



Physical - Chemical Profile of Kombuchas Marketed in the Governador Valadares City - Brazil

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Abstract

Kombucha is a drink typically made of green or black tea, as well as needs the fermentation coming from an association of microorganisms beneficial to health. The consumption of Kombucha has become more frequent, since it brings vast benefits to health, as well as preventing cardiovascular diseases, promoting digestive functions, stimulating the immune system, reducing inflammatory problems. The objective of this work was to evaluate the physical-chemical parameters of Kombucha samples sold in the city of Governador Valadares-MG. This was a qualitative and quantitative experimental type of analytical study, where pH, humidity, ashes, sugars and antioxidants were analyzed. The results obtained showed moisture values according to the standards required by current legislation, no indicative legislation was found for ash values, also showed ideal pH values for Kombucha according to current legislation, presence of ascorbic acid, small amount of reducing and non-reducing sugars and presented satisfactory results for consumption. Therefore, it is of great importance to encourage the consumption of the beverage since it presents benefits for health and quality of life.

Keywords: Kombucha; Fermentation; Microorganisms; Physical-chemical Analysis

Introduction

The search for a healthier lifestyle has grown, and has been showing more and more that the current consumer is no longer only prioritizing the sensory aspects of a given product, but is also interested in seeking the benefits and functionalities that these products can offer to their health [1].

With this it is possible to notice a growing search for foods with functional properties [2], that is, those that, according to the National Health Surveillance Agency (ANVISA), when consumed as part of a usual diet, produce metabolic and/or physiological effects beneficial to health, being considered safe for consumption without medical supervision [3].

Faced with this search for a healthier lifestyle, there was an increase in kombucha consumption by the world population [4].

Kombucha is a refreshing, sweet and carbonated drink obtained by fermentation of sugary tea with a probiotic culture of acetic bacteria and fungi, consumed for its beneficial effects on human health. Research indicates that the drink can prevent cardiovascular diseases, promote digestive functions, stimulate the immune system, reduce inflammatory problems [5].

Among the main functions are: antioxidant action, glycemic index control and cancer prevention; due to the presence of vitamins, polyphenols and acids such as lylucconic acid, glycanic acid and lactic acid, as well as amino acids and other micronutrients produced during fermentation [6].

The main organic acids present in the fermented beverage are citric, folic, glyconic, glyconic, lactic, malic, malic, oxalic, pyruvic, succinic, tartaric acids. These acids and the sugars present will give the characteristic flavor of the drink [7,8].

The acids and other products formed interact with the compounds of the tea used, thus producing new biologically active products, including amino acids and vitamins, predominantly of the B complex [9].

The B-complex vitamins found in higher concentrations are: Thiamine (B1), Riboflavin (B2), Pyridoxine (B6) and Cobalamin (B12), acting in metabolic reactions, collaborating as coenzymes in the process of energy production for the body [10]. In addition to these, it has vitamin C, which has a direct influence as antioxidants and anti-inflammatory drugs that will bring benefits to the body in the fight against free radicals [11].

Fermented tea also contains significant levels of antioxidants, substances with ability to action account for physiological oxidative stress and catechins, one of the few groups of flavonoid compounds that have significant bioavailability [6].

The sugar added to the tea serves as food for scoby crops, glucose being the preferred substrate of these crops. The final concentration of sugar in the ready product may be different between fermentations, noting that the metabolic pathways of fermentation do not occur in the same way in all Kombuchas [5].

Kombucha has symbiotic property because it presents beneficial living microorganisms (probiotics) and prebiotics, which are fundamental in the balance of the intestinal microbiota [12,13].

The microorganisms present in the drink will compete with pathogenic bacteria for sites of asion and nutrients, reduce the permeability of the intestine and control the intestinal microbiota, preventing food intolerances, bringing positive results to increased immunity, irritable bowel syndrome and other metabolic syndromes [14].

