	0.111 ± 0.01 ^{abA}	0.216 ± 0.00 ^{bbB}	0.213 ± 0.00 ^{bbB}
pH	5.750 ± 0.10 ^A	5.320 ± 0.10 ^{bB}	5.253 ± 0.13 ^B	5.887 ± 0.07 ^A	5.140 ± 0.04 ^{bbB}	5.113 ± 0.10 ^B	5.813 ± 0.15 ^A	5.283 ± 0.04 ^{abbB}	5.303 ± 0.10 ^B
total soluble solid (TSS)	6.200 ± 0.27 ^a	5.800 ± 0.10 ^a	5.800 ± 0.00 ^a	6.833 ± 0.29 ^a	6.667 ± 0.06 ^b	6.733 ± 0.25 ^b	8.667 ± 0.58 ^{baA}	7.233 ± 0.25 ^{cb}	7.167 ± 0.06 ^{cb}
density (g·m ⁻³)	1.034 ± 0.00 ^a	1.033 ± 0.00 ^a	1.036 ± 0.01 ^a	1.055 ± 0.00 ^b	1.061 ± 0.01 ^{ab}	1.070 ± 0.02 ^b	1.057 ± 0.00 ^b	1.089 ± 0.03 ^b	1.080 ± 0.01 ^b
total sugar concentration (g/100 mL)	17.356 ± 0.17 ^{aA}	17.152 ± 0.20 ^{abA}	16.562 ± 0.12 ^{ab}	16.458 ± 0.49 ^b	16.331 ± 0.47 ^b	16.298 ± 0.60 ^a	19.791 ± 0.11 ^{ca}	19.058 ± 0.04 ^{cb}	19.014 ± 0.21 ^{bb}
reducing sugar concentration (g/100 mL)	1.687 ± 0.01 ^{aA}	16.059 ± 0.20 ^{ab}	16.259 ± 0.18 ^{ab}	1.107 ± 0.00 ^{aA}	11.656 ± 0.07 ^{bbB}	11.356 ± 0.12 ^{bc}	1.328 ± 0.06 ^{ca}	13.004 ± 1.02 ^{bb}	12.902 ± 0.04 ^{cb}

^aLowercase letters denote differences between mixtures and the same treatment. Uppercase letters denote differences within the same mixture with different treatment. A (2% RF; 98% CW), B (5% RF; 95% CW), and C (8% RF; 92% CW). NHT: no heat treatment; S: sterilized; P: pasteurized.

total sugars.³¹ This effect is very well observed when the reducing sugars of the mixtures were measured. As can be seen, when heat treatments are used, the concentration of reducing sugars increases more than 100% in A, B, and C ($p < 0.05$) compared to mixtures without heat treatment (Table 1). The concentrations of reducing sugars are observed in higher amounts in A and C ($p < 0.05$), being these mixtures contain higher amounts of coconut water (mixture A) and higher amounts of rice flour (mixture C). Carbohydrates, by their very nature, are not stable under extreme heat treatment conditions.^{31,32} High heat causes dehydration, which leads large molecules,³² such as the starch present in rice flour, to form dextrins, which can hydrolyze in aqueous systems, increasing the concentration of reducing sugars such as fructose and glucose.^{30–32} As for coconut water, the main sugar is sucrose,³³ which, at temperatures above 55 °C, undergoes hydrolysis, generating more glucose and fructose in the medium and increasing the concentration of reducing sugars present in the mixtures.^{34,35} The mixtures obtained after being subjected to heat treatment show a gelatinous appearance and darken (Figure 2). The gelatinous appearance,

