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Kinetic study of carotenoids, anthocyanins and aroma compounds during loengo (*Anisophyllea boehmii* Engl.) nectar pasteurization and storage

Famorado João da Costa^{a,b}, Christian Mertz^{b,c}, Adrien Servent^{b,c}, Nawel Achir^b, Bettencourt Munanga^a, Manuel Dornier^{b,*}

^a Instituto de Tecnologia Agro-Alimentar, Rainha Njinga a Mbande University, Malanje, Angola

^b QualiSud, Université de Montpellier, Avignon Université, CIRAD, Institut Agro Montpellier, IRD, Université de La Réunion, Montpellier, France

^c CIRAD, UMR QualiSud, F-34398, Montpellier, France

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ABSTRACT

This work aimed to study the evolution of the color and content of anthocyanins, carotenoids, and aroma compounds of loengo nectars (*Anisophyllea boehmii* Engl.) subjected to different pasteurization treatments (from 70 °C/60 min to 100 °C/15 min) and storage conditions from 4 to 45 °C for up to 35 days. Spectrophotometric, chromatographic (HPLC-DAD and HS-SPME/GC-MS) and CIE-Lab* analyses were used to monitor the evolution of pigments, color and aroma compounds over time. First-order kinetics and Arrhenius models were applied to calculate the reaction rate constants and activation energies by non-linear optimization. The results revealed that pasteurization treatments did not greatly alter the pigments except at 100 °C for carotenoids which decreased of 23% within 15 min. However, the beverage color remained quite stable during the heating step. During storage, the anthocyanin content was strongly affected decreasing between 58 and 100% in 15 days depending on the temperature. Carotenoid degradation was also significant, with losses of around 30% in 35 days below 30 °C and close to 85% at 45 °C. Consequently, the color of the pasteurized product changed quickly during storage even at room temperature. The degradation rate of anthocyanins that depended on the geographical origin of the fruit, could not be mitigated by adding ascorbic acid or by deaerating the nectar. Both pasteurization and storage strongly modified the aroma profile of the beverage, with some aroma compounds degrading and others appearing. Further investigations are required to elucidate the reaction mechanisms involved and to identify solutions for optimizing the preservation of the nutritional and organoleptic qualities of loengo nectar.

1. Introduction

Loengo (*A. boehmii* Engl.), a fruit from the Miombo-type light forest [1,2], is widely consumed in Angola. It has an interesting nutritional and organoleptic potential because it is rich in minerals (Mg, Cu, Fe, Mn and Zn), carbohydrates, fibers, bioactive compounds, such as polyphenols [3,4] and carotenoids, and aromatic compounds that give it the characteristic aroma of the fruit [3]. Locally, it is widely consumed fresh or can be processed mainly into beverages such as nectars [3]. Processing into nectar allows the fruit to be stabilized, improving its availability throughout the year, in line with market trends for health-promoting beverages [5].

Large-scale production of this fruit processed into nectar would be a means of optimizing this local fruit resource by producing a popular beverage of good nutritional quality. In Angola, pasteurization of this

acidic beverage is the most suitable stabilization process. This unitary operation is simple to implement and produces products that can usually be stored for several months at room temperature. However, it is essential to assess the impact of heat treatment on the organoleptic and nutritional quality of the beverage, as it can lead to degradation of the raw material [6,7]. It is also necessary to study the chemical and physicochemical stability of the pasteurized product during storage in order to assess its shelf life [8].

Among sensory attributes, color plays a fundamental role as it is typically the first characteristic perceived by consumers. It can significantly influence their perception of quality and freshness of fruit beverages [9]. Flavor is another major organoleptic dimension whose intensity and volatile composition can significantly modulate the hedonic perception of fruit-based products, directly influencing consumer acceptability and preference [10]. Therefore, preserving the color,

* Corresponding author.

E-mail address: manuel.dornier@institut-agro.fr (M. Dornier).

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pigments and aroma compounds present in the product as much as possible during the stabilization process and storage, is crucial. From a health perspective, fruit beverages contain a multitude of bioactive compounds that offer a wide range of health benefits, such as antioxidant, anti-inflammatory, anticancer, and antimicrobial properties as well as cardiovascular and neuroprotective effects [11–13]. The degradation of these bioactive compounds such as vitamins, phenolics or carotenoids can be a key factor affecting the nutritional quality of fruit-based beverages, due to their inherent instability during processing and storage [14].

In this context, the work carried out proposes to study for the first time processing loengo fruit into nectar. It was based on the monitoring of nectar quality, mainly its color and concentrations of compounds of interest (phenolic compounds including anthocyanins and carotenoids), during heat treatment and storage of the stabilized product. In order to better understand the phenomena observed, a kinetic approach was chosen to determine the specific kinetic constants. The objective was to investigate quality modulation according to the intensity of heat treatment, storage conditions, the batch of fruit used and the formulation of the nectar.

2. Materials and methods

2.1. Fruit and nectar preparation

The fruits used in this study came from 3 provinces in the Miombo region of Angola: Malanje (AbM sample), Huambo (AbHb sample) and Huíla (AbHa sample). They were purchased in 2022 at the markets and picked by the local population from randomly selected trees. The fruits were chosen for their uniform ripening, without any visual defects,

diseases, pest attacks or mechanical injuries. Fig. 1 shows the various steps and main conditions for obtaining the loengo nectar. Firstly, the pulp was extracted from the fruits as described by da Costa et al. [3].

Loengo nectar was then formulated at 30% pulp mass fraction by diluting the pulp with distilled water and adding sucrose to reach a final total soluble solids (TSS) content of 12 g·100 g⁻¹. For 100 g, the nectars were formulated with on average 30 g of pulp, 62.1 g of water and 7.9 g of sucrose. In certain cases, additives (citric acid, sodium bicarbonate, or ascorbic acid) were used in order to modulate the pH of the product or as an antioxidant. After formulation, the mixture was homogenized using a Vorwerk Thermomix (Wuppertal, Germany) for 20 s, at room temperature. For some samples, a deaeration step was added in order to limit oxidation phenomena by dissolved O₂. The samples were then packaged in 25 mL polyethylene bags (20 g nectar per bag) which were vacuum-sealed for subsequent pasteurization and analysis.

2.2. Experimental methodology

The experimental design was developed to explore the effect of different factors of nectar production on its quality, specifically in terms of pigments, color and aroma compounds (Table 1). The factors studied were:

- The origin, i.e. the Miombo region,
- The use of additives and deaeration,
- The pasteurization conditions,
- The storage temperature.

Several process pathways were applied to assess their impact on the nectar stability trying to better understand the mechanisms involved.

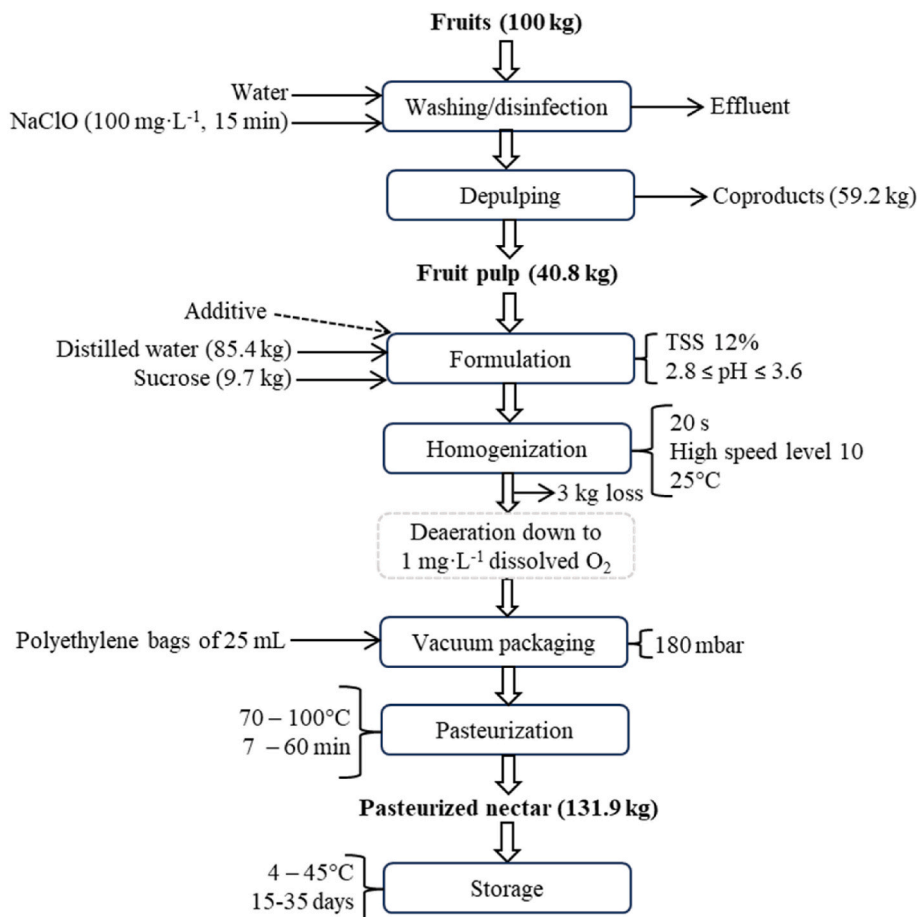


Fig. 1. Process used for loengo nectar production with 30% pulp and mass balance from 100 kg fruit basis.

