

Effect of Pasteurization on Physicochemical and Biochemical Stability of Dilute Extract of *Sclerocarya Birrea* During Storage at 4 °C

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Abstract

Sclerocarya Birrea, commonly known as African plum, is an oleaginous fruit with high nutritional value, the different parts of which have medicinal properties. Its pulp is used in particular in the production of juice. However, its high acidity induces rapid fermentation, hampering its conservation. This work aims to evaluate the impact of pasteurization on the stabilization of *S. birrea* extract to limit this fermentation. Three heat treatments were applied to separate batches: 65 °C/30 min, 75 °C/10 min, and 85 °C/10 min. The resulting extracts were stored at 4 °C for six weeks. The parameters monitored included: titratable acidity, pH, soluble solids, browning and yellowing indices, as well as polyphenol content. The analyses were carried out using standardized methods: titrimetry, pH-metry, refractometry, colorimetry, and spectrophotometry. The results show stability of pH, titratable acidity, and solids during storage. The colorimetric indices increased slowly at 65 °C and 75 °C, but more markedly at 85 °C. The polyphenol content decreased after heat treatment, with retention rates of 85.90%, 80.77%, and 78.53% for the 65 °C/30 min, 75 °C/10 min, and 85 °C/10 min treatments, respectively. The combination of pasteurization at moderate temperature and refrigerated storage thus proves effective in stabilizing *S. birrea* extracts, while limiting the degradation of bioactive compounds.

Keywords

Sclerocarya birrea; Stabilization; Pasteurization; Extract; Plants; Polyphenols

Introduction

Sclerocarya birrea, commonly known as African plum, is a fruit species of the *Anacardiaceae* family, widely distributed in the Sahelian and Sudanian zones of sub-Saharan Africa. Its fruits are valued for their richness in

bioactive compounds, including vitamins, minerals, dietary fiber, organic acids, and polyphenols [1, 2]. In addition to their nutritional value, the different parts of the tree (leaves, bark, roots) are used in traditional pharmacopoeia for their antimicrobial, anti-inflammatory and antioxidant properties [3]. The fruit pulp is frequently processed into artisanal or industrial beverages, but its high acidity, combined with a high content of fermentable sugars, leads to rapid fermentation after extraction, limiting its stability and complicating its conservation [4]. Several authors have emphasized the need to adopt suitable technological processes to preserve the functional qualities of the extracts while limiting their microbiological alteration [5]. Among the possible methods, pasteurization stands out as a mild heat treatment capable of inactivating unwanted enzymes and microorganisms without significantly altering sensitive compounds [6]. When combined with refrigerated storage, this technique can constitute an effective stabilization strategy for fruit matrices. The present study is part of this dynamic and aims to evaluate the impact of different pasteurization scales on the physicochemical and biochemical characteristics of an extract of *S. birrea* during storage at 4 °C, with a view to providing an optimized conservation approach.

1. Materials

1.1 Plant material

The fruits of *Sclerocarya birrea* (Figure 1) come from the Fatick region and were harvested in July 2024 and stored at 4 °C.



Figure 1. *Sclerocarya* fruit *birrea*.

2. Methods

2.1 Extraction of *Sclerocarya* fruit juice *birrea*

The fruit of *Sclerocarya Birrea* was sorted and washed before being peeled. It then underwent manual extraction by adding 1.5 L of dilution water for each kilo of pulp. The extract is then filtered (Figure 2).



Figure 2. Diluted extract of *Sclerocarya* fruit pulp *birrea*.

2.2 Heat treatments (pasteurization)

Three separate batches of diluted extract were subjected to heat treatments by pasteurization according to the following scales:

- ✓ Batch 1 : 65 °C for 30 minutes. Gentle pasteurization;
- ✓ Batch 2 : 75 °C for 10 minutes. Intermediate pasteurization;
- ✓ Batch 3 : 85 °C for 10 minutes. High pasteurization.

The nectars were pasteurized in a thermostatic water bath, according to established scales. The core temperature was monitored using a probe placed in the geometric center of a control bottle, ensuring precise control of thermal conditions.