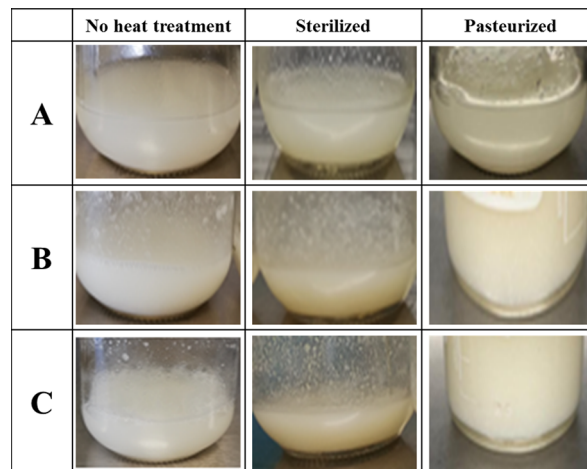


Figure 2. Appearance of the mixtures after being submitted to sterilization or pasteurization. A (2% RF; 98% CW), B (5% RF; 95% CW), and C (8% RF; 92% CW).

as mentioned above, is mainly due to the gelatinization process that occurs in the starch present in the rice flour.^{27,28} As for the color, the reducing sugars present in the mixtures when subjected to high temperatures, higher than 60 °C, can produce melanoidins as a result of the Maillard reaction, thus changing sensory aspects of the mixtures.^{30,36,37}

As mentioned above, sugars are the main component of the soluble solids in coconut water, highlighting the presence of sucrose, glucose, and fructose.³⁸ However, several studies have shown that the amount of sugar in coconut water tends to decrease due to storage conditions.^{39,40} This decrease is mainly due to the presence of enzymes in charge of metabolizing sugar.³¹ Among the enzymes of greatest interest are the invertases, such as acid invertase. This enzyme acts when the pH is between 4 and 5 and the temperature is between 35 to 50 °C.⁴¹ Therefore, we observed that the acidity and the concentration of reducing and total sugars are related. The conditions of the mixtures when subjected to heat treatment allow the activation of acid invertase, increasing the acidity of the medium, and in this way, this enzyme irreversibly converts

sucrose into glucose and fructose,⁴² decreasing the total sugars and increasing the reducing sugars of the mixtures (Table 1).

The physicochemical characteristics are important indicators of the quality and determine the different applications of these mixtures. Among the most outstanding applications for these types of beverages is their use as vegetable matrices for the maintenance of probiotics. Several studies have shown that both rice and coconut water are optimal materials for the growth of probiotics.^{43,44} Therefore, this study highlights that the physicochemical properties obtained from formulations based on coconut water and rice flour can be proposed as a symbiotic beverage (a mixture comprising live microorganisms and substrates used by these for their growth), which confers a health benefit.⁴³

In general, the results obtained in this study show the design and physicochemical characterization of formulations based on coconut water and rice flour. However, it is important to highlight the design and evaluation of the sensory properties in order to guarantee the commercial viability of these beverages. In this context, it is intended to subsequently evaluate the sensory properties by means of various tests, such as the check-all-that-apply (CATA),⁴⁵ questions that are applied to consumers to sensorially characterize the formulations of this study, as well as the different sensory tests by means of the profiling method (ISO 3972:1991; 11036:1994; 13299:2016; 6658:2017).⁴⁶

2.3. FTIR Spectrum Analysis of the Mixtures. Analysis of the infrared spectra did not show the formation of new functional groups in the mixtures (Figure 3). The typical

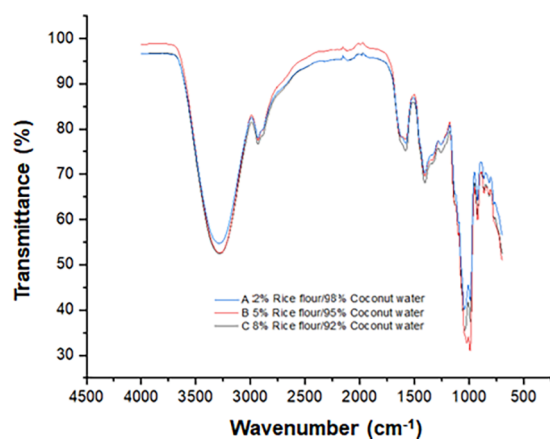


Figure 3. FTIR spectra of the different mixtures.

functional groups of coconut water and rice flour starch are observed.^{47,48} The band between 3393 and 3319 cm^{-1} is due to the stretching mode of the O–H groups. The adsorption band between 1648 and 1637 cm^{-1} is attributed to the intermolecular H bond involving the carboxyl group of starch or to the amide II band or N–H primary amines present in coconut water. The band between 1208 and 1155 cm^{-1} indicates the C–H vibrations of organic molecules.⁴⁸