Since it is a beverage that is available for sale, both in supermarkets and in stores of natural products, it is of paramount importance to make a physical-chemical analysis of antioxidants, pH, sugars and organic compounds present in Kombucha, thus allowing it to be possible to identify whether the product in question has a significant nutritional value [1].

Thus, the objective of this work was to evaluate the physical-chemical parameters such as moisture, ash, pH, glucose reducing sugar and non-reducing sugar in sucrose, ascorbic acid and galates in strawberry-based Kombuchas samples, commercialized in the municipality of Governador Valadares- Minas Gerais.

Material and Methods

Location and search period

The research was carried out at the Center for Health Sciences at Vale do Rio Doce University (UNIVALE), located in the city of Governador Valadares-MG, in the Pharmacognesia Laboratory. Kombuchas were collected and analyzed from September to October 2020.

Acquisition of raw materials

A survey was made of the available brands of Kombucha for sale in the trade of Governador Valadares, and later was punctuated all the flavors that each brand contained. From the list of flavors that each brand contained, a flavor was chosen that all presented in common. The base flavor chosen was strawberry.

Four types of different commercial trademarks of Kombuchas were collected, acquired in the shops of the Municipality of Governador Valadares-MG, using the consumer's availability criteria to find these Kombuchas. First, a survey was made in the hypermarkets of the city, and later in the stores of natural products. These samples were transported in ice coolers to the laboratory and stored in the refrigerator ($\pm 7^{\circ}\text{C}$) for possible analysis.

Physicochemical analyses

The physicochemical analyses were performed according to the methodologies of the Adolfo Lutz Institute [15] for pH, moisture, ash, glucose reducing glycidides and non-reducing sucrose.

Humidity

The Kombuchas samples were weighed in an analytical crucible, and soon after, they were transported inside the desiccator, with silica gel to the greenhouse (Nova Ética, model 404 - 3D) where they stayed for a period of 24 hours in direct drying at 105°C until the constant weight was obtained [15].

Ashes

The incineration method was used in Muffle at 700°C for one hour, when the residue was obtained from the determination of moisture [15]. After the samples had been in the greenhouse for 24 hours, they were transported inside the desiccator with gel silica to the Muffle (Digimec, Model SHM), where they stayed for a period of 1 hour, at a temperature of 700°C. After this period, they were kept in the desiccator with gel silica until they were at room temperature. They were weighed on analytical scale scans to determine the ash value.

PH determination

The pH values were measured using a digital pHmeter (KASVI, model K39-0014PA). Being, to perform the evaluation, the equipment was calibrated with known pH solutions, according to the manufacturer’s instructions. Between the exchange of one sample to another, the probe was cleaned with distilled water so that there was no change in the pH value [15].

Reducing glycidia in glucose and non-reducing glycidies in sucrose

The methods of glucose and non-reducing glycidies in sucrose were used to determine the sugars, according to fehling methodology, and the result was expressed in grams of glucose and sucrose per 100 g of the sample [15]. The samples were weighed in a beaker and subsequently supplemented with distilled water up to the 100ml mark. The ones in need were filtered and then transferred to a burette. In another beaker, a solution containing Fehling A and Fehling B was made, and 40ml of distilled water was supplemented. Soon after, they were taken to the heating plate to boil and titration. The faster the titration, the higher the content of sugars present, and the longer it takes, the lower the sugar content.

Antioxidant compounds

Ascorbic acid

Ascorbic acid was identified by weighing a quantity of the sample in a beaker and adding sodium bicarbonate and ferrous sulfa-

te. This reaction made the sample go from a rosy color, which is characteristic of the analyzed strawberry flavor, to a dark brown color [15]. The solution that had the presence of the antioxidant presented a transparent coloration after the addition of sulfuric acid at 10%.

Galatos

The gallates were identified from the extraction of these with 72% alcohol in a separation funnel, and later a reaction with ammonium hydroxide was made. The solution that had the presence of gallates, should present a rosy coloration [15].

