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SCLEROCARYA BIRREA (MARULA) AND STRYCHNOS SPINOSA (MASSALA) MIXED JAM: PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION

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ABSTRACT: Sclerocarya birrea (marula) and Strychnos spinosa (massala) are fruits with high nutritional value but are underutilized. Processing jam is an alternative for diversifying consumption and preserving these fruits. This study aimed to produce and characterize the mixed jam (marula and massala pulp). Three mixed jam formulations were defined, and the best one was selected. Physicochemical and microbiological analyses were conducted. The microbial stability of the jam was assessed over 84 days of storage at room temperature. Results of physicochemical analyses of jam were pH: 3.44, acidity: 2.86%, vitamin C: 45.57 mg/100g, soluble solids: 67.71 °Brix, ash: 0.89%, fibers: 0.32%, lipids: 0.39%, moisture: 21%, protein: 0.92% and carbohydrates: 76.48%. Microbial growth in the jam was only observed on the 84th day, with low aerobic bacteria counts $(3\times10^2 \, \text{CFU/g})$, molds, and yeasts $(10 \, \text{CFU/g})$. The results showed that the raw materials were of good quality, and the mixed jam was safe.

KEYWORDS: Wild fruit, processing, conservation, quality, microbiological stability.

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INTRODUCTION

Fruit plays an important role in the human diet and is considered a source of essential nutrients, as it contributes to the supply of energy, minerals, vitamins, dietary fiber, water, and bioactive compounds (Chiau et al., 2013; Dos Santos et al., 2010). Mozambique has a variety of edible wild fruits, widely distributed throughout the country, with high socioeconomic and cultural value (Bila & Vaz, 2017; Mashau et al., 2022). These fruits contribute significantly to the diet of many families, particularly in rural areas, where staple foods are scarce (Magaia et al., 2013; Mbhele et al., 2024; Omotayo & Aremu, 2021). However, some of these wild fruits, such as *Sclerocarya birrea* and *Strychnos spinosa*, are underutilized, and little attention has been paid to their commercial potential (Ngadze et al., 2017; Omotayo & Aremu, 2021).

Sclerocarya birrea, commonly known as marula, belongs to the Anacardiaceae family and is an edible wild fruit that grows naturally in various regions of sub-Saharan Africa (Mashau et al., 2022), including Mozambique (Bila & Vaz, 2017; Kamanula et al., 2022; Magaia et al., 2013; Zingara & Tivana, 2022). This fruit has high nutritional value and bioactive compounds (Mashau et al., 2022); it can be eaten fresh or used as a raw material in food industries to produce oils (Bila & Vaz, 2017; Kamanula et al., 2022; Magaia et al., 2013), juices (Magaia et al., 2013; Zingara & Tivana, 2022), alcoholic drinks (Magaia et al., 2013), jelly and jam (Mashau et al., 2022).

Strychnos spinosa (massala) is from the Loganiaceae family, an edible wild fruit in southern Africa (Mbhele et al., 2022; Tittikpina et al., 2020). It can be eaten fresh or used in the food industry to make juices and alcoholic drinks such as liqueur (Mbhele et al., 2022, 2024; Ngadze et al., 2017). It has high nutritional value, contributing vitamin and micronutrient levels to the diet. It has therapeutic effects and is considered medicinal (Omotayo & Aremu, 2021; Tittikpina et al., 2020).

Although marula and massala have high nutritional and economic value, these fruits are seasonal, with harvesting taking place between January to March and October to January, respectively (Magaia et al., 2013; Ngadze et al., 2017); so, most of these fruits are not utilized due to the limited harvesting time and poor storage conditions. In addition, these fruits are highly perishable, which means their availability is limited. It is therefore important to develop new products to diversify and guarantee the consumption of these fruits in times of scarcity. An interesting and promising alternative for preserving these wild fruits and adding their nutritional value is to produce products based on the pulp of these fruits, such as jelly or jam (Ngadze et al., 2017).

Jam is a product obtained by cooking whole or pieces of fruit, fruit pulp or juice, sugar, and water, concentrated to a jelly-like consistency. It can be made with one type of fruit or a mixture of two or more fruits. It makes it possible to create new flavors (Torrezan, 1998). According to Bellini et al. (2021), the production of jam makes it possible to preserve fruit and consume it in the off-season.

Therefore, this study aimed to produce jam made from a mixture of marula and massala pulps, to promote the consumption, and availability of these fruits, and guarantee product diversification, as well as to assess the microbiological stability of the jam to gauge its quality during storage in a room temperature.