2.3 Storage conditions

After pasteurization, the extracts were packaged in sterile bottles and stored at a constant temperature of 4 °C for a period of six weeks. Samples were taken weekly for analysis.

2.4 Physicochemical and biochemical analyses

The following parameters were monitored:

- **Physicochemical:** pH (pH-metry), titratable acidity (titrimetry), soluble dry extract (refractometry);
- **Biochemical:** browning index and yellowing index (colorimetry), total polyphenol content (spectrophotometry).

All analyses were performed according to standardized protocols in triplicate to ensure reproducibility of results.

2.4.1 Titratable acidity

Titrateable acidity was measured by titration with 0.1 N NaOH solution in the presence of phenolphthalein as an indicator. Results are expressed as g citric acid/100 mL nectar, according to AOAC 942.15 [7].

2.4.2 Determination of color parameters

The color of the samples of the products obtained was measured using a colorimeter (type: KONICA MINOLTA Japan) based on the CIELAB color system (L^* , a^* , b^* , and L^* , C^* , h , YI). The color parameters (L^* , a^* , b^* , and L^* , C^* , h , YI) were measured 3 times for each sample. L^* , a^* , b^* describe the colors black-white, Green-Red, and Blue-Yellow, respectively: L^* (0 = Black, 100 = White); a^* ($-a$ = Green, $+a$ = Red); b^* ($-b$ = Blue, $+b$ = Yellow) (Figure 3) [8].

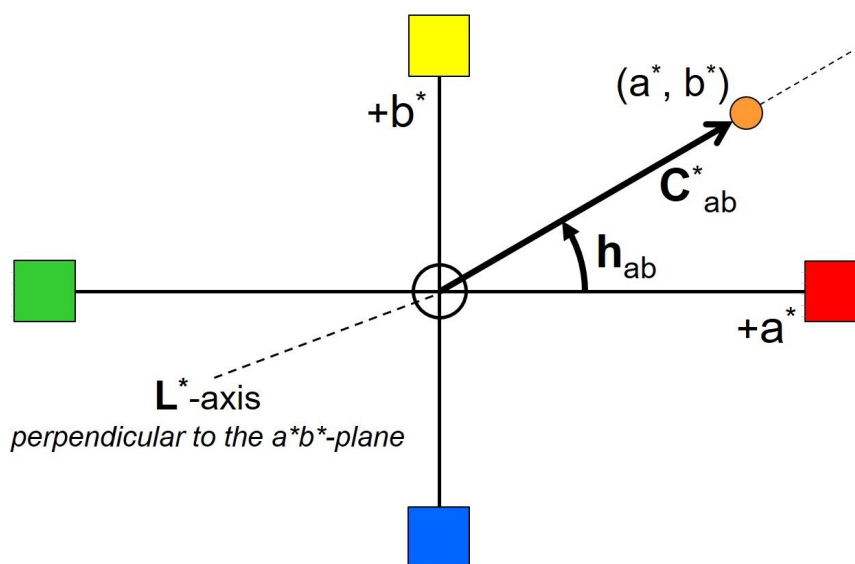


Figure 3. Representation of the CIELAB coordinate system.

2.4.3 Determination of the Brun index

The principle of determining the Browning Index in colored liquids is based on the spectrophotometric analysis of CIELAB colorimetric parameters, including L^* , a^* , and b^* values. These parameters are used to evaluate the color of a sample in terms of brightness (L^*), red-green hue (a^*), and yellow-blue hue (b^*). The Browning Index is calculated from the b^* value, which represents the yellow-blue component of the color [9].

2.4.4 Determination of total polyphenols

The content of these total phenolic compounds extracts of Combretum was estimated by the Folin-Ciocalteu method, which is based on the reduction in an alkaline environment of their phosphotungstic mixture of phosphomolybdate reagent of Folin by the oxidizable phenolic compounds, leading to the formation of blue reduction products in color. These have a maximum absorption at 760 nm. Whose intensity is proportional to the quantity of total polyphenols present in the extract [10].

