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Evaluation of soy milk quality and integrated analysis of physicochemical and mineral properties of soybean TGx 1904-6F Zamboane

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ABSTRACT

Soybeans are recognized for their high nutritional value being rich in proteins, fats, carbohydrates, vitamins and minerals. This study evaluated the physicochemical and mineral properties of the grains of the TGx Zamboane 1904-6F variety grown in the province of Zambezia, Mozambique, and the quality of the soy milk produced from these grains. The analysis of the grains revealed 34.7% protein, 21.3% fat, 29.3% carbohydrates, 4.85% ash and 9.3% moisture, in addition to minerals such as 0.52 mg phosphorus, 16.02 mg iron, 1,671.86 mg potassium and 67.16 mg sodium, with no calcium. Sensory tests of soy milk showed an unsatisfactory level of acceptance, with average scores of 4.69 ± 1.36 for aroma, 4.48 ± 1.5 for color and 3.94 ± 1.68 for taste, suggesting the need to add ingredients such as fruit and sweeteners to improve palatability. Physico-chemical analyses of the milk showed 2.39 ± 0.18% protein, 1.1 ± 0.14% fat, 1.88 ± 0.22% carbohydrates, 0.28 ± 0.35 ash and 94.3 ± 0.06% moisture. The microbiological quality was satisfactory, without the presence of pathogens such as *Staphylococcus aureus*, *Salmonella* and Coliforms. It is concluded that the Zamboane TGx 1904-6F variety has favorable characteristics and represents a viable alternative for human consumption, contributing to the diversification of the vegetable milk supply and serving as an affordable source of protein in resource-limited communities.

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1. Introduction

Soybean (*Glycine max*), originally from the East, is one of the most cultivated legumes in the world, standing out for its high nutritional and functional value (1, 2). With an average composition of approximately 40% protein, 20% lipids (oil), 5% minerals and 34% carbohydrates, soy is widely used in human and animal nutrition, in addition to having several applications in industry (3, 4). World soybean production grows annually at a rate of 4.68%, with four countries

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dominating 90% of production: the United States, Brazil, Argentina and China (2, 5). In Mozambique, soy is an emerging crop, especially in Alta Zambezia, where it stands out as a viable alternative for food and nutritional security and income generation for local farmers. The preservation of soybeans and the type of processing applied have a direct impact on the physical and chemical properties of the grain, which affects the quality of the final product (6). Soy is also recognized for its role in the prevention of diseases such as diabetes and certain types of cancer, due to its high fibre content and phytochemicals such as isoflavones (7). This nutritional versatility makes soy an important raw material in the production of various foods, including soy milk, which stands out as a healthy and affordable alternative (8, 9).

Soy milk is widely consumed in Asia because of its protein quality and because it is free of lactose, cholesterol and gluten (10, 11). It is becoming a valuable nutritional alternative for lactose-intolerant children, contributing to the prevention of child malnutrition and replacing cow's milk in cases of insufficient production (12, 9). However, its consumption can be limited by undesirable flavors such as bitterness and rancidity caused by the enzyme lipoxygenase. To overcome this problem, appropriate heat treatments and genetic improvement of varieties are needed.

In addition, the addition of flavorings or fruits, as well as methods such as peeling, hot water crushing and maceration, have been shown to be effective in eliminating these unwanted flavors (13). Therefore, in order to increase the acceptance of soy milk, it is essential to carry out an adequate heat treatment and, if necessary, to add flavorings to make the product more palatable (14, 9, 13).

Given the need to improve nutritional quality in developing countries, soy is a promising alternative as a rich source of plant protein (9). This study focuses on the characterization of TGx 1904-6F soybeans grown in the province of Zambézia, Mozambique, and the evaluation of the quality of soy milk produced from these grains. By analyzing the physicochemical and mineral properties of the soya beans and the quality of the soya milk obtained, the research will help to understand the potential of this crop in the region and explore its nutritional applications.

2. Materials and Methods

2.1. Sample collection

Soybeans of the TGx 1904-6F Zamboane variety purchased in the province of Zambézia were used in this study. A total of 10 kg of grain was supplied by a reputable company in the soybean production sector. This quantity was used both for the evaluation of the grains and for the production of soya milk, allowing a comprehensive analysis of the characteristics and applications of this specific variety.

2.2. Study location

The study was conducted at two distinct facilities. Soy milk extraction and sensory evaluation were performed in the Food Hygiene and Technology Laboratory of the Faculty of Veterinary at Eduardo Mondlane University. Subsequently, chemical and microbiological analyses were carried out at the National Laboratory of Hygiene, Food and Water.