2.4. Antioxidant Activity. Reactive oxygen species and free radicals have been of great interest because they can cause serious problems in health and in the food industry. Several studies have shown that excess free radicals can cause neurodegenerative and cardiovascular diseases.⁴⁹ Food oxidation causes changes in food quality, affecting nutritional and sensory qualities.⁵⁰ Therefore, it is important to look for

sources of antioxidant compounds that provide benefits both in the food system and in the health of the individual in order to reduce oxidative stress.⁵¹ In this study, antioxidant activity was evaluated by two methods ABTS and DPPH, both techniques involving the transfer of hydrogen atoms and electron transfer with loss of protons. We observed high % inhibition by mixtures A, B, and C (Table 2), values greater than 50% inhibition, with both methods. However, depending on the method used, it is observed that with ABTS, all the mixtures are equal ($p > 0.05$) and the thermal treatment decreased % inhibition in all the mixtures ($p < 0.05$). Regarding the DPPH method, in the mixtures without heat treatment, it was observed that mixture A presented the highest % inhibition compared to B and C ($p < 0.05$). When the samples were subjected to sterilization or pasteurization, only mixture A showed a significant effect ($p < 0.05$), decreasing its antioxidant activity, and this behavior was not observed with B and C (Table 2). Mixture A is observed to have the highest antioxidant activity and contains the highest concentration of coconut water compared to B and C.

The differences obtained by the type of method used to measure antioxidant activity are mainly due to the fact that they are different mechanisms: the DPPH method is based on radical scavenging activity and the ABTS method is based on cation radical scavenging activity.⁵² The ABTS radical reacts mainly with reducing sugars and melanoidins, products of the Maillard reaction,⁵³ in this case, mainly present in mixture A, with a higher concentration of coconut water. The DPPH radical reacts with polyphenols but not with sugars.^{52,53} Therefore, the % inhibition determined by DPPH and ABTS is partially different.

The values obtained by the ABTS method are higher, which is mainly due to the high reactivity of the radical,⁵⁴ with the ability to react with more antioxidant molecules present in the mixtures. However, it is also important to note that the preparation of the ABTS reagent is more complicated and less stable compared to DPPH.⁵⁵

Oxidation is a phenomenon that greatly affects the sensory attributes, nutritional value, and shelf life of food.⁵⁶ Therefore, it is important to highlight that the formulations A, B, and C showed high inhibition percentages compared to other beverages reported in other studies.⁴⁴ Therefore, it is interesting to see their application as beneficial beverages to reduce the damage caused by oxidation.

Both methods used in the determination of antioxidant activity correlate with each other, with Pearson's coefficients greater than $r > 0.80$ (Figure 4).

Although the mechanisms of radical scavenging may differ between the two assays, they both provide information about the antioxidant potential of the formulations A, B, and C. Generally, a substance that exhibits a high antioxidant capacity in one assay is likely to show a high antioxidant capacity in the other assay as well. However, it is important to note that the correlation may not be perfect and can vary depending on the heat treatment conditions, as can be seen from the results obtained in this study (Table 2).

2.5. Rheological Properties. It is essential to characterize the rheological properties of foods in order to determine the possible applications in science and in the food industry. It is important to emphasize that rheological behavior is complex to analyze due to the physical, chemical, and biological structures of food, so experimental measurements of rheological properties are indispensable for the characterization of new food

Table 2. Antioxidant Activity of Functional Beverages Formulated from Rice Flour and Coconut Water^a

method	A			B			C		
	NHT	S	P	NHT	S	P	NHT	S	P
%ABTS	69.594 ± 2.06 ^A	56.723 ± 1.93 ^B	59.664 ± 2.12 ^B	71.322 ± 2.46 ^A	58.637 ± 1.46 ^B	61.438 ± 0.80 ^B	69.454 ± 0.49 ^A	59.197 ± 1.54 ^B	62.885 ± 0.37 ^C
%DPPH	70.979 ± 0.46 ^{aA}	53.514 ± 2.42 ^B	55.982 ± 3.77 ^B	54.686 ± 0.25 ^b	54.069 ± 2.32	56.569 ± 2.64	56.970 ± 0.14 ^c	56.075 ± 0.56	57.587 ± 0.86