Table 1

Experimental design used to evaluate the effects of origin, formulation, pasteurization and storage conditions on loengo nectar stability (sample notation in brackets).

Origin	Additives/deaeration	Pasteurization conditions	Storage temperature
Malanje	Without (AbM22)	70 °C/60 min	30 °C
		85 °C/7 min	4 °C
			20 °C
			30 °C
			45 °C
			30 °C
Huambo	Citric acid (AbM22 A) Sodium bicarbonate (AbM22 BS) Ascorbic acid (AbM22 AS) Deaeration (AbM22 O) Citric acid & deaeration (AbM22 AO)	85 °C/20 min	
		100 °C/15 min	
		85 °C/7 min	
Hufla	Without (AbHb22)		
Hufla	Without (AbHa22)		

They included:

- the effect of origin on anthocyanins and color stability, through comparison of samples collected from the 3 regions chosen, Malanje, Huambo, and Hufla,
- the effect of additives and deaeration (reduction of dissolved O_2 from 6 to 1 mg L⁻¹ using gentle shaking under vacuum) on anthocyanins and color stability. The additives used were citric acid at 6 g L⁻¹ in order to decrease the pH to 2.8, sodium bicarbonate at 15 g L⁻¹ in order to increase the pH to 3.6 and ascorbic acid at 300 mg L⁻¹ as an antioxidant. Adding citric acid was also combined with deaeration in order to obtain a sample with low pH and low dissolved O_2 ,
- the effect of pasteurization, at different temperatures and durations, on the stability of pigments, and aromatic compounds,
- the effect of storage temperatures for up to 35 days, on the stability of pigments, aroma compounds and the color of loengo nectar.

2.2.1. Pasteurization and storage procedures

2.2.1.1. Pasteurization conditions. The stabilization of loengo nectar was achieved through pasteurization, using 20 g samples having been previously packaged in as many 25 mL polyethylene bags as the number of samples to be taken over time. Temperature control during the isotherm treatments were ensured using a digital temperature probe (Almemo ZA9020-FS thermo E4, France) mounted on a reference bag. It allowed accurate measurement of the temperature of the loengo nectar during experiments carried out by immersion in a water bath, under different conditions: 70 °C for 60 min (mild pasteurization), 85 °C for 7 or 20 min (standard or high pasteurization) and 100 °C for 15 min (strong pasteurization), leading to target pasteurization values of 0.14, 0.82, 2.33 and 85.06 min, respectively, calculated at a reference temperature of 93.3 °C [15] with a z-factor equal to 8.89 °C (*Byssoschlamys fulva* ascospore chosen as the microorganism of reference) [16]. The first three pasteurization values were defined in accordance with good industry practice. Indeed, the pasteurization values generally used in industry range from 0.1 min to 3 min with an average close to 1 min for acidic beverages such as fruit juices. Given the acidity of the nectar, these pasteurization values guaranteed the microbiological stability of the product in the vacuum-sealed polyethylene bags. The fourth treatment (pasteurization value of 85 min) was chosen in order to evaluate

the impact of a particularly strong heat treatment. Three to five points were used across treatment duration, quickly removing samples from the water bath and cooling them down in melting ice to 30 °C for approximately 35 s.

2.2.1.2. Storage conditions. After the treatment at 85 °C for 7 min (standard pasteurization), the polyethylene bags were stored under controlled temperatures of 4 °C (refrigerated storage), 20 °C (temperate climate), 30 °C (tropical climate) and 45 °C (accelerated aging temperature) for 15 to 35 days. For samples containing additives or that had been deaerated, the storage temperature was set at 30 °C. For all treatments, 3 to 5 monitoring measurements were evaluated during storage.

2.3. Study of color and pigment

2.3.1. Color measurement

The color of the nectar samples was measured using a CR-400 chromameter (Konica Minolta, Japan), previously calibrated with a standard white calibration plate (CIE standard D65 illuminant). Measurements were performed in the CIE Lab* color space. Hue angle (h*), chroma (C*) and total color difference ΔE were calculated using the raw nectar as the reference and using the equations described by Buvé et al. [17]. In order to represent overall color variations simply, we chose ΔE as the main indicator. It represents the distance in the Lab* color space between the initial product and the sample. A ΔE value of less than 5 is generally considered as undetectable by the human eye. For each sample, three independent measurements were taken at room temperature.

The polymeric color index (% PC) was also measured. For that, samples were stirred for 5 min at 8 rpm and centrifuged at 10,000×g for 10 min at 25 °C. The supernatant was recovered and filtered through 0.45 µm filter. Spectrophotometric measurements were performed using a SPECORD S600 spectrophotometer (Analytik Jena, Germany). The wavelengths were set at 420/510/700 nm, according to Sinela et al. [18]. The % PC corresponds to the ratio between the polymeric color and the total color density. To establish this ratio, 1 M sodium metabisulfite solution was added to the extract to selectively bleach monomeric anthocyanins. The residual color, referred to as polymeric color, is attributed to the presence of stable polymeric compounds. For each measurement, 2.8 mL of extract was used to determine the color density. To assess polymeric color, 0.2 mL of Milli-Q water or sodium metabisulfite was added to 2.8 mL of extract. The percentage of polymeric color was then calculated using equations (1) and (2):

$$\text{Color density} = [(A_{420} - A_{700}) + (A_{510} - A_{700})] \times \text{Dilution factor} \quad (\text{Equation 1})$$

$$\text{Polymeric color index (\%)} = \frac{\text{Color density with SO}_2}{\text{Color density without SO}_2} \quad (\text{Equation 2})$$

2.3.2. Pigment measurement

2.3.2.1. Carotenoids. For the extraction of carotenoids, 10 mL of loengo nectar was mixed with 10 mL of hexane and agitated for 20 min at 8 rpm using a rotary agitator (Reax 2, Heidolph, Germany). The extract was then centrifuged at 5000×g for 5 min at 4 °C. The resulting supernatant was carefully collected and this extraction step was repeated three times. The combined organic phases were transferred to a separatory funnel. The aqueous phase was removed and the hexane phase containing the carotenoids was recovered. The final volume was recorded and the absorbance of the extract was measured.

Total carotenoids were quantified at 451 nm using the method described by Peng et al. [19]. The value of the molar extinction coefficient (ϵ_{mol}) of β -carotene was 139,500 L·mol⁻¹·cm⁻¹ [20] and the results were expressed as mg eq β -carotene per 100 g of dry matter (DM).

2.3.2.2. Anthocyanins. Ten g of loengo nectar was agitated for 10 min at 8 rpm using a rotary agitator. The mixture was centrifuged at $10,000\times g$ for 10 min at 4°C . The supernatant was recovered and filtered through a $0.45\text{ }\mu\text{m}$ syringe filter. The filtered extract was then analyzed by HPLC-DAD, adapting the method described by Mertz et al. [21].

The HPLC system used was equipped with a diode array detector (Agilent Technologies, 1200 Series). The separation was carried out using an ACE 5C₁₈ column ($250\text{ mm} \times 4.6\text{ mm}$, $5\text{ }\mu\text{m}$, Avantor, USA) at 30°C . The mobile phase consisted of the solvents A water Milli-Q/HCO₂H (99/1, v/v) and B acetonitrile. The injection volume was $20\text{ }\mu\text{L}$ and the mobile phase flow rate was set at $0.7\text{ mL}\cdot\text{min}^{-1}$ under the following linear gradient: 95% A and 5% B at 0 min, gradually increasing the proportion of solvent B to 15% at 20 min and to 25% at 50 min. At 57 min, a 100% B phase was applied and maintained until 60 min for column rinsing. The system was then returned to its initial conditions (95% A and 5% B) at 62 min, followed by re-equilibration until 72 min. Detection was performed at 510 nm. Concentrations were determined using standard solutions between 1.56 and 50 mg L^{-1} of cyanidin 3-O-glucoside (C3G) purchased from Carlo Erba (Val de Reuil, France).