2.3. Sample preparation

The soybean grain samples were prepared following standardized procedures to ensure analytical accuracy. Initially, the grains were cleaned and milled using a hammer mill to obtain a homogeneous fine flour suitable for subsequent analyses. The ground samples were then stored in sealed plastic bags at room temperature to maintain their integrity prior to testing. Physical, chemical and mineralogical analyses were performed to evaluate the overall quality of the soybeans.

2.4. Determination of physicochemical properties of grains

The soybeans were analyzed for their physicochemical properties, including protein, fat, ash, moisture and carbohydrate content. These analyses were performed in triplicate, following the official methods of the Association of Official Analytical Chemists (15, 16), to ensure result accuracy.

Aliquots of the ground samples were used to determine protein, lipid, moisture and ash levels. For mineral analysis (calcium, iron, phosphorus, potassium and sodium), a new sub-sample was incinerated to obtain ash suitable for quantification.

2.4.1. Protein

Protein content was determined using the micro-Kjeldahl method, which involves quantifying the total nitrogen (N) present in the sample. For the analysis, 10 g of the sample were transferred to a Kjeldahl flask a long with a catalytic mixture containing concentrated sulfuric acid (H_2SO_4) and 30% hydrogen peroxide (H_2O_2) to facilitate mineralization. The mixture was then heated for 30 min.

During the distillation phase, the released ammonia was collected in a 4% hydrochloric acid (HCl) solution. The nitrogen content was subsequently determined by titration with 0.05 N hydrochloric acid. Total protein content was calculated by multiplying the nitrogen value by a conversion factor of 6.25, as recommended by the AOAC (15). Results were expressed as percentages (%) and rounded to one decimal place using the formula below.

$$\% \ N_2 = \frac{\text{N x 1.4 x VHCl x F}}{\text{m}}$$

N= normality of HCl, V= volume of HCl used in the titration, F= conversion factor and m= sample taken.

2.4.2. Fat

Fat content was determined by the Soxhlet extraction method, following AOAC (15). The procedure consisted of accurately weighing approximately 5 g of the sample on an analytical balance using a cellulose cartridge which was then covered with a layer of previously degreased cotton. Petroleum ether was then added to a flat-bottomed flask which had previously been dried in an oven for 30 min, cooled in a desiccator for the same period and tared.

The Soxhlet apparatus was set up and the fat was extracted by heating in a water bath for 8 h. After extraction, the light petroleum was evaporated in a rotary evaporator at a temperature ≤ 100°C. The flask containing the fat was then dried in an oven at 105°C for 1 h, cooled in a desiccator and weighed. This procedure was repeated, with the flask dried again in an oven at 105°C for 1 h before being cooled in the desiccator and weighed to a constant weight.

The results obtained were expressed as percentages (%), rounded to one decimal place, using the formula given below.

% Fat= $\frac{m2-m1}{m} \times 100$

m= mass of the sample taken for analysis, m_1= weight of the empty balloon and m_2= weight of the balloon with fat.

2.4.3. Ash

The ash content was determined by burning the sample in a muffle furnace. Approximately 3 g of the sample, previously crushed into fragments of approximately 1 mm, was accurately weighed into a porcelain crucible which had been placed in a muffle furnace at 550± 2°C for 1 h for heating and taring.

The sample was carefully carbonized with a Bunsen burner and then the crucible was transferred to a muffle furnace at 550°C until white ash was obtained. This process took approximately 4 h.

% Ashes = $\frac{m2-m}{m1-m} \times 100$

m= weight of crucible, m_1= weight of crucible with sample taken for analysis and m_2= weight of crucible with ash.

2.4.4. Humidity

Moisture was determined by the gravimetric method using heat. Approximately 5 g of the sample, crushed into 1 mm fragments, was accurately weighed in a filter balance that had been previously dried and tared. The sample was then placed in an oven at 105°C for 2 h. After this time, the sample was transfer to a desiccator and allowed to cool before being weighed to a constant weight, according to AOAC guidelines (16). Moisture results were calculated using the formula below.

% Humidity = $\frac{m-m1}{m} \times 100$,

Where: m= mass in grams of the sample taken for analysis and m_1= mass of the sample after drying.

2.4.5. Carbohydrate

The carbohydrate content was determined as the difference between 100 and the sum of the percentages of the solid components (protein, fat and ash) plus the percentage of moisture.

% Carbohydrates = 100− S% of solid matter (protein, fat and ash) +% moisture.

2.4.6. Mineral analysis

Mineral analysis was performed on a subsample previously ashed in a muffle furnace at 550°C for 3 h. The concentrations of potassium (K), iron (Fe), sodium (Na), and calcium (Ca) were determined by atomic absorption spectrophotometry, with all measurements conducted in triplicate.