2.4.5 Determination of soluble dry matter (Brix)

Brix is defined as the concentration of soluble solids in an aqueous solution. This concentration, measured at 25°C by the refractive index, is then expressed as the percentage by mass (g/100 g), is measured according to a standardized method (NA 5669) using a universal refractometer. Abbe ATAGO type refractometer with digital reader and temperature correction.

2.5 Data processing

Statistical treatments were performed using one-way analysis of variance (ANOVA), implemented in R software (*version 3.2.4 Revised, 2018-03-16, R-70336*). Each analysis was performed on data from three replicates per sample. Mean values for each sample were compared using a multiple comparison method (e.g., Tukey HSD test or other post-hoc test) and are accompanied by superscript letters (X^a , X^b , X^c). Samples that share one or more letters in common do not show a statistically significant difference at the significance level of $\alpha = 0.05$ (equivalent to $p > 0.05$).

3. Results and Discussion

3.1 *Sclerocarya fruit birrea* before pasteurization

Sclerocarya extract *birrea* before pasteurization is recorded in Table 1. Analysis of unpasteurized crude extract of *Sclerocarya birrea* highlights several important physicochemical and biochemical characteristics. The observed Brix degree (1.00%) is significantly lower than that reported in the literature, notably by Ojokoh *et al.* (2013), who indicate values between 5 and 7% for fresh pulp extracts. This low content of soluble sugars results from the dilution of the extract [11]. The pH of the extract (3.69 ± 0.01), associated with a moderate titratable acidity (0.0735 Eq.L^{-1}), indicates an acidic environment conducive to relative stability with respect to certain pathogens, but favorable to the activity of yeasts and lactic acid bacteria.

Table 1. Chemical and biochemical characteristics of diluted unpasteurized *Sclerocarya birrea* extract

Brix degree %	Acidity Eq/L	pH	Yellowing index b	Brown index	Polyphenol concentration (g/L)
1.00±0.01	0.0735±0.003	3.69±0.01	25.010±0.002	45.960±0.002	0.312±0.002

These values are consistent with those reported by Mariod *et al.* (2015) and Glew *et al.* (2004), who place the pH of *S. birrea* between 3.2 and 3.8 [2, 3]. They confirm the need for technological treatments to limit natural post-extraction fermentation. Visually, the yellowing index (25.010 ± 0.002) and the browning index (45.960 ± 0.002) indicate a rapid alteration of the color, probably linked to enzymatic oxidation processes. Indeed, the presence of polyphenoloxidases (PPO), enzymes responsible for browning, has already been identified in plant matrices rich in phenols [12]. The absence of heat treatment allows these enzymes to remain active, explaining the pigment degradation observed from the first hours. The initial polyphenol content ($0.312 \pm 0.002 \text{ g/L}$) confirms the antioxidant richness of the extract. Although slightly lower than those reported by Lockett *et al.* (2000), this concentration remains significant and underlines the functional interest of this fruit [1]. However, the sensitivity of these compounds to uncontrolled storage conditions justifies the application of stabilization processes from the post-harvest phase. Figures 4, 5, and 6 show the extracts after pasteurization.



Figure 4. Pasteurized at 65°C/30 min.



Figure 5. Pasteurized at 75°C/10 min.



Figure 6. Pasteurized at 85°C/10 min.

3.2 Brix degree monitoring

Sclerocarya extract *birrea* throughout the heat treatments and during storage at 4 °C for six weeks (Figure 7).