2.4. Characterization of the aroma profiles

The determination of the aroma compounds was performed as described by Marin-Castro et al. [22] and da Costa et al. [3]. The volatile compounds were extracted from 3 g of homogenized loengo nectar using Headspace Solid-Phase Micro Extraction (HS-SPME). The samples were placed in sealed vials and equilibrated for 10 min at 50°C , followed by extraction under orbital agitation for 45 min using a $65\text{ }\mu\text{m}$ DVB (divinylbenzene), CAR (carboxen), and PDMS (polydimethylsiloxane) fiber (Supelco, Bellefonte, PA, USA). Analyses were performed on an Agilent 6890 series GC coupled with an Agilent 5973 MS (Agilent, USA) and with a Gerstel MPS-2 sampler. The separation was achieved on a DB-WAX capillary polar column of 60 m, 0.25 mm , and phase film thickness of $0.25\text{ }\mu\text{m}$ (J&W Scientific, Folsom, CA, USA) using H₂ as the carrier gas, set at a flow rate of $1.5\text{ mL}\cdot\text{min}^{-1}$.

The fiber was thermally desorbed at 250°C and the compounds were separated using the following program: initial oven temperature of 40°C (5 min), then increased by $2^{\circ}\text{C}\cdot\text{min}^{-1}$ to 140°C , and finally by $10^{\circ}\text{C}\cdot\text{min}^{-1}$ to 250°C (10 min). The transfer line and ion source were maintained at 150°C and 250°C , respectively. The data acquisition and identification were carried out using the MassHunter workstation software version B.06.00 (Agilent Technologies, USA) and the NIST 2011 (National Institute of Standard Technology) mass spectral library.

Among the 80 aroma compounds detected in the fruit pulp, 57 aroma compounds were tentatively identified by comparing their retention times and Kovats retention index with those in the aforementioned library. Only matches with a similarity score (match factor) $\geq 80\%$ were considered [3]. We then chose to only quantify the aroma compounds representing more than 0.5% of the total relative peak area. The main characteristics of the aroma compounds selected for this kinetic study are given in Table S1. Results were expressed as mg eq. (3)-heptanol·kg⁻¹ of fresh material (FM), a compound that was used as an internal standard. Limit of detection and quantification (LOD and LOQ) were calculated using the signal/noise ratio method as described by da Costa et al. [3].

2.5. Kinetic modeling of compounds during pasteurization and storage

The kinetics of carotenoids, anthocyanins and aroma compounds in loengo nectar were assessed assuming isothermal conditions and a first-order behavior. In all cases, the decrease in the logarithm of the concentrations over time could be represented by an affine function within the explored temperature-time range.

2.5.1. Basic equations

The isothermal evolution rate constants at temperature T (k_T) were determined from concentration data over time according to equations (4) and (5).

$$\text{For degradation: } \frac{C}{C_0} = e^{-k_T t} \quad (4)$$

$$\text{For production: } C - C_0 = (C_{\infty} - C_0)(1 - e^{-k_T t}) \quad (5)$$

$$\text{that can be written as } \frac{C}{C_0} = \left(\frac{C_{\infty}}{C_0} - 1\right)(1 - e^{-k_T t}) + 1 \text{ if } C_0 \neq 0 \quad (6)$$

where C_0 , C and C_{∞} are the initial concentration, the concentration at time t and the concentration at infinite time which was taken as the maximum concentration obtained during the trial.

The calculated k_T values were used to determinate the activation energy E_a and the rate constant at the reference temperature $k_{T_{ref}}$ according to the Arrhenius equation (7) [23].

$$k_T = k_{T_{ref}} \exp \left[\frac{-E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right] \quad (7)$$

where k_T is the rate constant at temperature T and $k_{T_{ref}}$ the rate constant at the reference temperature (min^{-1}), E_a is the activation energy ($\text{J}\cdot\text{mol}^{-1}$), R the perfect gas constant ($8.314\text{ J}\cdot\text{mol}^{-1}\text{ K}^{-1}$), T the absolute temperature (K) and T_{ref} the reference temperature chosen (343.15 K for pasteurization and 293.15 K for storage).

k_T value was only calculated in cases where a significant variation was observed at $p < 0.05$.

2.6. Statistical analysis and calculations

Statistical analyses were carried out using XLSTAT 2025 software (Addinsoft, Paris, France). Comparison between mean values were carried out using the Tukey HSD test ($p < 0.05$).

To identify the kinetic model parameters, non-linear optimization was chosen, involving minimizing the sum of squared deviations between the experimental and modeled values. This approach provides a more accurate estimation of the kinetic model parameters compared to the conventional logarithmic linearization methods [24]. This was performed using the Solver tool in Microsoft Excel with the “Generalized Reduced Gradient” non-linear method. To ensure rapid and reliable convergence of the calculations towards the solution, initialization was systematically performed using the values obtained by linear regression after logarithmic transformation. The robustness of the calculations was verified in a few randomly selected cases by varying the initial numerical value from 0.1x to 10x around the value determined by linear regression (in log). The rate constants (k_T) were first estimated from kinetics vs. time. Rate constants at reference temperature $k_{T_{ref}}$ and activation energies E_a were then assessed from the calculated k_T at different temperatures. The confidence intervals (CI) (at 95% probability) of the non-linear regressions were calculated using the macro bundle as described by De Levie (2001), according to Chivala and Munanga [25].

3. Results and discussion

3.1. Main characteristics of the fresh loengo nectars

The characterization of loengo nectars prepared in this study is described in Table 2.

The different nectar formulations presented physicochemical characteristics typical of fruit-based beverages. All samples had an acidic pH ($2.8 - 3.6$), within the expected range for nectars, which contributes to microbiological stability, color preservation, and sensory freshness. Total soluble solids were very close to the target value ($12\text{ g}\cdot 100\text{ g}^{-1}$).

Table 2

pH, Total Soluble Solids (TSS), color Lab*, polymeric color and anthocyanin contents of fresh loengo nectars.

Samples	pH	TSS (g·100 g ⁻¹)	L*	a*	b*	Polymeric color (%)	Anthocyanin (mg·100 mL ⁻¹)
Formulation							
AbM22	3.27	12.3	27.6 (0.5) ^d	26.9 (0.1) ^{bc}	9.2 (0.1) ^c	7.2 (0.1) ^c	7.4 (0.1) ^b
AbM22 A	2.84	11.9	28.0 (0.4) ^{cd}	29.2 (0.3) ^a	11.7 (0.3) ^a	7.9 (0.7) ^c	7.8 (0.3) ^{ab}
AbM22 BS	3.55	12.4	29.4 (0.2) ^a	26.5 (0.2) ^c	10.1 (0.1) ^b	13.3 (0.5) ^a	7.4 (0.1) ^b
AbM22 AS	3.24	12.3	29.0 (0.1) ^{abc}	28.8 (0.3) ^a	11.5 (0.2) ^a	9.3 (0.4) ^b	8.3 (0.1) ^a
AbM22 O	3.11	12.2	29.1 (0.6) ^{ab}	27.2 (0.1) ^b	10.2 (0.2) ^b	9.3 (0.3) ^b	6.8 (0.2) ^c
AbM22 AO	2.92	11.8	28.3 (0.1) ^{bcd}	28.9 (0.1) ^a	11.8 (0.1) ^a	7.8 (0.8) ^c	7.8 (0.4) ^{ab}
Origins							
AbM22	3.12	12.3	30.7 (0.2) ^a	31.1 (0.2) ^a	12.3 (0.1) ^a	12.7 (0.3) ^b	8.0 (0.3) ^b
AbHb22	3.20	12.8	30.6 (0.6) ^a	28.1 (0.2) ^b	10.5 (0.1) ^b	15.5 (0.4) ^a	6.1 (0.1) ^c
AbHa22	3.19	12.4	26.8 (0.2) ^b	28.8 (0.5) ^b	8.7 (0.3) ^c	11.3 (0.4) ^c	9.5 (0.1) ^a

Values with the same letter within the column were not significantly different, according to the Tukey test ($p < 0.05$). Mean and standard deviation in brackets with $n = 3$.

Color parameters (L*, a*, b*) showed slight variations, particularly in the intensity of red and yellow hues, reflecting differences in pigment composition. Anthocyanin content and polymeric color varied among formulations with average values of 7.7 (1.0) mg·100 mL⁻¹ and 10.5 (2.9) % respectively ($n = 9$).

