Innovative Food Science and Emerging Technologies 95 (2024) 103724

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Maximising the stability of anthocyanins and the physicochemical quality in pure blood orange juice using advanced Weibull-Log-Logistic modelling and ascorbic acid fortification during pasteurisation

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ARTICLE INFO

Keywords:
Anthocyanins
Blood orange juice
Weibull-Log-Logistic model
Ascorbic acid
Pasteurisation
Physicochemical quality

ABSTRACT

Anthocyanins are natural pigments plentiful in Blood Orange Juice (BOJ), that give juice colour with highly beneficial nutrients. However, pasteurisation can affect anthocyanin stability and quality. This study aimed to investigate the effect of Ascorbic Acid Fortification (AAF) (100 ppm, "0.01%") varying temperature/time pasteurisation (60 to 90 °C) on the kinetics of anthocyanins degradation and the physicochemical quality of pure BOJ. Anthocyanin content was determined using the pH-differential method. The degradation kinetics was well predicted using the Weibull model with optimized n values (fixed values) ($0.824 \leq R^2_{adj} \leq 0.952$; $0.10 \leq$ RMSE ≤ 0.01). Findings showed that AAF improved anthocyanins retention, but increased thermal sensitivity over time. Furthermore, the Log-Logistic model accurately explains temperature-dependent anthocyanin rate constants during pasteurisation for BOJ with and without added ascorbic acid ($0.828 \leq R^2_{adj} \leq 0.991$; $0.0002 \leq$ RMSE ≤ 0.0019). This study provides a novel approach for maximising pure BOJ pasteurisation parameters, using an advanced Weibull-Log-Logistic modelling approach and AAF.

1. Introduction

Citrus fruits are among the most notable acid fruits globally; organic acids and sugars primarily comprise the constituents of their soluble solids, which serve as measures of the index of flavour quality and maturity (Nour, Trandafir, & Ionica, 2010). Citrus fruits have experienced a considerable increase in production over the last few decades. From 2011 to 2019, it averaged an annual production of 126 million tonnes in 2011, which surged to roughly 144 million tonnes in 2019. Likewise, Algeria's production also witnessed a rise from 1.1 million tonnes to 1.6 million tonnes during the same period (FAO, 2021).

The 'Tarocco', 'Sanguinello' and 'Moro' blood orange varieties belonging to the *Citrus sinensis* (L.) Osbeck spices are extensively grown in Mediterranean regions; among these, the 'Moro' variety stands out as the most vibrant in terms of colouration (Legua, Modica, Porras, Conesa, & Continella, 2022). The dominant red (or burgundy) hue of blood oranges can be primarily attributed to the presence of the anthocyanin (ANC) pigment (Cebadera-Miranda et al., 2019). Fresh blood orange juice's market success can be attributed to its wonderful taste and high nutritional value (Licciardello, Rizzo, Fallico, & Biagio., 2018). Specifically, the elevated levels of vitamin C, flavanones, and hydroxycinnamic acids (Derossi, De Pilli, & Fiore, 2010); alongside the presence of citric acid, malic acid, and small quantities of tartaric acid, oxalic acid, succinic acid and benzoic acid (Nour et al., 2010).

Blood oranges grown in Algeria are primarily consumed as fresh fruit juice due to their bright red colour, which is an important factor

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https://doi.org/10.1016/j.ifset.2024.103724
Received 5 January 2024; Received in revised form 20 May 2024; Accepted 28 May 2024
Available online 31 May 2024

affecting consumer appeal and product marketability (Titta et al., 2009). Therefore, a minimum blood orange juice anthocyanins (*ANC*) level should be guaranteed for the entire commercial duration of a product (Licciardello et al., 2018). However, acquiring long-lasting colour in processed fruits and juices provides challenges during processing and storage (Yu, Lin, Zhan, He, & Zhu, 2013). In fruit juice technology, thermal treatment (pasteurisation) is applied most frequently to preserve and extend the shelf life of the products, although temperature is one of the most crucial factors affecting anthocyanin (*ANC*) stability (Fischer, Carle, & Kammerer, 2013). Numerous factors, including pH, temperature, anthocyanin and protein concentration, light, oxygen, enzymes, and metallic elements, affect the stability of anthocyanin (Patras, Brunton, O'Donnell, & Tiwari, 2010). Conversely, it is worth noting that heat pasteurisation of blood orange juice has been found to be effective in specific circumstances (Cisse, Vaillant, Acosta, Dhuique-Mayer, & Dornier, 2009; Kırca & Cemeroğlu, 2003). However, food producers must ensure minimal losses in food quality during processing steps like pasteurisation. Therefore, the time and temperature conditions used for pasteurisation need to be optimized, as studies show high degradation of anthocyanins at certain thermal processing conditions (Patras et al., 2010). For that, there is a need to re-examine current industrial thermal processing methods to better retain anthocyanins and quality (Patras et al., 2010).