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MATERIAL AND METHODS

Production of Mixed Jam

Twenty-five kilograms (25 kg) of massala were bought in the Bobole locality in Maputo province and 25 kg of marula in the Licilo locality, Limpopo district in Gaza province. The raw material was transported in raffia bags to the Post-Harvest and Food Technology Laboratory of the Faculty of Agronomy and Forestry Engineering at Eduardo Mondlane University (FAEF-UEM). Other ingredients were bought at the Shoprite supermarket (Maputo City - Mozambique).

The experiment was conducted using a completely randomized design. Three jam formulations were defined with 60% sugar concerning the weight of the pulp, differing in the proportion of fruit pulp:

- F1 50% massala and 50% marula;
- F2 60% massala and 40% marula; and
- F3 40% massala and 60% marula.

The fruit was sorted and washed in running water, disinfected by immersion in the sodium hypochlorite solution (NaClO) at 150 ppm for 15 minutes, then washed with running water to remove excess disinfectant. The seeds were removed by hand using a knife and spoon. The obtained pulp was separated into two parts: (1) used for the physicochemical, microbiological, and approximate composition analyses; and (2) frozen in the domestic freezer at approximately -18°C for 17 days until use.

The jam was processed according to the methodology proposed by Torrezan (1998), with some modifications. Briefly, the pulp was thawed in refrigeration (in the fridge at approximately 4°C for 24 hours), weighed, and mixed with sugar (previously weighed) for cooking for approximately 30 min until it reached 67.5 °Brix. The jam was then packed into a glass bottle previously disinfected with a 1% sodium hypochlorite solution (NaClO) and washed with boiling water. Once the bottles were closed, they were inverted and returned to their original position after 5 minutes, cooled in a basin of cold water (with about ¾ of the water), and then stored at room temperature.

Characterization of the Mixed Jam

The research group evaluated the three prepared formulations and chose the formulation with the best texture, reproduced and characterized in three repetitions of the experiment, making up three experimental plots.

The characterization of the mixed jam was done through physicochemical analyses and approximate composition, in all the repetitions of the experiment. All the analyses were carried out in triplicate.

Physico-chemical Analysis and Approximate Composition

Physico-chemical analyses were performed following the procedures described by the Adolfo Lutz Institute (2008). The pH was measured in a digital potentiometer (HANNA, Romania).

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Soluble solids in 'Brix were obtained using a digital refractometer (brand ACCSEN, People's Republic of China). Vitamin C was determined using the titrimetric method. Acidity was obtained using the titratable acidity method.

The approximate composition: moisture and ash (gravimetric method), fiber, lipids (Soxhlet method), proteins (Kjeldahl method), and carbohydrates obtained by the difference of the other components was also determined (AOAC, 2005).

Instrumental Color Analysis

The color was determined on pulp and jam samples using a digital colorimeter (color Reader, CR-10 Minolta, Japan). The parameters L^* , a^* , and b^* of the *CIELab* system were assessed, where L^* ranges from 0 (totally black) to 100 (totally white); a^* from -80 (green) to +100 (red), and b^* from -50 (blue) to +70 (yellow) (Wrolstad & Smith, 2015). Samples were placed on a white plate and readings were taken at three different points, with three repetitions.

Microbiological Analysis

All the microbiological analyses were carried out following the procedures recommended by the *International Commission on Microbiological Specifications for Foods* (ICMSF, 1983) and Silva et al. (2017), and following the recommendations of the Laboratório Nacional de Higiene de Água e Alimentos (MISAU, 1997) of Mozambique and the Agência Nacional de Vigilância Sanitária (BRASIL, 2001).

The jam was analyzed immediately after processing (time zero), and then its stability was assessed with periodic analyses carried out every 21 days for 84 days, stored at room temperature. For this purpose, six bottles were prepared with the jam sample, opening on the analysis day.

Mesophilic aerobic bacteria (MAB) were counted on plate count agar (PCA) at 37 °C for 48 hours; mold and yeast (MY) were counted on potato glucose agar (PGA) at 25 °C for 72 hours; total coliforms (TC) were counted on Mac Conkey agar at 37 °C for 24 hours; and *Staphylococcus aureus* was counted on mannitol salt agar at 37 °C for 48 hours. Analyses were carried out on duplicate plates for each parameter analyzed. Counting was carried out using a colony counter (Stuart-UK) and the results were expressed in Colony Forming Units per gram of sample (CFU/g).