3.2. Trends during pasteurization and storage of loengo nectar

The tests carried out in the most extreme operating conditions during pasteurization (100 °C/15 min) and storage (45 °C/15 days) showed that some compounds remained stable, while others degraded or increased in concentration. Table 3 presents a qualitative evolution of carotenoid, anthocyanin, and aroma compound contents, as well as the changes in CIE Lab* color parameters of loengo nectar during pasteurization and storage.

3.2.1. Trends during pasteurization

Pasteurization at 100 °C/15 min had an impact on the pigments present in the pulp. The treatment clearly damaged carotenoids and, to a lesser extent, anthocyanins. Nevertheless, color stability was observed

Table 3

Trends in the impact of pasteurization and storage on loengo nectar: pigment, color and main aroma compounds (AbM22 sample).

Criteria	Evolution trends	
	Pasteurization	Storage (after pasteurization)
	100 °C/15 min	85 °C/7 min 45 °C/15 days
Pigments and color		
Anthocyanins	−20%	−100%
Carotenoids	−50%	−30%
L*	not affected	+20%
a*	not affected	−60%
b*	not affected	+60%
C*	not affected	−30%
h*	not affected	+160%
% Polymeric color (% PC)	not affected	+900%
Aroma compounds		
Ethyl Acetate	not affected	−100%
Ethanol	+60%	−30%
Ethyl butyrate	−30%	−80%
Ethyl 2-methylbutanoate	−50%	−90%
Hexanal	+80%	not affected
Heptan-3-one	not affected	not affected
(E)-2-Hexen-1-al	not affected	−70%
Heptan-4-ol	not affected	not affected
Hexan-1-ol	+50%	not affected
5-Amino-1-methyl-1H-pyrazole-4-carboxamide	not affected	not affected
(Z)-hex-3-en-1-ol	−40%	not affected
(E)-hex-2-en-1-ol	−40%	−60%

when subjected to this heat treatment (Fig. S1). Anthocyanins are fairly thermostable due to the acidic pH of loengo nectar or even the presence of sugars, organic acids, and other phenolic compounds, which limit their degradation. Carotenoids, however, are sensitive to oxygen and temperature. This result suggests that the color of the product is mainly related to the anthocyanins and not so much to the carotenoids. It also affected aroma compounds, with the concentration of 7 of the 12 measured compounds changing: 2 esters and 2 unsaturated alcohols decreased while 2 other alcohols and 1 aldehyde increased.

The increase in the concentration of certain aroma compounds could result from the thermal degradation of volatile or non-volatile precursors, or from a better extractability of compounds linked to the heat treatment. Indeed, pasteurization induces an alteration of cell structures, in particular by the rupture of cell walls, thus facilitating the release of intracellular compounds initially bound to the matrix. The SPME extraction protocol used mainly targets free volatile compounds in the headspace. Heating, by breaking physical barriers, promotes the release of these compounds and may therefore explain the increase observed in the content of certain aroma compounds after pasteurization.

Surprisingly, one of the five stable compounds was 5-amino-1-methyl-1H-pyrazole-4-carboxamide. Indeed, the formation of this volatile compound is generally related to thermal reactions, notably the Maillard reaction, between reducing sugars and amino acids or other amino metabolites [26]. These reactions are known to generate several nitrogenous heterocyclic compounds, notably pyrazoles, pyrazines and imidazoles, particularly at acidic pH and in the presence of heat [27].

3.2.2. Trends in the impact of storage

Pigments were significantly damaged after 15 days storage at 45 °C (Table 3). Similar behavior was observed on color alteration. These results were in accordance with many studies available in literature as for example, Shi et al. [28] who showed that the rate of anthocyanin degradation increased during storage as the temperature rose, or Alighourchi and Barzegar [29] who observed that storage conditions significantly influence the color and pigment contents of pomegranate juice. During storage, these pigments may be susceptible to degradation, resulting in natural discoloration and also possible rearrangement such as condensation of phenolic compounds for instance, already demonstrated in roselle extracts [30]. It is generally accepted that non-enzymatic browning (Maillard's reaction) occurs at higher temperatures, so it is not likely that it occurs during storage.

Six aroma compounds were stable during storage while there was degradation of the other 6. This degradation can be explained by several mechanisms: alcohols and aldehydes are likely to undergo oxidation reactions in the presence of residual oxygen or metallic catalysts such as iron and copper naturally present in the matrices. As for esters, their reduction could result from acid hydrolysis, leading to the formation of alcohols and carboxylic acids. It was also showed that certain aroma

compounds can be generated during storage, such as aldehydes via the oxidation of lipids or carotenoids like in tomato [31], or certain esters, as in the case of Wujiuxiang pears [32]. Therefore, the diversity of the phenomena involved makes analyzing the results complex.

This first qualitative description enabled us to focus the subsequent kinetic study only on the compounds and criteria that varied during pasteurization and storage of the nectar.

At this point, we also observed that the stability of the pH of the pasteurized nectars, as well as the absence of gas production during storage, attested to the efficiency of the pasteurization process for microbiological stabilization, whatever the conditions used.

3.3. Kinetic study during pasteurization

Fig. 2 presents the evolution of anthocyanin and carotenoid contents of loengo nectar during pasteurization.

The results presented in Fig. 2a reveal a moderate degradation of anthocyanins only at 100 °C, with a loss of around 23% over 15 min. This result agreed with the work carried out by Brownmiller et al. [33] on blueberry juice, where a heat treatment at 95 °C for 3 min resulted in a 26% loss of total anthocyanins. For this family of compounds, several reactional pathways and mechanisms could be involved: hydrolysis of glycosidic bonds, opening of the pyrylium ring, oxidation of the flavylum nucleus, condensation with other phenolic compounds or Maillard products, and/or fragmentation of aglycones [34,35].

This behavior was also observed in carotenoids (Fig. 2b), which presented greater thermal sensitivity at 100 °C, with a loss of 54% after 15 min (only 14% at 70 °C for 60 min and only 2% at 85 °C for 20 min). This corroborates the observations reported by Becerra et al. [36] in food matrices, or Meléndez-Martínez et al. [37] in foods, that showed these pigments can be vulnerable to high temperatures due to their polyunsaturated structure. This thermolability makes them particularly unstable, especially through oxidation which is often linked to the presence of dissolved oxygen in food [38]. Nevertheless, heat sensitivity of carotenoids depends on their structure, the hydrocarbons often being more resistant than oxygenated xanthophylls. For example, Baç et al. [39] measured a loss of 26% and 30% of β -carotene respectively in tomato pulp and pink grapefruit juice, after a treatment at 80 °C lasting more than 2.5 h. The study reported by Dhuique-Mayer et al. [40] showed that the pasteurization treatment applied to citrus juice did not significantly alter provitamin A carotenoids (β -carotene and β -cryptoxanthin).

The representation of the experimental curves obtained using a first-order kinetic model was satisfactory with most confidence intervals between 10 and 18% for the pigment k_T (Table 4a). According to the review proposed by Pénicaud et al. [38], the calculated rate constants for carotenoids were within the lower range of values reported in the literature for citrus juices, which are often used as references due to their high carotenoid content. However, the activation energy obtained was higher, indicating that the rate of carotenoid degradation is more sensitive to temperature increases in loengo nectar than in citrus juices.

Fig. 3 shows some aromatic compounds that degraded and others that increased throughout the pasteurization process.

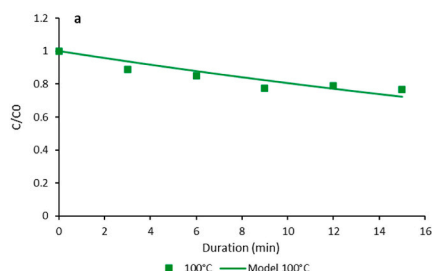


Table 4
Rate constants (k_T) and Arrhenius parameters corresponding to pigment degradation at different temperatures (a) and rate constants at 100 °C for aroma compound changes (b) in loengo nectars during pasteurization.

a				
Temperature (°C)	Anthocyanin k _T (min ⁻¹)	CI	Carotenoid k _T (min ⁻¹)	CI
70	-	-	2.93•10 ⁻³	4.34•10 ⁻⁴
85	-	-	2.56•10 ⁻³	9.36•10 ⁻⁴
100	2.16•10 ⁻²	2.28•10 ⁻³	7.66•10 ⁻²	1.36•10 ⁻²
k _{Tref} (min ⁻¹)	-	-	7.43•10 ⁻⁵	1.59•10 ⁻⁴
E _a (kJ•mol ⁻¹)	-	-	246.1	75.9

b		
Aroma compounds	k _{100°C} (min ⁻¹)	CI
Degradation		
Ethyl 2-methylbutanoate	5.14•10 ⁻²	4.66•10 ⁻³
Ethyl butyrate	1.61•10 ⁻²	5.45•10 ⁻³
(E)-hex-2-en-1-ol	3.56•10 ⁻²	5.58•10 ⁻³
(Z)-hex-3-en-1-ol	2.99•10 ⁻²	3.85•10 ⁻³
Production		
Hexanal	1.31•10 ⁻¹	3.79•10 ⁻²
Ethanol	2.73•10 ⁻¹	5.36•10 ⁻²
Hexan-1-ol	8.83•10 ⁻²	2.98•10 ⁻²

CI: Confidence interval.