For this reason, degradation kinetic modelling assumes a vital role in predicting and controlling physicochemical parameter changes that occur throughout the thermal processing. Moreover, the empirical approach of kinetic modelling, which relies on reaction order (yielding zero-, half-, first-, or second-order reaction modelling), is highly appropriate (Patras et al., 2010). Also, Cisse et al. (2009) have enhanced Arrhenius, Ball, and Eyring's capability as a secondary model to predict the effect of temperature on the anthocyanin (*ANC*) degradation rates from blood orange, blackberry, and roselle. In order to guarantee statistical precision, it is more desirable to employ the nonlinear regression technique for the estimation of kinetic parameters; which arises from the fact that transforming nonlinear equations into linear representations inherently alters the error distribution, thereby possibly breaching the assumptions of error variance and normality in the conventional least squares estimation (Chowdhury & Das Saha, 2011). For this purpose, the most well-known semi-empirical Arrhenius model (with his two experimental parameters, frequency factor and activation energy) in food chemistry, is a widely used tool for describing the kinetic constants' temperature dependence. Nevertheless, a thorough theoretical inquiry into the Arrhenius equation may unravel intriguing uncertainties (Van Boekel, 2008): (*i*) The association between the two approximated parameters usually exhibits a remarkably high correlation and in many cases, is practically equal to one, thereby rendering the precise determination of parameter values exceedingly challenging (Peleg, Corradini, & Maria, 2012). (*ii*) The assumption that all complex biochemical reactions, despite the several (and interacting) pathways that they can follow, should have a single 'energy of activation' and not a varying spectrum of energies of activation. Moreover, the reported energies of activation of biochemical reactions have rarely, if ever, been confirmed by independent tests such as calorimetry (Corradini & Peleg, 2006). (*iii*) The Arrhenius, Eyring-Polanyi and Williams-Landel-Ferry models (WLF model applied if the curvilinear plots of the Arrhenius relationship were obtained) all require that complex reaction rates (rates of the combination of all intermediate reactions) should be only temperature dependant but time-independent (Peleg et al., 2012; Peleg, Corradini, & Normand, 2004). However, for reactions and processes that have an optimal or threshold temperature (T_c), neither the original Arrhenius equation nor the Ball model are applicable and ought to be replaced (Peleg et al., 2012).

However, due to the complexity of the reactions in the food system and the low flexibility of the reaction order approach; especially when assuming that all the deterioration reactions are simple reactions in which both reactants and products remain unchanged (Amodio, Derossi, Mastrandrea, & Colelli, 2015); which might result in a decrease in the goodness of fit (Odriozola-Serrano, Soliva-Fortuny, Gimeno-Añó, & Martín-Belloso, 2008). Moreover, integer values of kinetic order are traditionally used to perform fitting procedures, but some degradation reactions could be better described by fractional values (Derossi et al., 2010). Consequently, the purpose of an alternative approach like 'Weibull distribution' may be a helpful tool to better pinpoint kinetic degradation parameters (Corradini & Peleg, 2006). According to Corradini's article, such models are no better or worse than regularly used models of a given order, and they are clearly worth investigating (Van Boekel, 2008). The main advantage of the Weibull model is its versatility, which enables it to represent a wide range of degradation reactions as cumulative forms of the temporal distribution of failure phenomena (Mizrahi, 2004). Therefore, the Log-logistic model (a purely empirical model) was advanced for describing the temperature dependence of the Weibullian rate constant ($b(T)$). The main reasons for choosing the Log-logistic model were (*i*) to show that it can be used for low as well as high temperatures and in regions, where the transition between the low and high rate degradation occurs, and (*ii*) that the assumption of a fixed energy of activation required by the Arrhenius model is unnecessary (Corradini & Peleg, 2006).