^aLowercase letters denote differences between mixtures and the same treatment. Uppercase letters denote differences within the same mixture with different treatment. A (2% RF; 98% CW), B (5% RF; 95% CW), and C (8% RF; 92% CW). NHT: no heat treatment; S: sterilized; P: pasteurized.

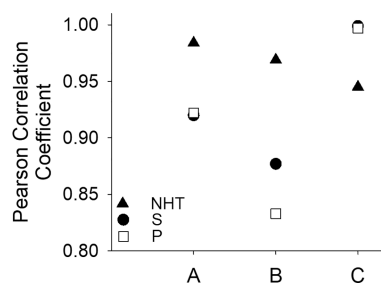


Figure 4. Correlation between the ABTS and DPPH findings of the formulations A, B, and C. NHT: no heat treatment; S: sterilized; P: pasteurized.

products.⁵⁷ Typical flow curves of mixtures are shown in Figure 5. It can be seen that the steady shear viscosity of mixtures A, B, and C decreased by increasing shear rate, indicating non-Newtonian shear-thinning behavior. When a shear force is exerted on the fluid, the apparent viscosity can increase or decrease over time. In mixtures A, B, and C, the viscosity presented a pseudoplastic behavior, which decreased with time of shear rate, presenting a thixotropic behavior.⁵⁷ As seen in Figure 5, mixture A exhibited a meaningfully lower decrease of apparent viscosity over shearing, suggesting more shear stability than mixture B and C. It is important to note that in all three mixtures, the apparent viscosity decreases rapidly at low shear rates, especially in mixture C, which contains the highest concentration of rice flour, demonstrating the strong pseudoplasticity of starch. This behavior is mainly due to the breakdown of the chemical structures of amylose and amylopectin that make up rice flour starch.⁵⁷

The Ostwald–de Waele power law obeys the behavior of dilating and pseudoplastic fluids,⁵⁸ which, in this case, (n) is dimensionless and (K) is dependent on (n). When $n < 1$, the fluid exhibits a pseudoplastic behavior, when $n > 1$, the fluid exhibits a dilatant behavior, and when $n = 1$, the fluid corresponds to a Newtonian behavior.⁵⁹ The results of n in mixtures A, B, and C were less than 1 (Table 3), which indicates that the mixes presented a behavior of the pseudoplastic fluid. Mixture A without heat treatment was the one that best fits the power model with an R^2 of 0.930 ± 0.04 (Table 3).

Several studies have shown that temperature and soluble solids have a significant effect on viscosity.^{58,59} This is observed with our results that the mixture C, which contains more soluble solids (Table 1), presented the highest viscosity value compared to A and B (Table 3, $p < 0.05$). It is observed that the samples decrease in viscosity when they are subjected to heat treatment. Viscosity is strongly dependent on the intermolecular forces between molecules and water-solute interactions.⁶⁰ As the temperature increases, these interactions break down, which causes an increase in the mobility of the molecules, increasing the intermolecular space and decreasing the resistance to flow.^{59,61}

3. CONCLUSIONS

The physicochemical properties of the coconut water and rice flour mixtures are affected depending on the heat treatment. The pH and acidity conditions also improve the rapid digestion of the starch present in the rice. In these mixtures A, B, and C, the viscosity presented a pseudoplastic behavior, which decreased with time of shear rate, presenting a thixotropic behavior. The observed physicochemical properties