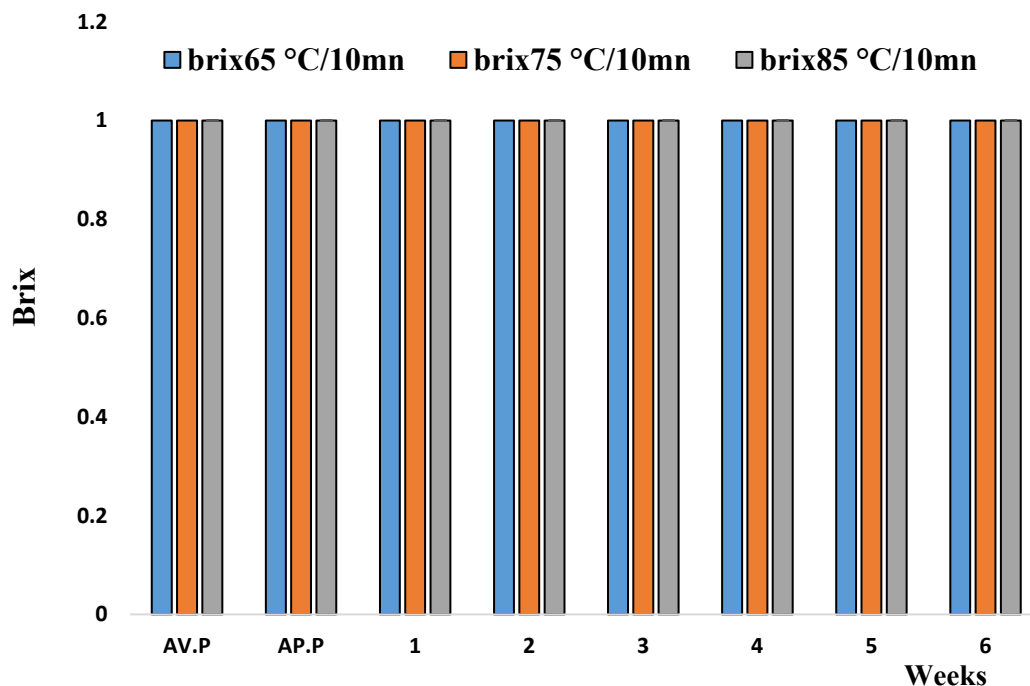


Figure 7. Brix degree before and after pasteurization and during storage at 4 °C for 6 weeks.

No significant variation was observed between the values measured before and after pasteurization, nor during the storage period. This stability suggests that the processing conditions were sufficiently moderate to avoid the evaporation of water contained in the extract, thus preserving the concentration of soluble solids. These observations are consistent with the work of Liu (2024), who emphasizes that well-controlled heat treatments can inactivate enzymes without altering the chemical structure of sugars or causing their artificial concentration by reducing the aqueous volume [13]. Similarly, in their study on the thermal stabilization of tropical juices, Fawole and Opara (2013) reported stability of °Brix after pasteurization at low temperature, confirming that mild processes allow the preservation of soluble constituents without significant alterations [14].

3.3 Evolution of the brown index

The brown index represents the proportion of yellow color to red color. The evolution of the brown index before and after pasteurization and during storage is shown in Figure 8.

The browning index (BI), a key indicator of phenolic compound oxidation and visual deterioration of plant extracts, evolved differently depending on the pasteurization scale applied. Extracts pasteurized at 65 °C for 30 minutes and at 75 °C for 10 minutes showed a moderate increase in BI, from $41.660^b \pm 0.001$ to 46.990 ± 0.002 (+12.8%) and from $37.980^a \pm 0.002$ to 44.47 ± 0.002 (+17.1%), respectively, during the six-week storage period. In contrast, treatment at 85 °C for 10 minutes resulted in a drastic increase, with BI increasing from $43.930^c \pm 0.001$ to 102.64 ± 0.001 (+133.7 %). This dynamic confirms that high temperature treatment promotes non-enzymatic browning reactions, such as the Maillard reaction, particularly in matrices rich in sugars and reducing compounds, as is the case for *Sclerocarya pulp birrea* [6]. This phenomenon was also observed by Fawole and Opara (2013) in pomegranate juices pasteurized at over 80 °C, where the formation of brown pigments was significant from the first days of storage [13]. On the other hand, more moderate thermal scales allowed a more effective colorimetric stabilization, limiting both enzymatic activity and undesirable thermal effects [16]. The relative stability observed at 65 °C and 75 °C could be explained by a partial or complete inactivation of oxidative enzymes (notably polyphenol oxidase), without, however, reaching temperature thresholds likely to induce major physicochemical transformations of the phenolic constituents. These results are in line with those of Oduro *et al.* (2009), who