The analysis of the experimental kinetics confirmed that pasteurization did not induce any significant variation for some aroma compounds such as heptan-4-ol, (E)-2-hexen-1-al, heptan-3-one and ethyl acetate (Fig. S2). However, a significative decrease in the contents of some esters and alcohols, such as ethyl 2-methylbutanoate, ethyl butyrate, (E)-hex-2-en-1-ol and (Z)-hex-3-en-1-ol was observed with losses at the end of the heat treatment of 49%, 33%, 42% and 39% respectively (Fig. 3a). These losses can be attributed to their known thermal instability, as shown by Cheng et al. [41]. What's more, some aroma compounds showed an increase in their concentration at 100 °C at the end of 15 min of treatment, including hexanal (82%) and ethanol (59%). This is surprising but it could be related to the degradation of the ethylic esters, and hexan-1-ol (47%) (Fig. 3b). In cashew apple juice, Zepka et al. [42] observed an increase in the concentrations of trimethylbenzene after pasteurization, reflecting thermal formation or release from matrix-bound precursors. These results confirm that heat treatment, although it leads to the degradation of some compounds, can also promote the formation of volatiles that can modify the sensory profile of the nectar.

The k_T values obtained (Table 4b) were fairly similar for the aroma compounds that degraded during processing (1.6 to $5.2 \cdot 10^{-2} \text{ min}^{-1}$). The k_T values were higher for the aroma compounds that were produced, particularly for hexanal ($13.1 \cdot 10^{-2} \text{ min}^{-1}$) and ethanol ($27.3 \cdot 10^{-2} \text{ min}^{-1}$).

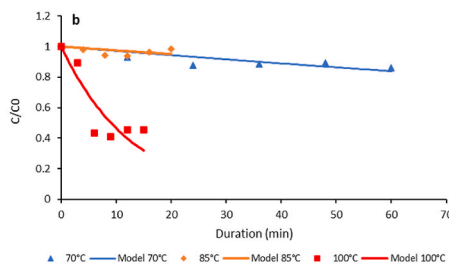


Fig. 2. Evolution of the pigment content during the pasteurization treatments: a) anthocyanins at 100 °C and b) carotenoids at 70 °C, 85 °C and 100 °C.

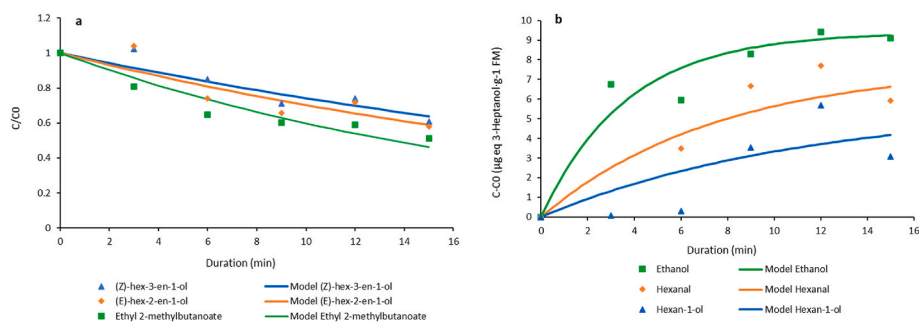


Fig. 3. Evolution of the content of some volatile compounds during pasteurization at 100 °C: a) degradation of (Z)-hex-3-en-1-ol, (E)-hex-2-en-1-ol and ethyl 2-methylbutanoate and b) formation of ethanol, hexanal and hexan-1-ol.

3.4. Kinetic study during storage

Anthocyanins degraded very strongly and quickly with losses from 58 to 100% over 15 days (Fig. 4a). Carotenoids underwent a reduction ranging from 23 to 86% after 35 days, with greater losses at elevated temperatures (Fig. 4b), while refrigerated storage showed a clear protective effect. Changes in color expressed as ΔE from the CIE Lab* parameters (Fig. 4c) were significant and aligned with pigment degradation. The color of the nectar gradually changed from red to brown, which occurred more quickly at higher storage temperatures (Fig. S3, Table S2). The increase in polymeric color (Fig. 4d) reflected the intensification of non-enzymatic browning reactions under higher temperatures. Overall, the results demonstrated that the origin of both chromatic and nutritional alterations lies in the instability of anthocyanins and carotenoids, with cold storage emerging as the most effective strategy to preserve loengo nectar stability over time.

Several studies have consistently demonstrated the influence of storage temperature and time on anthocyanin stability. Sinela et al. [18] conducted a study on *Hibiscus sabdariffa* extracts which revealed losses of cyanidin 3-O-sambubioside of 17% and 98% when the extracts were stored for 60 days at 4 °C and 37 °C respectively. Alighourchi and Barzegar [29] found losses of 94% and 98% for delphinidin 3,5-diglucoside and cyanidin 3-glucoside in pomegranate juice, stored at 37 °C for 210 days. Other studies confirmed low degradation in refrigerated conditions [43,44], in agreement with Touati et al. [14], who monitored anthocyanins in fruit nectars stored at 4, 25, and 37 °C for 28 days. Dündar and Akyildiz [45] further showed that storage time had a

significant effect on the total anthocyanin content of cloudy strawberry nectar, with approximately 50% of the degradation occurring within the first 20 days.

Once again, in the case of storage, the kinetics were described well by the first-order and Arrhenius models with confidence intervals ranging from 8 to 15% for the anthocyanin and carotenoid k_T (Table 5). For anthocyanins, the degradation rate doubled at 20 °C compared to 4 °C, was multiplied by almost 4 times at 30 °C and increased 15-fold at 45 °C (Table 5a). The increase in storage temperature therefore had a strong impact on the rate of degradation of these compounds. This result is consistent with studies conducted on plant extracts. However, in the case of loengo nectar k_T values were 10 times higher than those of *Hibiscus sabdariffa* extracts [18] or other fruit juices [44].

The carotenoids exhibit reduced stability during storage, which compromises their nutritional value in food matrices [46,47]. Processes such as heating and drying further accelerate carotenoid change through cis-trans isomerization and epoxide formation [47,48], with the heat, light, and molecular structure strongly influencing the extent of isomerization [49,50]. Jangir et al. [51] reported a decrease in the total carotenoid content that was observed during mango pulp storage, attributed to carotenoid oxidation.

For carotenoids, the degradation rate k_T increased 1.5 times at 30 °C and 5 times at 45 °C, compared to 4 °C. The rate constant at the reference temperature (20 °C) obtained for carotenoids was 20 times lower than that obtained for anthocyanins.