2.5. Soy milk extraction

A total of 9.0 kg of soybean grains was used for the extraction of soy milk (soy extract). The extraction process was conducted in the Food Hygiene and Technology Laboratory of the Faculty of Veterinary at Eduardo Mondlane University.

Initially, the grains were selected, weighed, and washed in potable water. Heat treatment was applied by immersing the grains in 10 L of water at 95°C for 30 min to inactivate the lipoxygenase, an enzyme associated with undesirable beany flavors. Subsequently, the grains were rinsed with cold water, drained using a plastic sieve, and manually dehulled to avoid off-flavors during extraction.

The warm, dehulled grains were blended with water at 95°C in a 1:10 (w/v) soy-water ratio for 3 min. The slurry was then filtered using a plastic sieve with multilayered gauze to separate the liquid extract from residual solids. Sixteen extractions were performed, based on blender capacity and volume requirements. The extracts were pooled into a single container to ensure sample homogeneity.

The combined extract was divided into three replicates and transferred into sterile 2 L glass bottles for sensory, microbiological, and physicochemical analyses. Throughout processing, strict hygiene and good manufacturing practices were followed.

Following homogenization, the soy milk was pasteurized at 98°C for 5 min in a stainless-steel vessel under constant stirring. It was then cooled, bottled in sterile containers, and stored at 5°C to until analysis to maintain microbial and enzymatic stability.

Fig. 1 shows a schematic of the soy milk extraction process. Extraction was performed using a 1.75 L blender at a fixed soy-to-water ratio of 1:10 (100 g soy per 1 L water), as recommended (17).

2.6. Sensory analysis

The sensory analysis of soy milk was carried out from an acceptance test through the hedonic scale of 1 (I disliked very much) to 7 (I liked much) points, for the evaluation of taste, color and aroma (18). The sensory team was composed of 18 trained examiners, consisting of individuals identified in the market, staff and graduate students of the Faculty of Veterinary Medicine including the Masters in Food Safety and Biotechnology students, respectively. The panel evaluated the sample using the specified sheet. The samples were served in disposable glasses of 20 mL, at cooling temperature (5°C) sequentially, in individual cabins.

2.7. Microbiological analyses

The microbiological analysis was conducted according to the method established by the International Commission on Microbiological Specifications for Foods (19), at the National Laboratory of Food, Water and Hygiene. The parameters evaluated were: *Salmonella* spp., *Staphylococcus aureus*, and coliforms, using the Most Probable Number (MPN) method.

2.7.1. *Salmonella* sp research

Salmonella research was carried out by aseptically measuring 25 mL of soy milk to 1000 mL sterile balloons containing 225 mL lactose broth. Shake and incubate at 37°C/24 h (pre-enrichment). Subsequently, 1 mL homogenized aliquots were transferred to a tube containing 10 mL of tetrathionate broth and 1 mL to a tubing containing 10 mL selenite broth. The tetrathionate broth was then incubated at 37°C/24h and the cystine-selenite broth at 42.5°C/24 h, respectively. After the incubation period of the same, strips were made with the help of sterilized ansa in the selective means of bright green agar (BGA) and *Salmonella-Shigella* agar (SS), whose plates were incubated inverted at 35°C/ 24 h. The results were then read out.

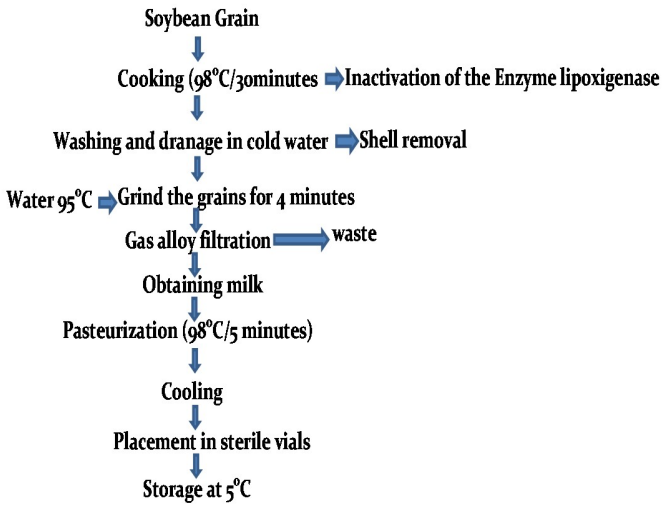

Figure 1. Process flowchart of soy milk obtain

2.7.2. Research of *Staphylococcus aureus*

The research for *Staphylococcus aureus* was carried out by aseptically weighing 10 mL of milk in 1000 mL sterile balloons containing 90 mL peptonated water. Then homogenized in the vortex agitator (initial dilution 10^{-1}). From the 10^{-1} mother dilution, series dilutions were prepared up to 10^{-2}. From each dilution, 0.1 mL aliquots were transferred to the surface of plates containing 25 mL of solidified and dry Agar Baird-Parker. The plates were prepared in duplicate. Afterwards, the plates were incubated inverted for 48 h in the greenhouse at 37°C. After this period the results were read out.