demonstrated that mild heat treatments ensure a good compromise between microbiological safety and preservation of visual attributes in plant juices rich in antioxidants [12]. Thus, treatments at 65°C/30 min and 75°C/10 min prove more effective in controlling browning during refrigerated storage, thus preserving the visual quality of the product. Conversely, treatment at 85 °C, although effective from a microbiological point of view, seems to accelerate pigment degradation mechanisms, potentially compromising the sensory acceptability of the final product.

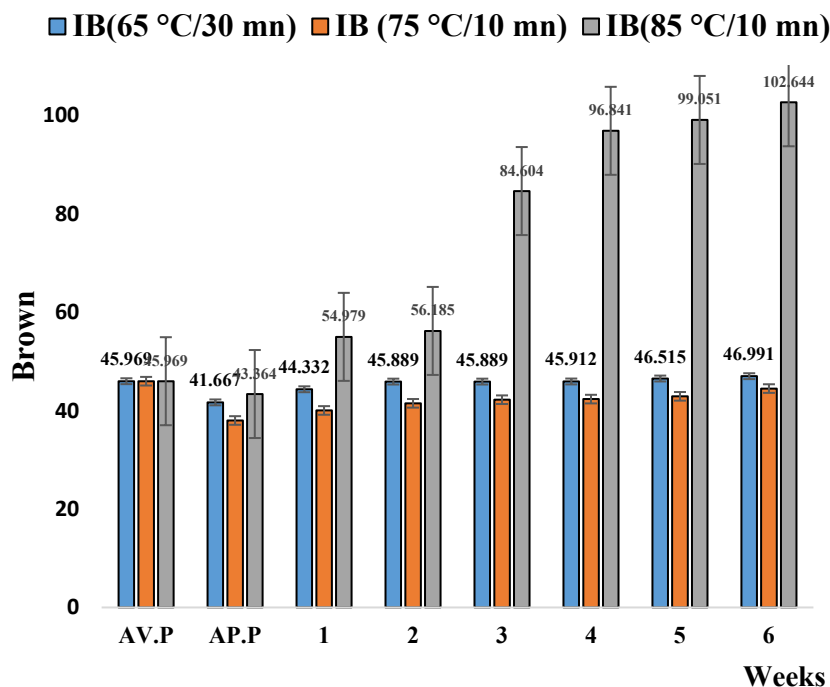


Figure 8. Evolution of the brown index before and after pasteurization and during storage at 4 °C for 6 weeks.

4. Evolution of the Yellowing Index

The evolution of the yellowing index before and after pasteurization and during storage is presented in Figure 9. The results show that the yellowing index (JI) increases gradually over storage for all pasteurized extracts, but with clearly different dynamics depending on the scales applied.

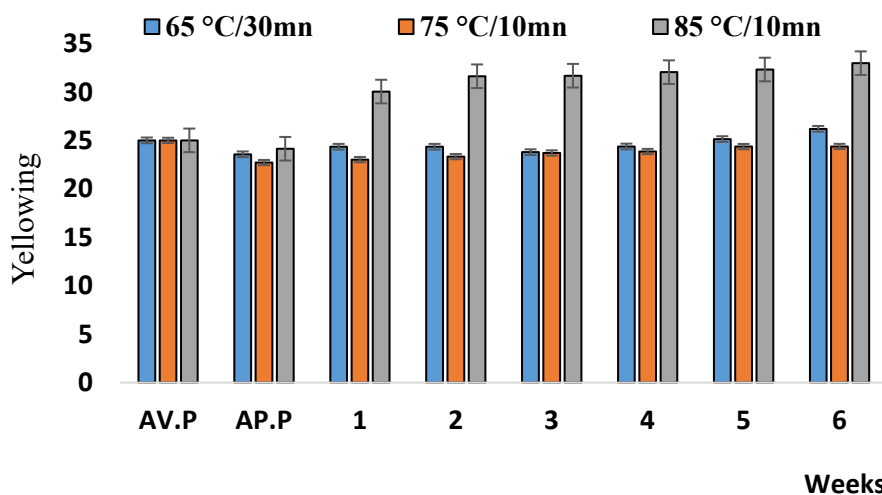


Figure 9. Evolution of yellow color before and after pasteurization and during storage at 4 °C for 6 weeks.