Despite the wide works devoted to studying the thermal degradation of anthocyanin (*ANC*) from blood orange juice (Cisse et al., 2009; Kırca & Cemeroğlu, 2003; Lo Scalzo, Iannoccari, Summa, Morelli, & Rapisarda, 2004) or model juice (Cao, Liu, & Pan, 2011), the effect of ascorbic acid fortification on the physicochemical as well as anthocyanins (*ANC*) stability content of blood orange juice during thermal processing (pasteurisation) has yet to be reported. Therefore, the study aimed (*i*) to evaluate the effect of the temperature/time pasteurisation and ascorbic acid fortification (at 100 ppm) on the kinetics anthocyanins degradation; (*ii*) to determine the physicochemical quality variation (pH, total dry matter, titratable acidity and soluble solid content) and anthocyanins (*ANC*) kinetic parameters from blood orange juice (*Citrus sinensis* [L.] Osbeck) during pasteurisation at various temperatures, by using Weibull-Log-logistic model (Well model). This approach will provide knowledge about the thermal stability of *ANC* content and physicochemical quality to provide the best conditions for developing more healthy pasteurised blood orange juice.

2. Materials and methods

2.1. Preparation of blood orange juice

Blood oranges "Moro" (*Citrus sinensis* [L.] Osbeck) were harvested at optimal maturity from agricultural fields in Algeria as outlined by Remini et al. (2015). Briefly, the freshly pressed juice was filtered and analysed for its physicochemical characteristics and total monomeric anthocyanin content, then frozen in HDPE (high-density polyethylene) plastic bottles at −20 °C.

2.2. Thermal treatment processing of blood orange juice

For the thermal processing (pasteurisation), frozen juice samples were thawed to room temperature ($T^{\circ} = 23 \pm 1^{\circ}C$). After homogenisation, the thawed squeezed juice (which represents the Non-Fortified Control Juice "NFCJ") was fortified with 100 mg L^{-1} of ascorbic acid. The two types of juice were equally divided (10 mL) into sealed Pyrex tubes (100 mm × 16 mm) to maintain an equal headspace. The juice samples were pasteurised under four isothermal conditions (60, 70, 80, and 90 °C) for different holding times (from 0 to 180 min) by immersion of sealed Pyrex tubes in a thermostatic and stirred oil bath (Memmert type-ONE 7, Schwabach, Germany) previously set at the desired temperature (Table S1).

Juice temperature variations (ΔT *(t)*, °C) of the cold spot (slowest heating point) within a sealed Pyrex tube (located in the middle of the thermostatic oil bath) were recorded every five seconds by a Type-K

external thermocouple probe. The external thermocouple probe is placed at the geometrical centre (considered to be the cold spot or slowest heating point in a cylindrical can with a minimal headspace) of one tube and connected to a temperature data logger (Testo 175-T3, Testo AG, Lenzkirch, Germany) to record the time-temperature history. Testo Comfort Software Basic 5.0 (Testo AG, Lenzkirch, Germany) retrieved the time-temperature profiles ($T\ (t)$), also called heat penetration data. The recorded three stages of thermal history (Fig. 1) included: (i) the heating up or come-up stage, during which the retort reaches processing temperature; (ii) the maintaining or set-up stage, during which the retort was held at the same processing temperature level for a designated time and (iii) cooling dawn stage, during which the retort temperature was lowered to 50 °C. Each time-temperature profile ($T\ (t)$; Fig. 1) was fitted with a cubic smoothing spline (Matlab ver. 9.4 "2018 a", The MathWorks Inc., Natick, Massachusetts, USA). The duration required for the juice to attain the set-up temperature was <2.5 min, whereas the period of cooling amounted to approximately 1 min. Consequently, the duration for heat transfer was potentially negligible, thus allowing for the possibility of an isothermal treatment.

After each temperature-time processing, Pyrex tubes (three replicates at the same time, randomly chosen) were quickly cooled down in an ice water bath to minimise the thermal effect during cooling. The thermally processed juices were stored in amber-sealed vials of 20 mL capacity volume and kept frozen (at $T^{\circ} = -20 \pm 1^{\circ}C$) until analysed as described below.

2.3. Calculation of pasteurisation severity parameter

The severity parameter of thermal treatment, based on Biglow equation (Eq. (1)), for Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg L^{-1} (AAFJ-100 "0.01%") was determined. The calculation of the Pasteurisation Value (PV), a crucial indicator of the thermal load in relation to microorganisms, can be achieved by utilizing Eq. (2). with a z-value of 10 °C and a reference temperature of 70 °C (Heinz, Toepfl, & Knorr, 2003). The z-value signifies the temperature change required to alter the decimal reduction time (D) by one order of magnitude.