In loengo nectar, color is highly dependent on the concentration of anthocyanins, which are prone to degradation [52,53]. As observed in

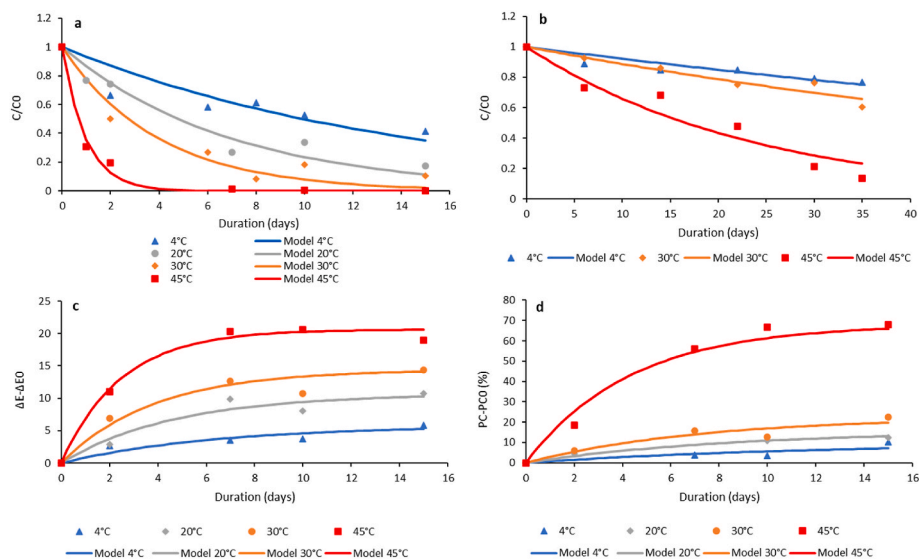


Fig. 4. Effect of storage temperature after pasteurization at 85 °C for 7 min on: a) anthocyanins, b) carotenoids, c) color difference (ΔE) and d) polymeric color (% PC). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 5
Rate constants (k_T) and Arrhenius parameters corresponding to pigment and aroma compound degradation in loengo nectars during storage.

a										
Temperature (°C)	Anthocyanins k_T (d ⁻¹)	CI	Carotenoids k_T (d ⁻¹)	CI	ΔE k_T (d ⁻¹)	CI	% PC k_T (d ⁻¹)	CI		
4	6.97•10 ⁻²	1.08•10 ⁻²	8.20•10 ⁻³	8.44•10 ⁻⁴	1.56•10 ⁻¹	3.50•10 ⁻²	8.08•10 ⁻²	2.56•10 ⁻²		
20	1.45•10 ⁻¹	1.90•10 ⁻²	-	-	2.14•10 ⁻¹	4.50•10 ⁻²	1.16•10 ⁻¹	8.43•10 ⁻³		
30	2.54•10 ⁻¹	3.60•10 ⁻²	1.20•10 ⁻²	1.03•10 ⁻³	2.64•10 ⁻¹	5.57•10 ⁻²	1.39•10 ⁻¹	2.71•10 ⁻²		
45	10.4•10 ⁻¹	8.62•10 ⁻²	4.17•10 ⁻²	5.05•10 ⁻³	4.04•10 ⁻¹	4.75•10 ⁻²	2.31•10 ⁻¹	3.00•10 ⁻²		
k_{Tref} (d ⁻¹)	1.13•10 ⁻¹	2.58•10 ⁻²	6.71•10 ⁻³	3.70•10 ⁻³	2.18•10 ⁻¹	1.03•10 ⁻²	1.16•10 ⁻¹	7.89•10 ⁻³		
E_a (kJ•mol ⁻¹)	68.6	7.3	56.2	17.9	18.4	0.1	20.6	2.7		
b										
Temperature (°C)	Ethyl acetate k_T (d ⁻¹)	CI	Ethanol k_T (d ⁻¹)	CI	Ethyl butyrate k_T (d ⁻¹)	CI	Ethyl 2-methylbutanoate k_T (d ⁻¹)	CI	(E)-2-hexen-1-ol k_T (d ⁻¹)	CI
4	1.46•10 ⁻²	7.09•10 ⁻³	1.25•10 ⁻²	3.67•10 ⁻³	1.20•10 ⁻²	4.21•10 ⁻³	1.18•10 ⁻²	4.76•10 ⁻³	1.42•10 ⁻³	1.98•10 ⁻³
20	6.36•10 ⁻²	1.49•10 ⁻²	1.42•10 ⁻²	3.58•10 ⁻³	6.71•10 ⁻²	8.85•10 ⁻³	5.42•10 ⁻²	6.63•10 ⁻³	2.77•10 ⁻²	2.66•10 ⁻³
30	9.01•10 ⁻²	9.77•10 ⁻³	-	-	2.60•10 ⁻¹	9.97•10 ⁻²	2.72•10 ⁻¹	1.09•10 ⁻¹	7.75•10 ⁻²	8.74•10 ⁻³
45	2.21•10 ⁻¹	1.01•10 ⁻²	2.27•10 ⁻²	3.60•10 ⁻³	-	-	3.52•10 ⁻¹	3.55•10 ⁻²	1.34•10 ⁻¹	1.66•10 ⁻²
k_{Tref} (d ⁻¹)	5.36•10 ⁻²	5.85•10 ⁻³	1.32•10 ⁻²	3.06•10 ⁻³	6.86•10 ⁻²	5.34•10 ⁻³	1.12•10 ⁻¹	5.05•10 ⁻²	3.55•10 ⁻²	1.50•10 ⁻³
E_a (kJ•mol ⁻¹)	43.8	3.7	12.3	9.9	98.3	6.0	37.3	15.8	42.1	3.4

i: Confidence interval.

CI: Confidence interval.

roselle extracts by Cisse et al. [54], elevated storage temperatures accelerate this process, reducing the characteristic red color and leading to browning.

The k_{Tref} values associated with color variation (ΔE) and polymeric color (PC) were similar to those of anthocyanins. It therefore confirmed that the degradation of anthocyanins was mainly responsible for color changes during storage. In terms of activation energy (E_a), the values obtained for the two pigment families were similar. However, they were about three times lower for color indicators. This showed that color sensitivity to temperature is less pronounced than anthocyanin content sensitivity.

During storage, some aroma compounds experienced a significant decrease in their concentrations (Fig. 5) such as ethyl acetate (47 to 100%), ethanol (19 to 52%) and ethyl butyrate (25 to 76%). Ethyl 2-methylbutanoate saw its concentrations decrease by 95%, (E)-2-hexen-1-al from 3 to 85% and (E)-hex-2-en-1-ol from 23 to 83%. These variations were mainly observed between 18 and 30 days of storage.

Regarding the aroma compounds (Table 5b), k_{Tref} values range from 0.13 to 1.12 d⁻¹. The three esters exhibited the highest rates, particularly ethyl 2-methylbutanoate, while alcohols exhibited the lowest rates. The activation energy ranged from 13 kJ•mol⁻¹ for ethanol to 98 kJ•mol⁻¹ for ethyl butyrate.

3.5. Evolution of the anthocyanin content and color of loengo nectar during storage according to the raw material origin

After 15 days of storage at 30 °C, more losses were observed in the sample from Malanje (99.7%), compared to those from Huíla (90.5%) and Huambo (82.1%) (Fig. 6a). The k_T for the sample from Malanje (AbM22) was approximately 2 times higher than that from Huambo (AbHb22) and 5.6 times higher than that from Huíla (AbHa22) (Table 6), indicating significant increased instability of anthocyanins in sample AbM22. This pigment loss resulted in an increase in the total color difference (ΔE), higher for Malanje (26.8) than for Huíla (13.7) and Huambo (10.9) (Fig. 6b). Color change was visually detected within the first 24 h ($\Delta E > 5$).

The percentage of polymeric color (% PC) reached 59.6% for Malanje, compared to 23.4% and 13.4% for Huíla and Huambo, respectively (Fig. 6c). The nectar underwent condensation of monomeric anthocyanins, which leads to the formation and increase of polymeric compounds responsible for browning [18]. The results described by Li et al. [55] indicated that condensation reactions of anthocyanins form other phenolic compounds, that absorb at low wavelengths in the visible spectra and therefore appear brown.

The stability of anthocyanins is influenced by several parameters, including the specific nature of the anthocyanins, the storage temperature and the origin of the juice, as reported by Hellström et al. [56]. This differential sensitivity between samples could be attributed to factors such as climate, soil, genotype, harvest season and maturity stage, influencing the initial composition and stability of the anthocyanins.

Sinela et al. [30] showed in *Hibiscus sabdariffa* extracts that monomeric anthocyanins are lost while polymeric color increased during storage, which is consistent with our observations. These authors demonstrated that anthocyanins were mainly degraded through condensation reactions following oxidation catalyzed by certain metal ions. These reactions are strongly influenced by the composition of the extracts, especially their metal-ion content.

Based on the detailed composition of the three fruit samples used, already determined in a previous study [3], we applied principal component analysis (PCA) to investigate correlations between the rates of anthocyanin degradation or color changes (ΔE and polymeric color PC), and nectar composition, considering the main sugars (sucrose, glucose and fructose), organic acids (citric and malic acids) and minerals (P, K, Ca, Mg, Na, Cu, Fe, Mn, Zn and B).