The extract pasteurized at 65°C/30 min shows a moderate change in the IJ, increasing from 23.570 ± 0.001 (S0) to 26.200 ± 0.001 at the sixth week (+11.1%), indicating relative chromatic stability under the effect of mild heat treatment. Similarly, the extract pasteurized at 75°C/10 min shows a lower progression, from 22.710 ± 0.002 to 24.380 ± 0.001 (+7.3%), suggesting better visual preservation at this temperature. On the other hand, the treatment at 85°C/10 min results in a more marked increase in the IJ: from 24.140 ± 0.001 to 32.910 ± 0.002 (+36.4%). This rapid evolution could be attributed to a more significant thermal degradation of natural pigments (flavonoids, carotenoids) and heat-induced side reactions. These results confirm the observations of Ajila *et al.* (2007), who reported that high heat treatments can alter thermosensitive pigments present in plant matrices rich in colored compounds [15]. Similarly, Ramaswamy and Marcotte (2006) emphasize the importance of finding a compromise between enzymatic inactivation and visual preservation, as increased color degradation is often correlated with functional or organoleptic losses [6]. Furthermore, the continuous increase in IJ during storage, even at refrigerated temperature (4 °C), could also reflect residual oxidation of pigments or the slow but persistent activity of partially inactivated polyphenoloxidases. Fawole and Opara (2013) observed similar trends in thermized juices, where a slight change in the yellowing index was inevitable, even under controlled conditions [14].

5. PH Monitoring

The evolution of pH before and after pasteurization and during storage is presented in Figure 10. The study highlighted a significant decrease in pH immediately after pasteurization, regardless of the intensity of the heat treatment applied. This trend could be explained by the degradation of certain heat-sensitive components, such as simple carbohydrates or amino acids, leading to the formation of acidic compounds [16]. However, this decrease remains limited and seems to stabilize from the beginning of storage.

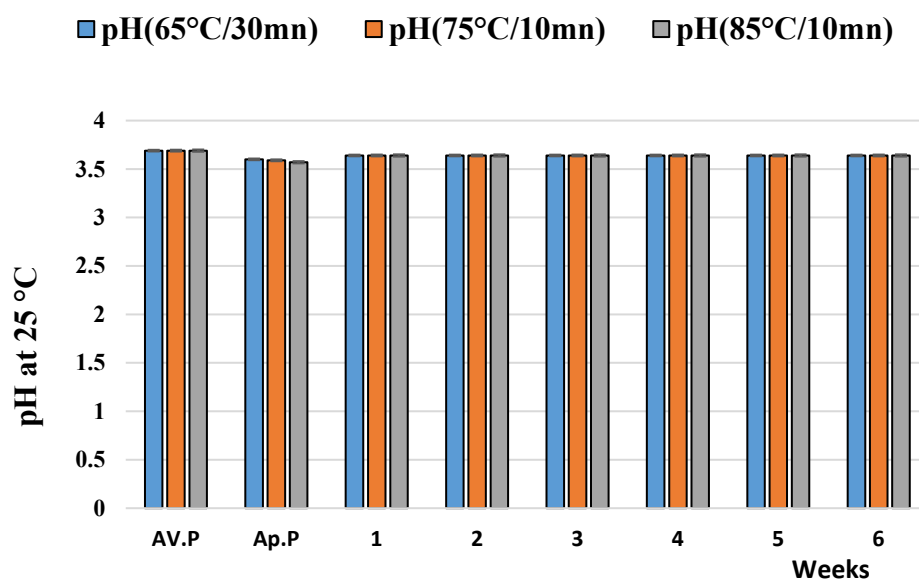


Figure 10. Monitoring of pH before and after pasteurization and during storage at 4 °C for 6 weeks.