2.7.3. Coliform search by most likely probable number (MPN) method

The determination of coliforms was done by aseptically weighing 10 mL of milk and adding 90 mL of 1% peptoned water in the balloon in order to obtain the initial dilution (10^{-1}), which was homogenized by the vortex agitator. Subsequently, 1 mL of this dilution was placed in a tube containing 9 mL 0.1% peptone solution, obtaining the 10^{-2} dilution and this procedure was repeated to obtain the 10^{-3}. For the determinations the method of multiple tubes, with three series of three tubes was used. From each dilution (10^{-1} to 10^{-3}) of the sample, three servings of 1 mL each were taken and inoculated, respectively, in three tubes with 10 mL Brilliant Green Bile Broth (BVB). The tubes were incubated at 37°C for 48 h. After this period the results were read out.

2.7.4. Physicochemical analysis of soymilk

The physicochemical evaluation of soy milk was carried out at the National Laboratory of Hygiene, Water and Food (LNHAA), as recommended by AOAC International. In these analyses, the following components were determined: fat, protein, moisture, ash, carbohydrates, pH and total acidity.

The moisture content was determined by evaporation method (240°C, 2 h) in sea bath, until removal of humidity, followed by drying in the greenhouse at 105°C for 2 h, up to constant weight (16). The protein content was determined by Kjeldahl's method for the quantification of total nitrogen (N). The fat content was determined by Mojonnier's method (20). The carbohydrates were obtained by the difference between 100 and the sum of the other solid constituents (protein, fat and ash) + % of moisture.

Acidity

The acidity of the sample was determined by adding 10 mL of the sample to a 200 mL beaker containing 100 mL of distilled water, followed by the addition of 1 mL of phenolphthalein. The solution was stirred while being titrated with a 0.1 N sodium hydroxide (NaOH)

solution. The titration was continued until the color of the solution changed from colorless to pink, indicating the end point of the titration. The volume of NaOH used was recorded during the experiment. To calculate the total acidity in terms of lactic acid, the following formula was used % of lactic acid = volume of NaOH consumed x 0.09.

pH

The pH of the milk sample was measured directly using a digital pH meter (WTW pH-720 series). The meter was first calibrated with pH 4 and 7 solutions. The milk sample was placed in the beaker and adjusted to room temperature. The pH meter's electrode was immersed in the milk sample, and the reading was recorded after the pH meter stabilized.

2.8. Statistical analysis

To organize and validate the experimental data from the sensory analyses, the data were subjected to analysis of variance (ANOVA) followed by comparison of means using the Tukey test at a 5% level of statistical significance (p>0.05). For the physicochemical properties, the data were analyzed using means and standard deviations. The evaluation of the parameters studied was carried out by comparing means and standard deviations, which allowed a more rigorous analysis of the data.

3. Results

The physicochemical properties were analyzed using means and standard deviations, which allowed a rigorous evaluation of the data. The sensory data were then subjected to an analysis of variance (ANOVA), followed by a comparison of means using the Tukey test, with a statistical significance level of 5% (p>0.05). The comparison of means and standard deviations allowed a detailed analysis of the parameters studied.

3.1. Physicochemical properties of soybeans

The centesimal composition of soy beans (Zamboane TGx 1904 - 6F) used in the production of soy milk (water-soluble soy extract) is shown in Table 1.

Table 1. Average composition of soybeans, dry matter (g/100 g).

Compostional parameter	Mean values
Protein	34.7
Fat	21.8
Ash contente	4.85
Moisture	9.32
Carbohydrate	29.3

3.2. Mineral analysis

Table 2 shows the mineral content found in Zamboane TGx 1904-6F soybeans.

Table 2. Mineral content

Parameters (mg/100g)	Value found
Phosphorus	520
Iron	16.020
Potassium	1.671,86
Sodium	6.716
Calcium	nd

*nd: not detected

The results of the mineral content analysis of the soybean sample indicate a notably high concentration of potassium, reaching 1,671.86 mg/100 g. Iron also showed a considerable level at 16.02 mg/100 g, highlighting the sample as a relevant source of this mineral. Calcium was not detected in the analyses.

3.3. Sensory analysis of soy milk

Our study demonstrated that there is no significant difference (p>0.05) regarding the responses attributed by the evaluation panel for each analyzed parameter.