Data Analysis

The results obtained from the physicochemical composition and approximate composition were entered into the *Microsoft Excel* spreadsheet version 2013, and the average values and standard deviation were calculated and tabulated. The data obtained from the microbiological analysis was analyzed descriptively, and the averages were obtained and tabulated in Microsoft Excel (version 2013). These results were compared to the standards established by the LNHAA. In cases where there was no standard in Mozambican legislation, they were compared to the standards established by ANVISA (BRASIL, 2001).

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RESULTS AND DISCUSSION

Evaluating the three jam formulations, the research group chose formulation T3 (40% massala and 60% marula, Figure 1), which was then reprocessed and subjected to characterization. Thus, the results of the physical-chemical characterization and approximate composition refer to formulation T3 (Figure 2).



Figure 1: Different mixed jam formulations

F1 - 50% masala pulp and 50% canhú pulp; F2 - 60% massala pulp and 40% canhú pulp; and F3 - 40% massala pulp and 60% canhú pulp.



Figure 2: Samples of mixed jam (F3) storage at room temperature

Characterization of Mixed Jam

The results of the physical-chemical analysis and approximate composition of the raw material (massala and marula pulp samples) and the mixed jam are shown in Table 1. In general, the results of the physical-chemical assessment, and centesimal composition of the pulps were similar to those obtained in other studies (Magaia et al., 2013; Zingara & Tivana, 2022). It is essential to know the approximate composition of the raw material as well as its physical-chemical characteristics, because, in the processing industries, these results will indicate which treatment can be given to a given raw material and can also estimate the characteristics of the final product and its quality (Tivana, 2022).

Table 1: Physicochemical analysis and proximate composition of the pulps and the mixed jam

Parameter	Marula pulp	Massala pulp	Mixed Jam
pН	3.4 ± 0.00	4.3 ± 0.0	3.4 ± 0.02
Soluble solids (°Brix)	12.1 ± 0.05	20.7 ± 0.05	67.7 ± 0.16
Acidity (%)	1.6 <u>+</u> 0.06	2.2 ± 0.02	2.9 ± 0.06
Vitamin C (mg/100g)	105.6 ± 0.00	52.8 ± 0.00	45.6 ± 0.01
Moisture (%)	87.8 ± 0.01	79.4 ± 0.02	21.0 ± 0.03
Protein (%)	1.5 ± 0.00	2.3 ± 0.00	0.9 ± 0.00

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Lipid (%)	0.4 + 0.05	0.5 + 0.00	0.4 + 0.00
Ash (%)	1.6 ± 0.00	3.2 ± 0.03	0.9 ± 0.01
Fibers (%)	0.3 ± 0.00	3.2 ± 0.03	1.3 ± 0.00
Carbohydrates (%)	8.67 ± 0.00	13.58 ± 0.00	76.48 <u>+</u>
			0.00^{-}

Results are expressed as mean + standard deviation of three replicates.

Concerning pH, the value obtained for the jam sample is close to what was recommended. According to Celestino (2013), the pH value influences the processing and final quality of the product. The ideal pH for fruit jam should be around 3.4, because at pH values below this, there is a tendency for syneresis, and at pH values above 3.45, gel formation is weak. Similar pH results were obtained in other studies. Caetano et al. (2012), observed values between 3.42–3.48 in jam formulations of acerola juice and pulp. Tivana (2020), on the other hand, in a study of mixed jam produced with local wild fruits *Vangueira infausta* and *Adansonia digitata* with added pectin, determined a pH of 3.47.

The concentration of sugar in jam influences the quality of this product. It was observed that the average value of total soluble solids in the mixed jam was close to 67.5 °Brix (Torrezan, 1998). Above this value, crystals may form during storage, and if it is below, the jam will have a very soft consistency. The minimum value of total soluble solids for a common jam must be 62 °Brix, and 65 °Brix for extra jam. These results corroborate those previously reported by Caetano et al. (2012) in acerola and passion fruit jam formulations observed values between 66.92 and 67.97 °Brix. Tivana (2020) obtained values ranging from 67.14 to 67.51 °Brix in mixed jam.