Indeed, no notable variation in pH was observed throughout the storage period, which demonstrates good physicochemical stability of the finished product. This behavior is consistent with the observations of Diallo, Sarr, and Ndiaye (2020), who emphasize that thermal inactivation of acidifying flora plays a key role in preserving the pH of plant extracts [17]. The absence of fermentation, generally marked by pH stabilization, supports this hypothesis and confirms that pasteurization is an effective means of ensuring product stability throughout its shelf life.

6. Monitoring of Titratable Acidity

The evolution of titratable acidity before and after pasteurization and during storage is shown in Figure 11.

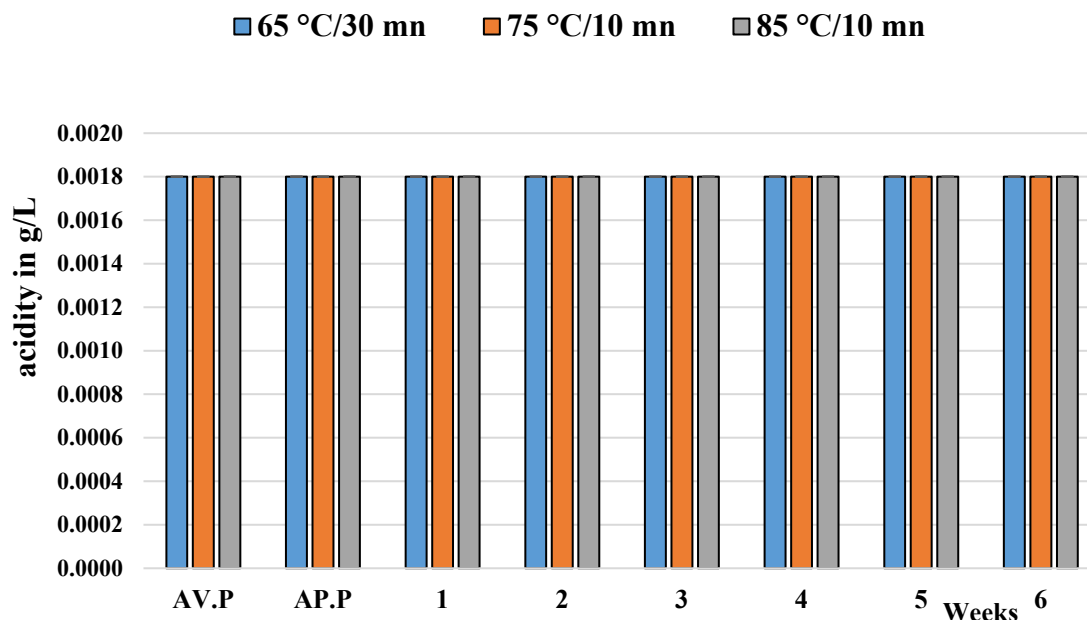


Figure 11. Monitoring of titratable acidity before and after pasteurization and during storage.

The results obtained indicate that the titratable acidity of the extract remains generally stable before and after pasteurization, as well as during storage at 4 °C for six weeks. This stability can be explained by the effect of the heat treatment applied upstream, which contributes to the inactivation of microorganisms likely to cause fermentation, a phenomenon often associated with an increase in acidity [26]. Indeed, pasteurization plays an essential role in the preservation of the physicochemical properties of products by limiting microbial development [17]. In addition, maintaining the storage temperature at 4 °C seems to have no significant impact on the titratable acidity, a result in agreement with the conclusions of Ndao, Sagna, and Ba (2019), who also observed stability of this parameter in plant extracts preserved in the cold [18]. This consistency confirms the effectiveness of the combination of heat treatment and the cold chain in controlling the quality of extracts during storage.