Fig. 7 presents the PCA results of anthocyanin degradation rates in loengo nectars relative to the overall loengo composition, revealing that

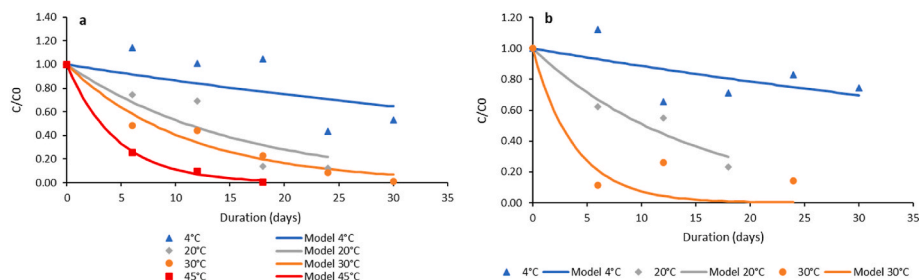


Fig. 5. Effect of storage at different temperatures for 30 days, after pasteurization at 85 °C for 7 min: a) evolution of ethyl acetate and b) ethyl butyrate.

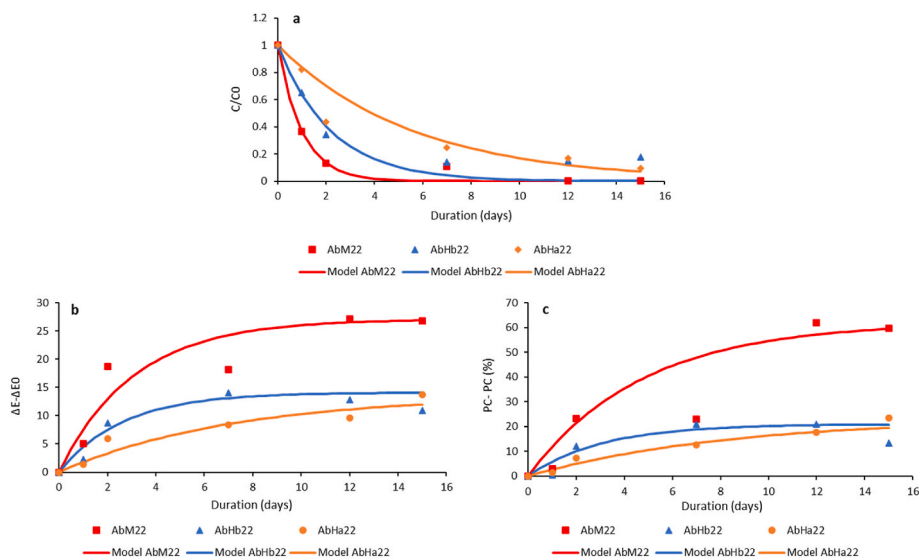


Fig. 6. Effect of storage at 30 °C/15 days, after pasteurization at 85 °C/7 min: a) anthocyanin evolution, b) total color difference (ΔE) evolution and c) polymeric color (% PC) evolution, according to the origin of the raw material. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 6

Rate constants (k_T) of pigment and color degradation in loengo nectars from raw materials of different origins during storage at 30 °C for 15 days.

Origin	Anthocyanins k_T (d^{-1})	CI	ΔE k_T (d^{-1})	CI	% PC k_T (d^{-1})	CI
Malanje (AbM22)	$10.00 \cdot 10^{-1}$	$1.05 \cdot 10^{-1}$	$3.23 \cdot 10^{-1}$	$9.65 \cdot 10^{-2}$	$2.11 \cdot 10^{-1}$	$2.93 \cdot 10^{-2}$
Huambo (AbHb22)	$4.54 \cdot 10^{-1}$	$1.07 \cdot 10^{-1}$	$3.80 \cdot 10^{-1}$	$7.19 \cdot 10^{-2}$	$3.26 \cdot 10^{-1}$	$8.88 \cdot 10^{-2}$
Hufla (AbHa22)	$1.78 \cdot 10^{-1}$	$1.00 \cdot 10^{-2}$	$1.39 \cdot 10^{-1}$	$2.33 \cdot 10^{-2}$	$1.19 \cdot 10^{-1}$	$1.02 \cdot 10^{-2}$

CI: Confidence interval.

the first two principal components account for exactly 100% of the variance.

The first component (PC1) was positively correlated with polymeric color production rate, total color difference production rate, concentrations of sucrose, copper, iron, calcium, malic acid, fructose, glucose, and anthocyanin degradation rate. It is anti-correlated with magnesium, pH and citric acid that act as stabilizing agents for anthocyanins. The second component (PC2) was positively related to only three minerals (phosphorus, sodium and zinc).

It was possible to observe that the projection of the individuals showed a tendency towards agglomeration of compounds coming from the same area. However, zinc, sodium, boron, and magnesium remained isolated, indicating the absence of significant differences between the samples for these specific compounds.

These results agreed with previous studies revealing that anthocyanins are highly reactive to some metals and can be oxidized by copper and iron [30]. Other studies on the interactions between metals and anthocyanins in plants mentioned that stable blue colors are produced through the formation of complexes between anthocyanins and certain

metals, such as aluminum, copper, iron, molybdenum and tin [57,58]. Sinilal et al. [59] also found that magnesium increased the accumulation and stability of anthocyanin in red grapes. Therefore, it can be seen that metals, particularly copper and iron, could have a harmful or stabilizing effect on anthocyanins. However, few recent studies have addressed this issue, and metal-anthocyanin interactions warrant further investigation. According to Hubbermann et al. [60] fructose has been shown to accelerate anthocyanin degradation due to the formation of sugar degradation products. In the present study, calcium content was correlated to the degradation of anthocyanins, which was also reported by Asare et al. [61] on strawberry puree fortified with calcium.

Finally, reducing sugars can also react with anthocyanins through Maillard-like reactions and promote non-enzymatic browning [17,62, 63]. Anthocyanin degradation is also triggered by metal ions (Cu and Mg), which can catalyze oxidation, thus accelerating nectar discoloration. Samples from Hufla have higher levels of citric acid and pH. The influence of high concentrations of citric acid on anthocyanin degradation, as well as that of low pH is confirmed by Buvé et al. [17,64] and Weber and Larsen [65]. Furfural compounds, which are byproducts of

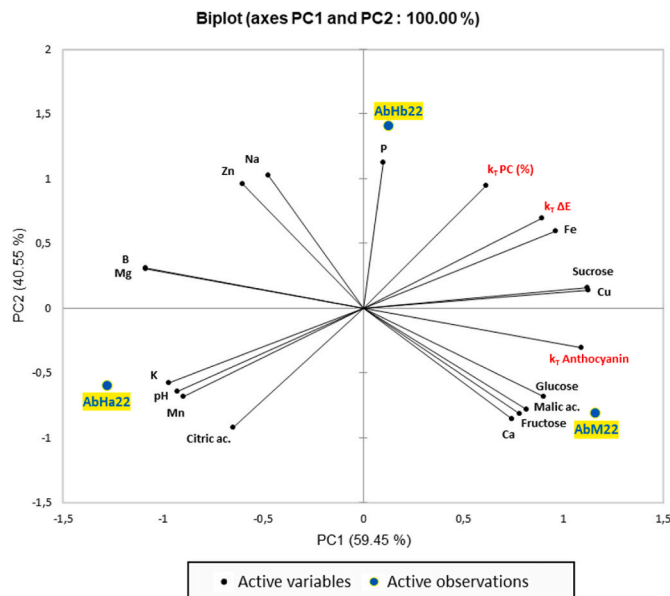


Fig. 7. Principal component analysis (PCA) based on anthocyanin degradation in nectars relative to the overall composition of loengo.

sugar degradation, are created during processing and storage of anthocyanin-rich juices. Therefore, there is a correlation between the anthocyanin degradation constant and the amount of sugar in the juices as supported by Song et al. [66].

3.6. Evolution of anthocyanin content and color of loengo nectar during storage according to the formulation

Fig. 8 presents the evolution of anthocyanin concentration and color during storage at 30 °C for 15 days using different additives or deaeration.