$$PV = F_0 = F_{70^{\circ}C}^{10^{\circ}C} = \int_0^t 10^{\left(\frac{T-T_{ref}}{z}\right)} dt = \int_0^t 10^{\left(\frac{T-70}{10}\right)} dt \text{ with } T = f(t) \quad (1)$$

$$PV = F_0 = F_{70^{\circ}C}^{10^{\circ}C} = \sum_{i=1}^{n} 10^{\left(\frac{T_i-70}{10}\right)} \Delta t \quad (2)$$

Where: $PV = F_0 = F_{70^{\circ}C}^{10^{\circ}C}$ = the integrated pasteurisation lethality (at the coldest point) (min); t = time of thermal processing (min); $T = f(t) = T\ (t)$ = processing temperature (°C) at time t (thermal history); T_{ref} = 70 °C reference temperature. z = 10 °C = slope of the logarithm of the decimal reduction time, D versus temperature to destroy pathogenic and most, but not all, spoilage microorganisms.

2.4. Analytical methods

Triplicates of the freshly extracted juice (0 min) and/or heat-processed juice were analysed for their physicochemical characteristics and total monomeric anthocyanins content at various isothermal conditions (60, 70, 80, and 90 °C) over varying durations. Standard methods (AOAC, 1998) were employed to analyse the samples for overall titratable acidity, pH, overall soluble solids, and dry matter. The total anthocyanin content was assessed utilizing the pH-differential method detailed by Lee, Durst, and Wrolstad (2005).

2.4.1. Physicochemical analysis

a) *Titratable acidity and pH*

The total titratable acidity of juice samples was determined potentiometrically by titration with 0.1 N sodium hydroxide (NaOH) until the pH endpoint of 8.2 and expressed as 'g citric acid L^{-1} juice' (AOAC, 1998). pH measurements (at $T^{\circ} = 20 \pm 1^{\circ}C$) were carried out using a pre-calibrated microprocessor pH meter (HI pH -211, HANNA