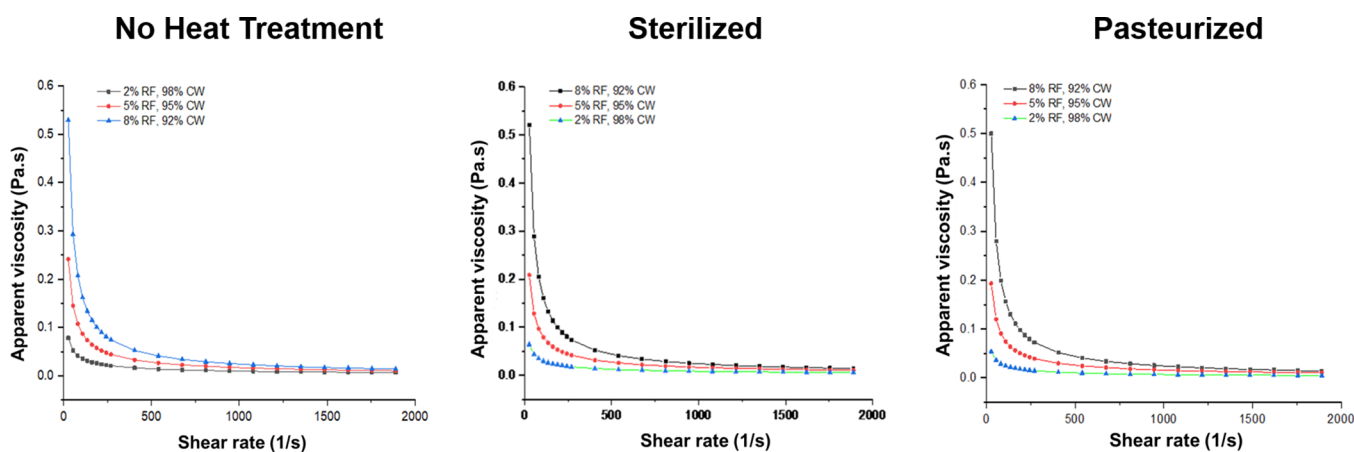


Figure 5. Shear thinning behavior in a plot of apparent viscosity versus shear rate.

in these matrices also position them as potential candidates for producing nondairy fermented beverages that facilitate the growth and maintenance of lactic acid bacteria and that can be consumed by vegans or lactose-intolerant people.

4. MATERIALS AND METHODS

4.1. Chemicals. Sodium hydroxide (98%), 3,5-dinitrosalicylic acid (98%), phenol (99%), sulfuric acid (98%), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (98%), and 1,1-diphenyl-2-picrylhydrazyl (97%) were purchased from Sigma-Aldrich, Mexico.

4.2. Biological Material. Hybrid green coconut water from Donaji, 7 months old, was used, which is a cross between yellow dwarf Malay coconut from Acapulco with the tall criollo of the Pacific and from the town of Tecolutla, Veracruz state. The rice used in this work was a commercial brand, Verde Valle, originating in Mexico. It belongs to the long grain variety and is the most consumed variety in Mexico.

4.3. Extraction and Preparation of CW and RF. The CW used in this work was extracted by hand drilling. The total water from the 20 selected fruits was homogenized, filtered through a filter paper of 1:11 μm (Whatman, Maidstone, UK) and maintained at $-70\text{ }^{\circ}\text{C}$ (Thermo Scientific 89000 Series, Massachusetts, USA) to prevent any microbial or enzymatic activity. A 1 kg sample of white rice grain was pulverized in an electric mill (Hamilton Beach 80350R, Virginia, USA) for 5 min, and to obtain a fine rice flour with homogeneous particle size, a MONTINOX # 60 sieve was used, equivalent to 250 μm of light passage.

4.4. Preparation of the Mixtures. The beverage formulations used in this study were designed on the basis of the linear programming method.⁶² Linear programming is a mathematical technique for generating optimal solutions that can satisfy several constraints at the same time. This mathematical approach optimizes (minimizes or maximizes) a line function of a set of decision variables, respecting linear constraints. The linear function, known as the objective functional, used was as follows:

$$M_i = X_i M_{\text{CW}} + X_i M_{\text{RF}} \quad (1)$$

where M_i is the mass of carbohydrate, fat, protein, or fiber, M_{CW} is the mass of coconut water, X_i is the fraction of carbohydrate, fat, protein, or fiber, and M_{RF} is the mass of rice flour.