Data analysis

With the aid of software R [16], the data were tabulated to variance analysis (ANOVA) for repeated measurements, and thus verify the homogeneity of the means. The means that were homogeneous (p>0.05) were submitted to duncan’s test. The p values were considered significant when less than 0.05. Four treatments were formulated, one for each kombucha sample (A, B, C and D). The analyses were performed in triplicates.

Results

The analysis of variance shows that all analyses showed significant differences between kombuchas samples (Table 1).

| Sources of variation | GL | Middle Square | | | | |
|----------------------|----|---------------|--------------|-----------|-----------------------------|---------------------------------|
| | | pH | Humidity (%) | Ashes (%) | Reducing sugars (% glucose) | Sugars not reducers (% sucrose) |
| Treatment | 3 | 0,22* | 49,36* | 46,11* | 24,99* | 274,68* |
| Residue | 8 | 0,005 | 0,13 | 0,07 | 0,16 | 1,93 |
| CV (%) | | 2,26 | 0,38 | 4,39 | 10,90 | 11,54 |

Table 1: Summary of variance analysis, by mean square, referring to pH, moisture, ash, glycidies in glucose and sucrose of different kombucha brands.

Source: Search data.

CV= Coefficient of Variation; GL= Degree of Freedom; ns= Not Significant, *=Significant at p < 0.0001.

As shown in figure 1, the samples showed significant differences for moisture and ash, differing from each other. Sample B (99.39%) presented a higher humidity value than the other patients. Sample C (89.82%) presented lower moisture value compared to other samples. Regarding the ashes, the C sample (10.16%) presented higher values and the B sample (0.90%) lower values.

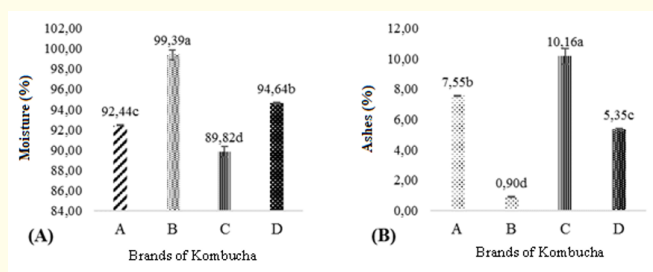


Figure 1: Average values (\pm EP) values of moisture (A) and ash (B) of different kombucha brands.

Source: Search data.

^{a,b,c} Averages followed by different lowercase letters differ according to the Duncan test ($P < 0.05$).

Figure 2 shows the mean pH values of the samples. Samples B and C presented similar pH values, and higher than samples A and D.

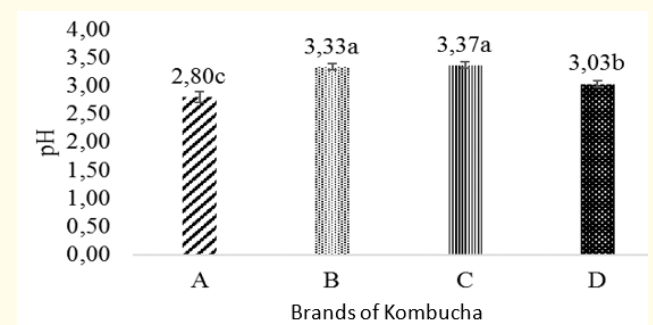


Figure 2: Mean pH (\pm EP) values of different Kombucha brands.

Source: Search data.

^{a,b,c} Averages followed by different lowercase letters differ according to the Duncan test ($P < 0.05$).

The analysis of variance presented in Table 1 shows that there was a significant difference between the samples for the analysis of glucose reducing sugars and non-reducing sugars in sucrose (Figure 3). All samples differed significantly from each other in terms of reducing sugar content, with sample C (7.77%) presenting higher values, while sample A (1.23%) lower values. For non-reducing sugars, there was also a significant difference between all samples, with sample C (23.78%) presenting higher sucrose values and sample B (1.05%) lower values.