7. Evolution of the Polyphenol Concentration

Figure 12 shows the evolution of the polyphenol concentration before (AVP) and after pasteurization (APP) and during storage.

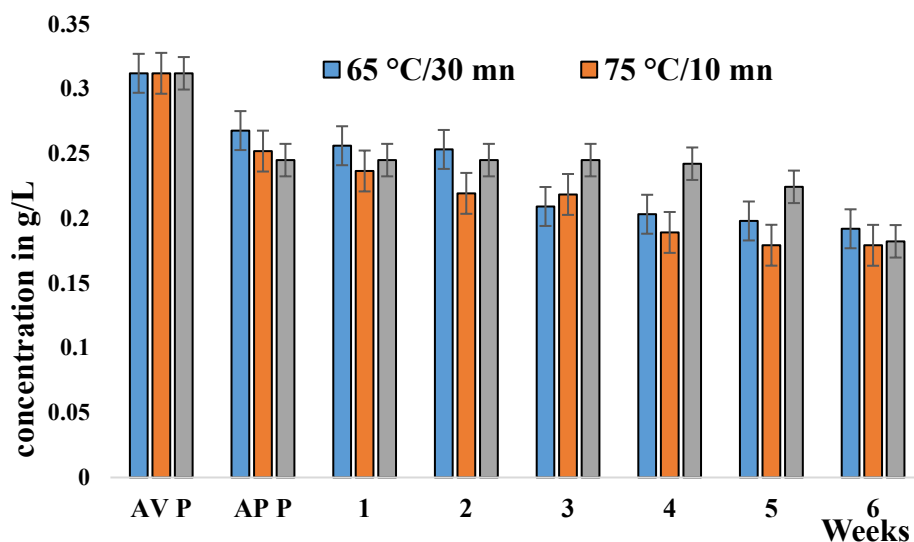


Figure 12. Evolution of the quantity of polyphenols before and after pasteurization and during storage at 4°C for weeks.

Immediately after pasteurization (APP), a significant decrease in polyphenols is observed for all treatments. The loss is more marked for high temperatures, reaching -19.2 % at 75 °C and -21.5 % at 85 °C compared to -14.1% at 65 °C. This decrease is consistent with the observations of Madrau *et al.* (2009), who attribute this degradation to the thermal sensitivity of phenolic compounds [19]. During storage, a notable difference appears between treatments. At 85 °C, the polyphenol content remains stable until the third week, suggesting more efficient enzymatic inactivation, particularly of polyphenoloxidase. This hypothesis is supported by the work of Metlef *et al.* (2022), who highlight the interest of high temperatures in slowing down enzymatic oxidation [20]. In contrast, samples pasteurized at 65 °C and 75 °C showed a progressive decrease in polyphenols throughout storage, indicating continuous degradation due to incomplete enzymatic inactivation. Pasteurization at 85 °C for 10 min appeared to be the most favorable condition for polyphenol stability during the first three weeks of storage. However, a significant loss was observed after six weeks, regardless of the heat treatment applied. These results highlight the need to combine optimized heat treatment with rigorous management of storage conditions to ensure the preservation of bioactive compounds.

8. Conclusion

Sclerocarya birrea fruit extract showed that pasteurization combined with storage at 4 °C allowed samples to be stabilized for six weeks. Pasteurization at 85 °C allowed better short-term stability of polyphenols, which induced significant browning and yellowing, essential for consumer acceptance. Therefore, pasteurization at 75 °C/10 min is recommended as an optimal compromise, ensuring adequate microbial safety, better color retention, and the preservation of more than 80% of the initial polyphenols. Indeed, for better preservation of the extract, the fruit must be processed immediately after harvest to avoid fermentation. During extraction and pasteurization, compliance with good hygiene practices (wearing gloves, masks, and protective equipment, disinfecting the environment, equipment, and raw materials) is essential to avoid any contamination of the extract. Using suitable packaging (plastic) helps to better preserve the color of the extract during storage at 4°C. Pasteurization remains a good preservation method.

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