The goal of optimization was to find the optimal solution or the correct formulation with the desired nutritional composition of carbohydrates, fat, protein, and fiber. Table 4 shows the minimum (min) and maximum (max) values of carbohydrate, fat, protein, and fiber used for the linear programming method. These values were established from data previously reported in other studies to produce vegetable beverages.^{63–67} The linear programming of carbohydrates, fat, protein, and fiber was carried out with the software Octave 6.4.0.

Each formulation (A, B, and C), resulting from applying the linear programming method, was divided into three samples, one without heat treatment (control, NHT) and the other two for heat treatment, pasteurized (P) or sterilized (S). For thermal pasteurization, the samples were placed in a precision water bath (Precision-Scientific TSGP05, Chicago, USA) and maintained at $60\text{ }^{\circ}\text{C}/60\text{ min}$ and subsequently $73\text{ }^{\circ}\text{C}/15\text{ s}$.⁶⁸ The temperature of beverages was continuously monitored. For sterilization, an electric autoclave (All-American, 24 L, 50X-120 V, California, USA) at $121\text{ }^{\circ}\text{C}/15\text{ psi}/15\text{ min}$ was used. Controls (NHT), sterilized (S), and pasteurized (P) samples were stored at $4\text{ }^{\circ}\text{C}$ to analyze their physicochemical properties.

4.5. Physicochemical Characterization of the Mixtures. The titratable acidity was determined by titration with 0.1 N sodium hydroxide solution using phenolphthalein (1% w/v) as an indicator.⁶⁹ The pH of the mixtures was measured with a pH meter (Oakton Acorn series pH 6-meter, Illinois, USA) at ambient temperature. Total soluble solids (TSS) concentration was determined directly using a digital hand refractometer (Grand Index RHB-90ATC, Esslingen, Germany) at ambient temperature. Density was measured with pycnometer of 50 mL at ambient temperature.⁶⁹ The concentration of reducing sugars was measured using the 3,5-dinitrosalicylic acid (DNS) method,⁷⁰ and total sugar content was measured using the phenol–sulfuric acid method.⁷¹ Each physicochemical property of the formulations (A, B, and C) was measured in triplicate, determining their average and standard deviation.

4.6. Determination of Antioxidant Activity. The antioxidant activity was evaluated by the two most common radical scavenging assays using 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS)⁵² and 1,1-diphenyl-2-picrylhydrazyl (DPPH)⁵² radical. Each sample was measured in triplicate. Mean and standard deviation ($n = 3$) were calculated. A working solution was prepared of cation radical

Table 3. Ostwald–de Waele Parameter^a

parameters	A			B			C		
	NHT	S	P	NHT	S	P	NHT	S	P
K (Pa · s ⁿ)	33.067 ± 1.51	32.417 ± 0.52 ^a	35.830 ± 2.17	41.140 ± 5.56 ^A	32.352 ± 1.94 ^{AB}	26.866 ± 5.36 ^B	37.823 ± 0.81 ^{AB}	47.206 ± 6.67 ^{BA}	30.378 ± 5.05 ^B
n	0.107 ± 0.01 ^{abA}	0.131 ± 0.00 ^{ab}	0.0431 ± 0.01 ^c	0.0767 ± 0.02 ^{aA}	0.141 ± 0.01 ^{ab}	0.115 ± 0.03 ^{baB}	0.113 ± 0.01 ^b	0.0964 ± 0.02 ^b	0.135 ± 0.02 ^b
R^2	0.930 ± 0.04	0.844 ± 0.02	0.813 ± 0.12	0.863 ± 0.09	0.920 ± 0.02	0.848 ± 0.05	0.904 ± 0.01	0.861 ± 0.08	0.850 ± 0.05
η_{27} (Pa·s)	1.733 ± 0.05 ^{aA}	1.849 ± 0.02 ^{ab}	1.528 ± 0.05 ^C	1.973 ± 0.15 ^{ba}	1.908 ± 0.06 ^{aA}	1.440 ± 0.17 ^b	2.030 ± 0.04 ^{ba}	2.392 ± 0.19 ^{bb}	1.746 ± 0.16 ^A

^aConsistency coefficient (K), flow behavior index (n), coefficient of determination for the Ostwald–de Waele model (R^2), and apparent viscosity at 27 s⁻¹ (η_{27}). Lowercase letters denote differences between mixtures and the same treatment. Uppercase letters denote differences within the same mixture with different treatment. A (2% RF; 98% CW), B (5% RF; 95% CW), and C (8% RF; 92% CW). NHT: no heat treatment; S: sterilized; P: pasteurized.