Regarding acidity, Morais et al. (2021) stated that this is a fundamental physicochemical parameter in the processing and quality control of jams, as it helps to develop the right texture. According to Torrezan (1998), the acidity values for jam are in the range of 0.5% to 0.8%, as in cases of acidity above 1%; there is a tendency for syneresis to occur in jam samples. In this study, it was found that the acidity (2.9) was above the recommended level. However, these values can be justified by the nature of the wild fruits used (massala and marula) which had an average acidity of around 2.24 and 1.59, respectively (Table 1). On the other hand, it should be noted that during the processing of the jam, the acidification step took place with lemon juice, to lower the pH to obtain adequate gelling. During this process, no acidity regulator was used. However, it is important to highlight that despite the high acidity values, there was no liquid exudation or syneresis.

About the vitamin C parameter, the average value obtained for jam in this study (45.57 mg/100 g) was high. This value can be explained, on the one hand, by the nature of the pulps used, which had high amounts of vitamin C, and, on the other hand, by the acidification with the use of lemon juice during processing to correct the pH. The vitamin C value obtained is above the values reported by some authors in different studies. Leão et al. (2012) in their work on the formulation of papaya jam obtained an average of 41.33 mg/100 g of vitamin C.

Regarding the approximate composition of the mixed jam sample, the values of the parameters (proteins, lipids, fibers, and ashes) found in the jam of the present study are close to the values obtained in the fruit pulps since what influences these parameters is the composition of the raw material used during the processing of the jam.

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In this study, moisture was found to be below the recommended level (21%). According to Brazilian legislation, jam moisture values range from 35.0 to 38.0% (BRASIL, 1978). Caetano et al. (2012) obtained values ranging from 29.79% to 32.56%. Tivane (2020) obtained moisture values between 32.80% and 35.67%.

For the carbohydrate parameter, the average value (76.48%) found in the present study is above the values reported by some authors in different studies. This result may be due to the low moisture of the jam; since the carbohydrates were obtained by difference, the moisture and carbohydrate values are inversely proportional. Oliveira et al. (2018) obtained carbohydrate values ranging from 57.09 to 62.94% in *Spondias tuberosa* jam formulations. Celestino (2013) obtained values ranging from 62 to 68% in their *Mauritia Flexuosa* jam formulations.

Instrumental Color Analysis

The average results of the instrumental color analysis on pulp and mixed jam samples of massala and marula are presented in Table 2. In general, the luminosity values obtained showed that both massala and marula pulps presented a darker color (low L^* values, close to zero "0", which represents black), the a and b intensities were positive, towards red (+a*) and yellow (+b*); however, the intensity towards yellow was greater. Thus, the color of the pulps tended more towards dark yellow, with the massala pulp having a darker hue than the marula pulp.

Table 2: Results of instrumental color analysis

Sample	Parameter			
	L	a	b	
Marula	31.20	+1.70	+13.80	
Massala	25.96	+5.20	+19.30	
Mixed Jam	23.20	+4.84	+17.67	

Results are expressed as mean \pm standard deviation of three replicates. Luminance (L^*) , Chromaticity (a^*) , and (b^*) parameters in the colorimetric analysis.

Evaluating the luminosity value of the pulps, it was noted that the marula had a lighter color when compared to the massala pulp. The luminosity value of the mixed jam was lower, that is, darker in tone, and the a^* and b^* values remained positive with greater yellow intensity. These values indicate that the pulps were lighter in color than the jam. This darkening of the jam can be explained by the occurrence of Maillard and caramelization reactions during the evaporation process in jam processing, since, according to Fellows (2006), during evaporation processing, the temperature and soluble solids content increase, while the water activity decreases, providing a perfect environment for the occurrence of non-enzymatic darkening reactions. Similar results were found by other authors such as Damiani et al. (2008) when they evaluated mango jam formulated with different levels of peel replacing the pulp.

Microbiological Analyses

Microbiological Analysis of the Raw Material

The results of the microbiological analysis of the raw material (massala and marula pulps) are presented in Table 3. Quality indicator microorganisms were tested. The average results of the total coliforms (TC), molds and yeasts (MY), and mesophilic aerobic bacteria (MAB) were expressed in Colony Forming Units per gram of sample (CFU/g).

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Table 3: Results (average) of the microbiological analysis of raw material

Pulp		Parameter*			
	TC	MY	MAB		
Massala	0.00	$4,5 \times 10^{1}$	4.1×10^{2}		
Marula	7.5×10^{1}	$3,95 \times 10^2$	$2,65 \times 10^2$		

^{*}CFU/g - Colony Forming Units per gram of sample.