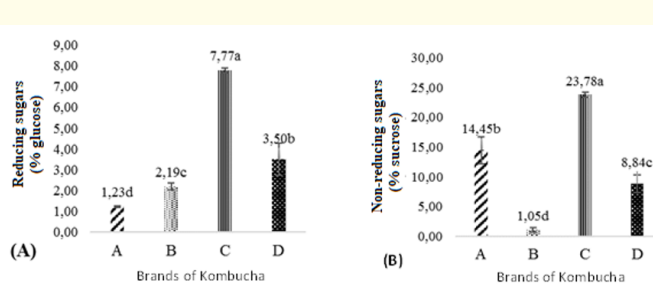


Figure 3: Mean values (\pm EP) of glycidic (A) and sucrose (B) of different brands of Kombuchas.

Source: Search data.

^{a,b,c} Averages followed by different lowercase letters differ according to the Duncan test ($P < 0.05$).

Table 2 presents the results for the antioxidant analyses of the four Kombuchas samples. It can be observed that none of the samples present the antioxidant gallates in their composition. However, all samples showed the antioxidant ascorbic acid.

| Antioxidant | Brand | Result |
|---------------|-------|---------|
| Ascorbic acid | A | Present |
| | B | Present |
| | C | Present |
| | D | Present |
| Galatos | A | Absent |
| | B | Absent |
| | C | Absent |
| | D | Absent |

Table 2: Presence of Antioxidants in Kombuchas samples.

Source: Search data.

Discussion

Humidity

Figure 2 (A) shows that all the analyzed brands showed significant differences in relation to the respective humidity values. Brand A (92.44%) presented a value closer to the D mark (94.64%) and the B mark (99.39%) presented the highest humidity values.

According to Resolution 12 CNNPA 12 of 1978, the minimum humidity for green tea-based beverages is 28%. Therefore, the

humidity values of the Kombuchas analyzed were within the standards required by the current legislation [17].

Ashes

Ash refers to the content of inorganic residues in food or fixed mineral residue (sodium, potassium, magnesium, calcium, iron, phosphorus, copper, chloride, aluminum, zinc, manganese and other mineral compounds) remaining from the burning of organic matter in muffle at high temperatures [18].

No indications were found in the legislation regarding the ash content for fermented beverages, but the results indicate that the B mark (0.90%) contains the lowest ash content and the c mark (10.16%) obtained the highest ash value. One of the reasons for the high ash content in the C mark is the fact that the fresh fruit was used, as shown in the ingredients on the drink label and the other brands used the fruit juice, thus presenting less residues.

The ash content in the beverage is caused by the presence of tea and the addition of the fruit in order to flavor and improve the palatability of the product. According to the data from the table of chemical composition of the food, the ash content found in the strawberry is 0.4g per 100g [19,20].

Another possibility would be due to a possible contamination of the products by impurities of inorganic origin, which may result from the production process or even contamination stemming from the fact that the strawberry is grown outdoors, being exposed to dust particles, leaves and insects [21].

pH

pH is one of the most important parameters affecting kombucha's fermentative microenvironment. Some acids formed during the process, such as acetic acid and glyconic acid are responsible for some of the biological properties and antimicrobial pharmacological activities against *Staphylococcus aureus*, *Salmonella* sp, *E. coli*, *Streptococcus* sp, in addition to inhibiting the growth of molds and yeasts [22].

According to authors [22], the pH should not be below 3.0 so that the drink can be ingested without causing damage to the gastrointestinal tract. The ideal pH for the beverage to have good palatability and biological properties is between 4.0 and 5.0. The period for obtaining this pH is mainly dependent on the ambient

temperature and the fermentation conditions of the culture medium [22].