Table 4. Maximum and Minimum Carbohydrate, Fat, Protein, and Fiber for Coconut Water and Rice Flour-Based Vegetable Beverage Formulations

variables	component: CW (g/100 g)		component: RF (g/100 g)	
	min	max	min	max
carbohydrate	2.61	77	0.84	17.94
fat	0.2	2.21	0.187	8.87
protein	0.72	6	0.74	8.38
fiber	1.1	3.5	0.11	0.70
water	94.99	10.37	3.15	91.23
others	0.38	0.92		
total	100	100		

ABTS⁺ to achieve an absorbance of 0.700 ± 0.02 at 734 nm. For the absorbance measurements of the samples, 180 μL of diluted ABTS⁺ and 20 μL of sample was used. The calibration curve was prepared using Trolox at 250 μg/mL. The absorbance was acquired at 734 nm after 10 min. A working solution of DPPH was prepared at 1 mM. Fifty microliters of the DPPH working solution was added to 200 μL sample. Similarly, the calibration curve was prepared using 100 μL of Trolox at a concentration of 1 mM. The absorbance was measured at 515 nm after 30 min. The inhibition percent of DPPH or ABTS⁺ was calculated according to the following equation:

$$\% \text{inhibition} = \frac{A_{\text{negative control}} - A_{\text{sample}}}{A_{\text{negative control}}} \times 100 \quad (2)$$

4.7. Characterization of FTIR of the Mixtures. The mixes were previously lyophilized (Labconco 7,740,021 FreeZone, Kansas City, USA) for 24 h and subsequently analyzed by Fourier transform infrared (FTIR) (Spectrum Two FTIR, PerkinElmer spectrometer, Massachusetts, USA) by using the method of transmission. Samples were measured with a beam splitter of KBr and a DTGS detector of KBr between 500 and 4000 cm⁻¹, with a resolution of 4 cm⁻¹ at ambient conditions.

4.8. Rheological Properties of the Mixtures. The mixes were measured in a viscosimeter (Thermo Haake Fisons RV-20, Massachusetts, USA) with strain rate percentages from 1 to 98%, and then the shear rate ($\dot{\gamma}$) (eq 3) and the shear stress (τ) (eq 4) were calculated. After, the apparent density value in units of Pa·s was obtained (eq 5).

$$D = M \times \%D \left[\frac{1}{s} \right] \quad (3)$$

where M is the shear rate factor and depends on the system sensor and $\%D$ is the current shear rate.

$$\tau = A \times \% \tau \text{ [Pa]} \quad (4)$$

where A is the sensor-dependent shear stress factor of the system and $\% \tau$ is the shear stress value on the equipment display.

$$\mu = \frac{\tau}{D \text{ [Pa} \cdot \text{s}]} \quad (5)$$

where τ is the shear stress and D is the shear rate.

The experimental results were submitted to the mathematical model of the Ostwald–de Waele power law to predict the behavior of the fluid of each of the mixes, the model constants (k) and (n), and the coefficient of determination R^2 (eq 6).

$$\tau = K\gamma^n \quad (6)$$

where k is the consistency index and n is the flow behavior index.

4.9. Statistical Analysis. Data were reported as mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey test were used to access statistical differences by the SigmaPlot 11.0 at 0.05 significance level.

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Author Contributions

N.S.V.P., G.R.-S., J.Y.-F., and D.C.C.-R. researched data. J.Y.-F. and D.C.C.-R. were responsible for preparing the manuscript, as well as the design of the present study. All authors have approved the final version of the manuscript and agree to be responsible for all aspects of the work. All persons designated as authors are eligible to be authors.

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Notes

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