The averages and default deviation of the values found in the assessment of the three attributes are expressed in Table 3.

Table 3. Averages and Default deviation

Attributes	Averages and default deviation
Aroma	4.69 ± 1.36
Color	4.48 ± 1.55
Flavor	3.94 ± 1.68

3.4. Microbiological analysis of soy milk

The microbiological results, demonstrate the absence of pathogenic microorganisms and quality indicators in all tested samples. None of the analyses revealed the presence of total coliforms, *Staphylococcus aureus* or *Salmonella*. The microbiological data are presented in Table 4.

3.5. Physicochemical analyses of soy milk

The results of the physicochemical composition of soy milk (water soluble soy extract) are given in Table 5.

Mean value of pH of soy milk was 7.2 (Table 5). It is direct influence on the flavor perception in the dairy products. Product pH is influenced by biochemical changes and its composition.

Acidity

Means for acidity of soy milk was 0.04%. The result pertaining to mean value given in Table 5.

Table 4. Result of microbiological analyses.

Parameters	Analysis 1	Analysis 2	Analysis 3
Total coliforms (MPN/mL)	0	0	0
Stapylococcus aureus	Absence	Absence	Absence
Salmonella (Absence/ 25 mL)	Absence	Absence	Absence

Table 5. Mean values of physicochemical analysis of soy milk.

Compositional parameter	Mean values ±SD
Protein	2.39±0.18
Fat	1.1±0.14
Ash	0.28±0.35
Moisture	94.3±0.06
Carbohydrate	1.88±0.22
pH	7.2
Acidez	0.04%

pH

Mean value of pH of soy milk was 7.2 (Table 5). It is direct influence on the flavor perception in the dairy products. Product pH is influenced by biochemical changes and its composition.

Acidity

Means for acidity of soy milk was 0.04%. The result pertaining to mean value given in Table 5.

4. Discussion

4.1. Physicochemical properties of soybeans

The analysis of the centesimal composition of Zamboane TGx 1904-6F soybeans (Table 1) revealed a nutritional profile with significant levels of protein, fat and carbohydrates, highlighting a nutritional its potential as a valuable source of nutrients for the formulation of nutritional products, especially in soy milk processing. The protein content (34.7 g/100 g) reflects the high-quality protein characteristics of soybeans, known for its complete amino acid profile. The fat concentration (21.8 /100 g) underscores the energy contribution of the grain and its role in providing caloric value to the final product. Additionally, the carbohydrate content (29.3 /100 g) supplies a readily available source of energy. The mineral content, as indicated by the ash value (4.85 /100 g), suggests the presence of essential minerals that contribute to the grain's nutritional value.

The composition of a food is essential to determine its nutritional quality and its possible uses in the diet. Knowing the composition of the source material helps guide manufacturing processes, such as the production of soy milk, and allows comparisons with other varieties. It is important to evaluate how our results relate to or differ from similar studies, taking into account genetic, environmental and agricultural management factors.

The recorded protein content was 34.7 g, within the range observed in previous studies. This value is slightly lower than that found in study (21), which used the variety BRS 213, and also lower than that reported in studies (22) and (23), and which analyzed the variety BRS 284, obtaining 37.03 g, 37.3%, and 38.3 g, respectively. On the other hand, the protein content found in this study is significantly higher than the 33.29 g reported in study (17), which evaluated common soybean (SC), but much lower than the 46.8 g found in study (23) for the variety BRS 216.

Variations in protein content can be attributed to differences in genetic material, geographic origin, climatic conditions, processing methods, and analytical techniques used.

The fat content (21.8 g) was higher than the values reported by Brunelli et al. (21), who found 18.79 g; (17), with 15.57 g; and (9), who recorded 19.87 g. The obtained fat values are similar to those reported by Bowles et al. (22), which was 21.2 g, but lower than the range of 22.2 g to 28 g reported by Felberg et al. (23).

The fat content was 21.8 g. This value differs from the results obtained by Felberg et al. (23), who observed values ranging from 22.2 g to 28 g. These differences in fat content can be attributed to the cultivation conditions of the soybean, which influence the final lipid content, as well as the specific composition of the samples analyzed.

The observed ash content was 4.85 g, higher than the 4.57 g reported in another study (21), representing an increase of 6.14%. Compared to the 4.7 g documented (22), the increase was 3.19%. Additionally, the measured value significantly exceeded the 3.64 g reported by Ciabotti et al. (17), yet remained below the 5.2 g and 5.7 g reported by Felberg et al. (23). It is important to note that ash, which reflects the mineral

fraction in the food, can vary depending on soil mineral content and the analytical techniques employed.