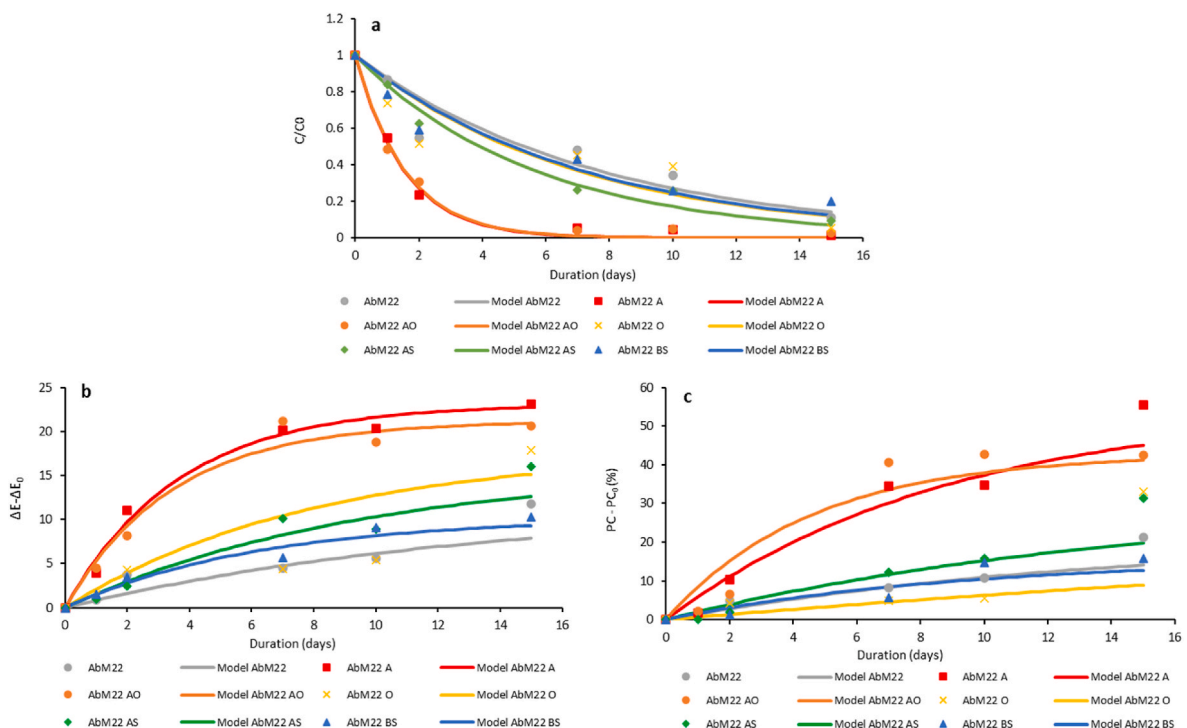


Fig. 8. Effect of storage at 30 °C of different formulations after pasteurization at 85 °C for 7 min, a) anthocyanin, b) total color difference (ΔE) and c) polymeric color (% PC) evolutions. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The kinetics of anthocyanin degradation obtained in alkalinized (AbM22 BS) and deaerated (AbM22 O) nectars were very close to the reference (AbM22) (Fig. 8, Table 7). Higher pH or a lower dissolved O_2 content therefore did not slow down their degradation. The addition of ascorbic acid did not have much effect either on the kinetics and even tended to slightly accelerate degradation. When the product was acidified, the degradation of anthocyanins was much faster regardless of the dissolved O_2 content of the samples (AbM22 A and AbM22 AO). The rate constants were thus multiplied by 5 in the presence of exogenous citric acid compared to the reference. These results are surprising because, in theory, lower pH values shift the equilibrium towards the flavylium ion, the most stable form of anthocyanins [64,67]. The same trends found for anthocyanins were logically found with the color indicators, ΔE and PC.

These results contradicted those presented in other studies. For example, according to Walkowiak-Tomczak and Czapski [68], after 30 days at 20 °C, anthocyanin losses from red cabbage were significantly higher under aerobic conditions (47% at pH 3 and 70% at pH 5) than under anaerobic conditions (7% at pH 3 and 33% at pH 5), highlighting the degradative effect of oxygen on anthocyanin stability, especially at higher pH. The presence of oxygen can lead to rapid degradation of anthocyanins by oxidation mechanisms [69,70].

Gérard et al. [71] reported that ascorbic acid, a powerful antioxidant, can also accelerate the thermal degradation of anthocyanins in an acidic medium depending on its concentration. According to Patras [72], ascorbic acid can activate molecular oxygen, generating free radicals that degrade the pyrylium nucleus of anthocyanins. Walkowiak-Tomczak and Czapski [68] reported that the destabilizing effect of ascorbic acid on anthocyanins in red cabbage could be due to the formation of hydrogen peroxide during its oxidation to dehydroascorbic acid. Furthermore, Ojwang and Awika [73] observed that, under the influence of ascorbic acid, red cabbage anthocyanins suffered a color loss of 85%, 82%, and 30% after 21 days at pH 2, 3.2, and 5, respectively.

However, it is difficult to provide additional explanations, given the variety of mechanisms involved in anthocyanin degradation and the complexity of the reaction medium in which they occur. One likely

Table 7

Rate constants (k_T) of pigment and color degradation in loengo nectars of different formulations during storage at 30 °C/15 days.

Additives/deaeration	Anthocyanins k_T (d ⁻¹)	CI	ΔE k_T (d ⁻¹)	CI	PC (%) k_T (d ⁻¹)	CI
AbM22 (reference)	1.30•10 ⁻¹	2.32•10 ⁻²	1.10•10 ⁻¹	2.84•10 ⁻²	7.22•10 ⁻²	6.93•10 ⁻³
AbM22 A	6.55•10 ⁻¹	4.59•10 ⁻²	2.72•10 ⁻¹	2.50•10 ⁻²	1.12•10 ⁻¹	9.45•10 ⁻³
AbM22 AO	6.45•10 ⁻¹	4.67•10 ⁻²	2.89•10 ⁻¹	4.09•10 ⁻²	2.19•10 ⁻¹	5.77•10 ⁻²
AbM22 O	1.42•10 ⁻¹	3.36•10 ⁻²	1.25•10 ⁻¹	3.13•10 ⁻³	2.12•10 ⁻²	2.94•10 ⁻³
AbM22 AS	1.76•10 ⁻¹	1.63•10 ⁻²	1.04•10 ⁻¹	1.14•10 ⁻²	6.66•10 ⁻²	4.54•10 ⁻³
AbM22 BS	1.40•10 ⁻¹	2.11•10 ⁻²	1.60•10 ⁻¹	1.99•10 ⁻²	1.10•10 ⁻¹	2.84•10 ⁻²

CI: Confidence interval.

hypothesis is that the fruit's high content of certain multivalent cations, such as iron or aluminum, could generate condensation reactions after quinone formation by oxidation, as has already been shown in the case of bissap [30]. Of course, a more in-depth analysis of the mineral composition of the beverage and of the reaction products would be necessary to explore this chemical pathway. Another way to verify the role of metal ions in the reaction mechanisms could be to use a specific chelating agent to lower the concentration of free multivalent cations. Among the chelators available, EDTA is excluded because it is not active at the low pH of the nectar. The use of phytic acid could be considered, but it must be verified that this compound does not interfere with other elements present in the product.

4. Conclusions

Under standard pasteurization conditions ($T \leq 85$ °C), anthocyanins and carotenoids exhibited minimal degradation, preserving the original color of the beverage. Additionally, the aromatic profile of the nectar was altered by heat treatment, some aroma compounds degraded, while others were newly formed. During storage, temperature significantly influenced pigment stability. Nectar color evolution was correlated to anthocyanin concentration and to the formation of brown compounds involving polyphenols. Aroma compound stability varied, with certain esters showing heightened sensitivity. The degradation kinetics of anthocyanins and of color changes were well represented by first-order and Arrhenius models. Kinetic parameters calculated during storage indicated that anthocyanins in loengo nectar degraded much more rapidly than in other fruit-based beverages, suggesting matrix-specific effects. Neither the antioxidant addition, deaeration nor acidification improved anthocyanin stability. The degradation rates of anthocyanins and color were correlated with Fe and Cu content that suggested the involvement of metal cation-catalyzed condensation reactions.

The instability of anthocyanins and color degradation during storage at ambient temperature present significant challenges for the industrial-scale production of loengo nectar. Therefore, refrigerated storage at 4 °C is recommended. A complementary study is required to assess the efficacy of varying preservative concentrations in delaying color evolution. Further research should focus on more detailed characterization of the metal ion composition of the nectar and of the anthocyanin degradation products in order to better understand the reaction pathways involved. The impact of changes in composition during storage on the organoleptic and nutritional quality of the product should also be quantified. Finally, the Weibull model could be compared to the classical first-order model, using a larger experimental dataset, with the purpose of better forecasting the evolution of the product over time.

CRedit authorship contribution statement

Famorado João da Costa: Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation. **Christian Mertz:** Writing – review & editing, Visualization, Validation, Methodology, Data curation. **Adrien Servent:** Writing – review & editing, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Nawel Achir:** Writing – review & editing, Visualization, Validation, Software, Methodology, Formal analysis, Data curation,

Conceptualization. **Bettencourt Munanga:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Manuel Dornier:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jafr.2026.102810>.

Data availability

Data will be made available on request.

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