According to legislation [23], the ideal pH for Kombuchas is at least 2.5 and maximum 4.2. Thus, as shown in the results, the four brands (A, B, C and D) obtained pH values considered ideal for fermented beverages such as Kombucha, by legislation. Thus, the results showed that the pH of brand A presented a value of 2.80, being the only brand to obtain a value lower than the ideal pH so that the beverage is ingested without causing damage to the gastrointestinal tract. Samples B, C and D obtained a pH value above 3.0, thus being considered the ideal drink for consumption [21]. The C brand (3.37%) was kombucha that presented the highest pH value becoming close to ideal [21]. Kombuchas presented low pH values due to the fermentation process, temperature, preparation time [7].

Glucose reducing glycidies

It can be observed that sample C presented higher content of glycidies (7.77%) glucose reducers when compared to the other samples. This may be due to a greater addition of the sugar used for its production. There is not yet a following parameter of reducing sugars for Kombucha, therefore the present work obtained the result between 1.23 g/100 mL and 7.77 g/100 mL.

According to the research carried in 2018 [24], the values of reducing sugars in Kombuchas ranged from 0.28 g/100 mL to 3.17 g/100 mL. A difference was identified in the values obtained, compared to the value of reducing sugars in Kombucha C (7.77%), which presented a higher amount of these sugars. The lack of legislation indicating minimum or maximum values allowed makes it difficult to compare.

Non-reducing glycidia in sucrose

It was observed that the samples showed a significant difference regarding non-reducing sugars. Kombucha C (23.78%) has a higher content of non-reducing glycidies in sucrose compared to other samples. The kombuchas labeling did not inform the quantity of nutrients present in three samples (A, C and D), i.e. only cited the ingredients used in their preparation, not presenting nutritional information. According to research of Richter and Lannes [25] due to sucrose being an ingredient that makes the products tastier, it is incorporated in larger quantities in food, to make them more

attractive. As Kombuchas have more acidic flavors, the incorporation of sucrose causes this flavor to be masked.

Sample B, in turn, presented the lowest level of sucrose, showing that it was less used in the preparation of Kombucha, being a healthier option.

Antioxidants

Ascorbic acid (vitamin C) is a water-soluble vitamin, of great importance for collagen synthesis and tissue repair. It also plays a significant role in the metabolism of tyrosine, carbohydrates, iron, in the conversion of folic acid into folinic acid, in the synthesis of lipids and proteins, resistance to infections and in cell respiration. In addition to supporting the immune system, contributing to antioxidant properties, which helps neutralize free radicals in cells [26]. The recommended dose of ascorbic acid is given by DRI (Dietary Reference Intakes), which recommends the recommendation of 90 mg for men and 75 mg for adult women [27].

Samples A, B, C and D present the presence of ascorbic acid due to the use of strawberry, a fruit that is rich in vitamins C, A, E, B5, B6, and minerals such as calcium, potassium, iron, selenium and magnesium [28].

For gallates, the absence of this antioxidant was found in all samples. According to some authors [29,30], one of the explanations for this absence is the preparation mode, the fermentation period and the low concentration of strawberry, a raw material used in order to aid in fermentation and provide flavor to the drink.

Another possible explanation of gives by the non-use of the fruit but, industrialized strawberry juice, which can also explain the light red presented in the drinks. In the strawberry is anthocyanins, composed of great concentration and responsible for characterizing the red color. This compound is highly sensitive and can be affected by the processing and fermentation method, which can alter the chemical and sensory physical characteristics [29-31].

Conclusion and Final Considerations

Kombucha is gaining more and more space in the Brazilian market for adapting to the interests related to the healthiness that are on the rise. All Kombuchas analyzed in the study presented the presence of the antioxidant ascorbic acid, a small amount of reducing and non-reducing sugars, besides the pH being in accordance

with the legislation, the Kombuchas presented satisfactory results for consumption.

According to the results obtained, sample B obtained better results, with a lower percentage of sugar compared to the other samples, thus being a healthier option for consumption. Only one brand presented nutritional information on the label, so it is important to highlight the need to add nutritional information to food labels, since they inform all nutrients and their quantities, which helps in the act of consumer choice to a given food.

Another important factor is the need to add more information in current legislation, such as the physical-chemical and sensory characteristics of the product.

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