The moisture content was 9.32 g, a value lower than those reported by Felberg et al. (23) as 9.2 g and 9.0 g, (21) as (9.87 g), and (17) as (9.59 g). The study (22) did not measure moisture content. Variations in moisture may may be attributed to differences in storage, processing conditions, and environmental factors at the time of sampling.

The carbohydrate content was 29.3 g, aligning closely with the 29.76 g reported by Brunelli et al. (21) and the 28.8 g found by Bowles and Demiat (22). Differences in carbohydrate levels can be associated with soybean variety and the analytical methods used.

The proximate composition results of the studied variety are consistent with data for common soybean varieties (SC), as well as BRS 213, BRS 216 and BRS 284. Although slight variations were observed, these differences are likely due to factors such as soybean genotype, environmental conditions during cultivation, and analytical procedures. Overall, the data suggest that Zamboane TGx 1904-6F possesses promising nutritional properties, indicating its potential for future cultivations and relevance to soybean research.

4.2. Mineral analysis

The results of the present study show significant mineral content in soybeans, with phosphorus at 520 mg/100 g, iron at 16.020 mg/100 g, potassium at 1,671.86 mg/100g, and sodium at 1,671.86 mg/100 g. Calcium was not detected (ND).

In comparison with other studies, lower values reported in a study (17), which analyzed the BR 133 variety, with phosphorus (0.0664 mg/100 g), iron (7.2266 mg/100 g), potassium (0.1696 mg/100 g), and calcium (0.017 mg/100 g). In (4), phosphorus (580 mg/100 g), iron (9.4 mg/100 g), potassium (1900 mg/100 g), sodium (1 mg/100 g), and calcium (230 mg/100 g) were observed, showing similar or higher values for phosphorus and potassium, but considerably lower sodium content than in the present study.

In a study (24), mineral levels were much lower, with phosphorus (4.4735 mg/ 100 g), iron (0.0186 mg/100 g), potassium (0.28574 mg/100 g), sodium (0.11792 mg/100 g), and calcium (1.1342 mg/100 g). These differences may be attributed to distinct analytical methods, varying cultivation conditions, or genetic diversity among the soybean cultivars evaluated.

The absence of calcium in the sample analyzed may indicate a low concentration of this mineral, supporting (11), which highlights the naturally low calcium content in soybeans.

These variations observed in mineral content reinforce the influence of soybean variety, environmental conditions, agricultural practices, and analytical methodologies, factors that are crucial for nutritional evaluation and the industrial use of soybeans grains.

4.3. Sensory analysis of soy milk

The results of the sensory analysis of soy milk (Table 3) indicate that the evaluators' perception of aroma, color and flavor was uniform, suggesting a general acceptance of the product. The attributes were evaluated individually and compared to the results of other authors, which enriches the interpretation of the data. Although the attributes presented varied averages, the absence of significant differences indicates consistency in the sensory characteristics, which is positive for the marketing of soy milk. This

homogeneity may indicate that the product meets consumer's expectations regarding its quality and may be a viable alternative in the vegetable beverage market.

The flavor attribute showed a mean of 4.69 with a standard deviation of 1.36, indicating moderate acceptance. This suggests that the inclusion of fruit or other ingredients could increase the attractiveness of soy milk. In comparison, values between 6.6 and 7.1 were (25) when using EHS and blackberry juice, indicating that formulation affects sensory perception.

Higher values, between 5.29 and 6.67, were also obtained (26), confirming the importance of combinations in acceptability. A mean of 7.9 was highlighted (27) when combining soy extract with passion fruit pulp and sugar, suggesting that intense flavors improve perception. Means from 6.44 to 6.58 were presented (21), suggesting that even in mixed formulations, flavor acceptance was not ideal, with other factors, such as the type of ingredient, possibly playing a more relevant role.

For the color attribute, the mean was 4.48, reflecting a mixed acceptance between "slightly liked" and "indifferent", with a standard deviation of 1.55, suggesting divergent opinions among evaluators. This variability is confirmed (18), were the ideal combination of 25% strawberry and 5% sugar had the highest acceptance of the water-soluble soy extract, while a mixture of 15% strawberry and 15% sugar was the least appreciated, highlighting the influence of formulation on color perception.

The taste attribute showed a mean of 3.94 and a standard deviation of 1.68, indicating lower-than-expected acceptance, ranging from "slightly disliked" to "indifferent", which is concerning, especially compared to aroma and color attributes. Studies report higher averages in beverages with added fruit and sugar (18, 21), suggesting that the lack of flavorings or sweeteners contributed to the negative taste perception in this study. Additionally, the combination of EHS with fruit juice did not negatively affect the flavor (25), emphasizing the importance of ingredients that can improve product acceptability.