In general, pulps showed microbial growth, but with counts below the established microbiological standards (MISAU, 1997). For the MY parameter, the microbiological standard recommended by the LNHAA is 10^3 CFU/g (MISAU, 1997) and 10^4 CFU/g recommended by ANVISA (BRASIL, 2001). For the TC parameter, a limit of 10^2 UFC/g was established for fruit pulps (BRASIL, 2001). The results obtained in this study demonstrate that during the fruit pulp processing, Good Manufacturing Practices (GMP) were followed, minimizing the risk of compromising the quality of the final product. Similar results were observed by Gonçalves (2014) when evaluating the microbiological safety of *Theobroma grandiflorum* pulp, which presented counts of 3.3×10 CFU/g of MY, below the limits.

Microbiological Stability of Mixed Jam

The results of the microbiological stability of the mixed jam during storage at room temperature for 84 days are presented in Table 4.

Table 4: Microbiological stability of mixed jam during storage

Parameter (CFU/g)		Storage time (days)			
	0	21	42	63	84
TC	0.00	0.00	0.00	0.00	0.00
\mathbf{MY}	0.00	0.00	0.00	0.00	10^{1}
AMB*	< 10/g	< 10/g	< 10/g	<10/g	3×10^{2}
S. aureus	0.00	0.00	0.00	0.00	0.00

TC - Total coliforms; MY- molds and yeasts; MAB - mesophilic aerobic bacteria. CFU/g - Colony Forming Units per gram of sample.

No microbial growth was observed in the jam immediately after processing (day 0). This may be due to the influence of the chemical composition, intrinsic characteristics of the jam and the fact that this product had undergone the cooking and concentration process, an operation that preserves foodstuffs and contributes to reducing microorganism counts through exposure to heat (Lima et al., 2018).

Similar results were reported by Priya and Prakash (2017) in their microbiological assessment of blueberry jam (*Vaccinium corymbosum*), which linked the presence of sugar to a reduction in microbial growth. Therefore, high sugar concentrations cause a decrease in water activity, thus creating unfavorable conditions for bacteria growth, molds, and yeasts. The absence of these microorganisms was evidence of efficient hygienic conditions during the processing of the jam, combined with the use of good quality raw materials (with a low initial microbial load).

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^{*&}lt;10/g plates that did not show colonies.

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Regarding the microbiological stability of the jam, it was possible to observe that until the 63rd day of storage, there was no microbial growth (AMB, *S. aureus*, total coliforms, and molds and yeasts) (Table 4). On the 84th day of storage, there were low counts of molds, yeasts, and AMB, which were within established standards. These results were within microbiological standards of 10⁴ CFU/g for molds and yeasts, and the absence of Salmonella in 25g of the sample, as recommended by ANVISA (BRASIL, 2021).

Brasil and Góis (2016) obtained results lower than 10⁴ CFU/g for MY in banana, guava, papaya, and orange jam, and within the microbiological standards required by current legislation. Several studies have shown that jam has good microbiological stability for between 6 and 12 months when stored in suitable conditions (Assis et al., 2007; Cunha et al., 2020; Oliveira et al., 2018).

The results obtained in this study show that the jam was processed under good hygienic conditions. It can be stated that the jam made from the mixture of massala and marula pulp fruit can be stored for longer at room temperature.

CONCLUSIONS

The massala and marula pulps present good physicochemical and microbiological quality and can be used as raw materials to produce a quality product such as jam. Mixed jam of massala and marula is a safe product and has good physicochemical and microbiological quality. Jam made from a mixture of massala and marula pulp is microbiologically stable and can be consumed within 84 days when stored at room temperature.

FUTURE RESEARCH

From the findings of this research, further research could be carried out regarding:

- Analysis of micronutrients, bioactive compounds, and other parameters not covered.
- Study of mixed jam syneresis.
- Sensory analysis and product acceptance.
- Study of the economic viability of setting up a jam-processing agroindustry.

Author Contributions

*Q. M. G. conceptualization, methodology, writing - original draft, *E. L. C. conceptualization, methodology, writing - original draft, J. S. M. S. methodology, visualization, S. I. C. conceptualization, methodology, writing-review & editing, supervision, E. O. SR. conceptualization, methodology, formal analysis, data curation, writing-review & editing, supervision.

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Conflicts of Interest

The authors declare that they have no conflict of interest regarding this manuscript.

Data availability

The corresponding author will provide all data generated or analyzed during this study upon reasonable request.

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