4.4. Microbiological analysis of soy milk

According to the National Sanitary Surveillance Agency (28) and the International Commission on Microbiological Specifications for Food (19), soy milk must meet the following microbiological standards: no more than 10 coliforms per 45°C/mL; no more than $5x10^2$ Staphylococcus coagulase positive bacteria/mL; and absence of *Salmonella* spp in 25 mL. It should be noted that all analyses conducted in this study were within the required standards, as demonstrated by the results in Table 4.

The results obtained (Table 4) show that the total coliform count of the soy milk produced was below the established limits, confirming the observance of good hygiene and sanitary conditions during the milk extraction process.

In the same analysis, since no tubes were found to be positive for total coliforms, faecal coliforms were not detected.

In the case of *Salmonella*, there was no formation of colonies characteristic of this type of micro-organism,

which would make the milk unfit for consumption, thus indicating a product of safe and good microbiological quality.

Regarding the research on *Staphylococcus aureus*, no growth and no suspected colonies of this type of microorganism were detected in the samples analyzed, since in these cases less than 10 bacteria per gram (<10/g) were applied, but the sample is within the established microbiological limits.

Similar analyses were carried out in this work (29), evaluating the hygienic-sanitary quality of soy milk processing at UNISOJA through the study of E. coli and the determination of the most probable number (MPN) of total and faecal coliforms (45°C). Their results showed that total and faecal coliforms were present in 42% of the samples analyzed, but contamination of the final product with faecal coliforms was below the levels established Brazilian legislation (28). The same study did not detect *E. coli* in any of the 79 surface samples.

4.5. Physicochemical analyses of soy milk

When comparing our results (Table 5) with the existing literature, the protein content obtained, quantified at 2.39 ± 0.18, aligns well with the value reported in (29), which documented 2.81%. Conversely, our protein levels surpass those reported in (30) for the soybean varieties CD 206 and BR 232 which exhibited protein contents of 1.85% and 1.76%, respectively. Higher protein values recorded (8) and (21), at 3.18% and 3.40% respectively, underscore the influence of genetic heterogeneity, extraction techniques, and processing parameters on soybean protein composition. These variations likely reflect the combined effects of genetic diversity and environmental factors impacting protein synthesis and accumulation.

Regarding lipid content, the value determined in this study, approximately 1.1 ± 0.14, is notably lower than the 3.29% and 1.95% reported in (30) for the soybean varieties CD 206 and BR 232, respectively. Our lipid content closely approximates the 1.0% reported in (18). Values slightly higher were reported in (8) and (29), at 1.62% and 1.48%, respectively. The observed lipid variability can be attributed to intrinsic differences in soybean lipid profiles, modulated by cultivation practices, environmental conditions, and post-harvest handling procedures.

The ash content, measured 0.28 ± 0.35, is lower than the 1.0% found in (18) and the 1.02% ± 0.05% documented in (30) for the variety CD 206. Our findings are consistent with the 0.26% value reported in (29), suggesting similar mineral profiles. Since ash content reflects mineral constituents, its variation may be indicated differences in soil mineralization, fertilization regimes, and post-harvest processing methods. The moisture content of 94.3 ± 0.06 observed in this study exceeds the values reported in (30), which documented 87.85 ± 0.37 and 88.48 ± 0.09% for the respective soybean varieties. This elevated moisture level suggests incomplete dehydration during processing, which could compromise the stability and shelf life of the soy milk bay fostering microbial proliferation and spoilage.

The carbohydrate content, measured at 1.88±0.22, was significantly lower than the values reported (30) for varieties such as CDC and BR 232, which exhibited carbohydrate levels of 7.96±0.49 and 8.28±1.23, respectively. This disparity indicates substantial varietal differences in carbohydrate composition, likely driven by genetic factors, environmental conditions, and agronomic management. The lower carbohydrate

content aligns with the phenotypic profiles of the specific soybean varieties analyzed in this study.

Furthermore, the compositional analysis of soy milk derived from the BR-16 soybean variety, as evaluated (31), revealed a protein content of 2.86%, a fat content of 1.53%, and a moisture level of 9.3%. These data corroborate the general trend observed in the literature, wherein variations in the physicochemical properties of soy milk are attributable to factors such as raw material composition, cultivation conditions, climatic influences, and agricultural practices associated with different soybean genotypes.

It is important to consider that processing methodologies, including extraction procedures and pasteurization techniques, significantly influence nutrient bioavailability and concentrations in soy milk. Variability in analytical results across studies can thus be ascribed to differences in soybean characteristics and technological approaches used during production.

According to the Brazilian Technical Regulation for Vegetable Protein Products, specifically Resolution RDC no. 268 of 22 September (32), liquid water-soluble soy extract must contain a minimum of 3% protein. In our study, the measured protein content fell below this threshold, potentially due to the soy to water ratio 1:10. Increasing the protein concentration could be achieved by adjusting this ratio to 1:9 or 1:8, thereby enhancing the nutritional profile (33).

Resolution no. 14/1978 states that unflavored soy extracts should contain approximately 93% moistures, 0.6% ash, at least 3% crude protein (calculated as Nx6.25), and 1% lipids on a wet basis (34). The observed compositional values observed in this study are consistent with these specifications, considering permissible variation as reported (35). Overall, the results demonstrate compliance with established quality standards, confirming the product's adequacy within regulatory frameworks.

pH

In our study, the pH of soymilk was measured at 7.2, which is higher than the values reported (36), who documented a pH of 6.50 ± 1.50. Additionally, our pH values exceed those reported (30), which found pH values of 6.49 and 6.47 for the CD 206 and BR 232 varieties, respectively. A pH between 6.5 and 7 is considered ideal for soy milk consumption, providing a neutral and safe environment, as noted (33). This range is essential to preserve specific sensory characteristics and ensure microbiological safety, avoiding undesirable fermentation and texture changes.

Lower pH values were observed (37), which can be attributed to the exclusive use of soy in our study. In contrast, the addition of fruit juices in those studies contributed to increased acidity, resulting in a lower pH. Specifically, (37) reported pH values between 4.01 and 4.25 in apple-flavored beverages, while (38) highlighted that the inclusion of flavorings affects beverage properties. Furthermore, (30) emphasized that soybean variety also influences pH. Therefore, pH analysis is critical in formulating products that meet consumer expectations.

Total Acidity

Titratable acidity is a critical factor in soy milk quality, directly affecting its flavor and consumer acceptance. In this study, the titratable acidity was 0.04%, a value significantly lower than the 0.67% ± 0.12% reported in (36). This discrepancy highlights the differences in acidity levels across various studies and formulations. Similar values were reported in (38), particularly for the Ades Original® product and in (33), where acidity of 0.06% was observed. Conversely, higher acidity levels were documented in (21), (39) and (40), at 0.13%, 0.1%, and 0.46%, respectively. These variations may result from differences in extraction methods, formulations, and the absence of fruit juice, which can reduce acidity. Soy milks with lower acidity, such as that observed in the present study, are generally considered more palatable and microbiologically stable, potentially inhibiting the growth of spoilage organisms. Additional factors influencing acidity include harvesting conditions, grain quality, and storage practices, making the monitoring of this parameter essential for maintaining production standards (41- 43). Therefore, the 0.04% value obtained here is acceptable when contextualized within the specific processing and formulation conditions of this study.

5. Conclusion

The integrated analysis of the physicochemical and mineral properties of the Zamboane TGx 1904-6F soybeans variety reveals a high nutritional profile, with elevated protein and fat contents alongside considerable variability in mineral content, particularly phosphorus and iron. Despite these favorable attributes, the soy milk derived from this variety exhibits suboptimal protein levels relative to recommended standards and processes a high moistures content, indicating a need to optimize the soybean to water ratio to enhance product quality and shelf stability.

Microbiological assessments confirmed that the soy milk complies with established hygiene and safety standards, ensuring its microbiological safety. However, the sensory evaluation results indicated low consumer acceptability, underscoring the potential benefit of incorporating additional ingredients, such as fruits and sweeteners, to improve the taste, color, and overall perception of the soy milk produced from this soybean variety.

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The resources for conducting the research were provided by the company Lozane Farm, whose purpose was to establish a soy milk processing line. To this end, request that the study be carried out with the aim of evaluating the nutritional value of soybeans and soy milk, as well as understanding consumer perceptions of soy milk.

Author's contributions

Amade Dauto Ibramugy: Conducted the research, analyzed and interpreted the data, drafted the first version of the manuscript, and revised the different versions of the manuscript.

Eduarda Zandamela Mungói: Acquired funding for the research, oversaw the overall study, reviewed the microbiological data on soy milk, and reviewed subsequent versions of the manuscript.

José da Cruz Francisco: Reviewed the data on the chemical part, including the physicochemical composition of the soybeans and soy milk, and oversaw the study. He also contributed to the review of the different versions of the manuscript.

Declaration of competing interest

The authors declare that they do not have any financial or personal relationships with other people or organizations that could influence or compromise the impartiality of this work.

Data availability

The data used in this work are available upon request to the authors. There is no public database accessible to the general public at the time of submission. We guarantee transparency and access to the data for validation and reproducibility purposes.

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