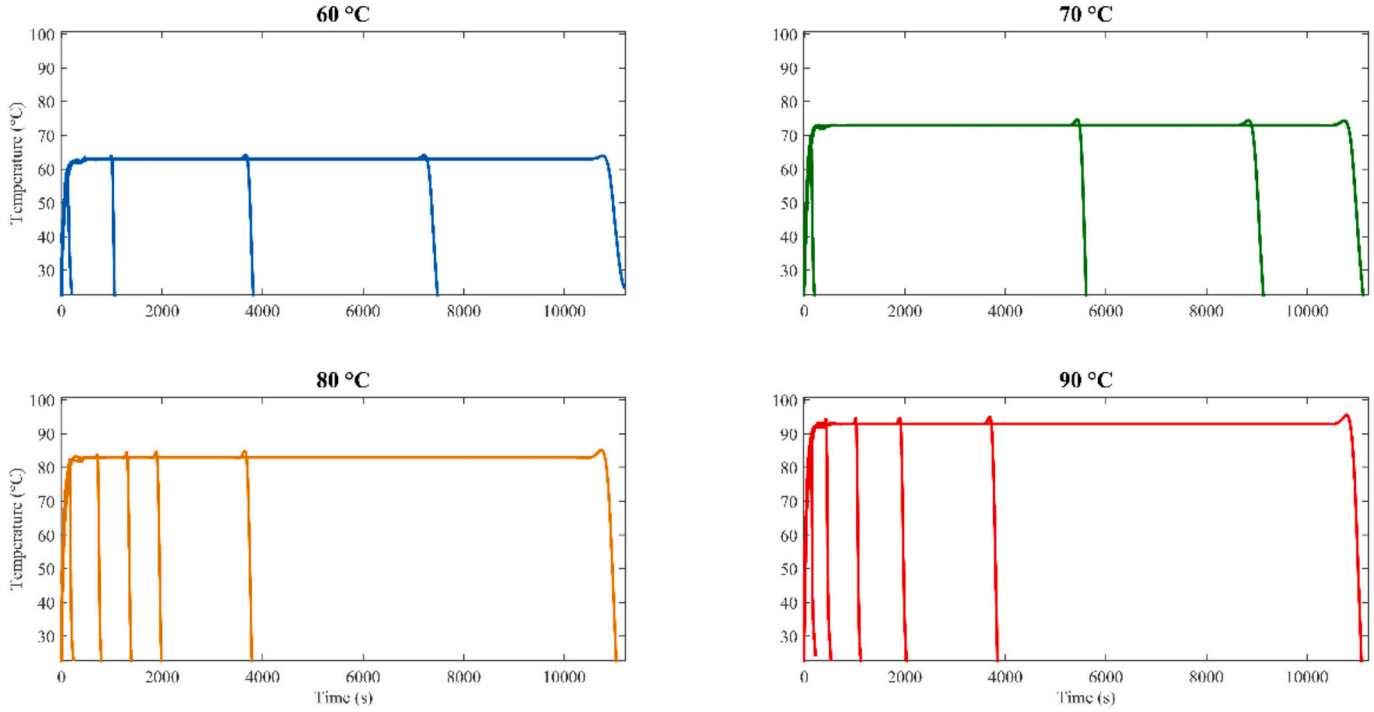

Fig. 1. Time-temperature profiles, $T\ (t)$, for the fortified and non-fortified blood orange juice samples pasteurised at 04 isothermal temperatures (60, 70, 80 and 90 °C) in a Pyrex sealed tube.

Instruments, Woonsocket, Rhode Island, USA).

b) *Total soluble solids (TSS) measurement*

The total soluble solids (TSS) content of juice samples was measured at $T^{\circ} = 20 \pm 1^{\circ}C$ with an Abbe-type refractometer (AR 12, SCHMIDT+HAENSCH GmbH & Co., Berlin, Germany) the results were reported in degrees Brix.

c) *Total dry matter (TDM) determination*

The total dry matter *(TDM)* determination of juice samples was carried out by the gravimetric method using the oven-drying method (AOAC, 1998). The juice samples underwent exposure to a drying temperature of $T^{\circ} = 103 \pm 2^{\circ}C$ until complete dryness (no weight change). The TDM is expressed in percentage in grams per 100 mL of the initial juice sample (%, *w/v*).

2.4.2. Determination of the total monomeric anthocyanins (ANC) content

The pH-differential method, as proposed by Lee et al. (2005), was employed to track total monomeric anthocyanins (ANC) content in the juice samples.

Monomeric anthocyanin (ANC) pigments exhibit reversible colour changes in response to variations in pH; at a pH of 1.0, the pigments assume the form of coloured oxonium, while at a pH of 4.5, the predominant form is the colourless hemiketal. The disparity in the absorbance of the pigments at a wavelength of 520 nm is proportional to the concentration of the pigments. The findings are presented as cyanidin-3-glucoside compound basis (the most prevalent anthocyanin found in blood orange juice). Polymeric anthocyanins demonstrate colour stability independent of the pH level and are excluded from the measurements due to their absorption capabilities at both pH 4.5 and pH 1.0 (Lee et al., 2005).

UV/Visible spectra (250 to 600 nm) absorption of blood orange juice samples were recorded using a UV–visible spectrophotometer (Shimadzu UV-1605 UV–Visible Spectrophotometer, Tokyo, Japan). The anthocyanin maximum absorbance (A_{ANCmax}) of juice samples carrying visible wavelengths ($\lambda_{visiblemax}$) was at 515 nm.

After the addition of potassium chloride buffer (0.025 M, pH =1.0) and sodium acetate buffer (0.040 M; pH = 4.5), the juice samples were diluted and allowed to equilibrate for 20 min. For absorbance determination of the equilibrated juice samples, a blank cell filled with distilled water was used as a reference for both pH 1.0 and 4.5 at the maximum absorbance wavelengths ($\lambda_{visiblemax}$ = 515 nm) and at 700 nm to correct for any haze. Measurements were performed in triplicates using a UV–visible spectrophotometer (Shimadzu UV-1605 UV–Visible Spectrophotometer, Tokyo, Japan) and the *ANC* concentrations (C_{ANC}) were expressed as cyanidin-3-glucoside equivalents, as depicted in Eq. (3).

$$C_{ANC}\ (\text{cyanidin-3-glucoside equivalents, mg L}^{-1}) = \frac{A_{ANC} \times MW \times DF \times 10^3}{\varepsilon \times l} \quad (3)$$

Where $A_{ANC} = (A_{515nm} - A_{700nm})_{pH1.0} - (A_{515nm} - A_{700nm})_{pH4.5}$; *MW* (molecular weight) = 449.2 g.mol^{-1} for cyanidin-3-glucoside (cyd-3-glu); *DF* = dilution factor of the filtered juice samples; *l* or pathlength = 1 cm; ε or molar extinction coefficient for cyd-3-glu = 26,900L.mol^{-1}.cm^{-1}; and 10^3 = conversion factor from g to mg.

2.5. Degradation kinetics modelling of ANC change during thermal processing (pasteurisation)

When discussing nutrient degradation in food due to heat treatment or preservation, it is common in food science to use reaction orders such as zero, half, first, or higher-order kinetics (Corradini & Peleg, 2006). The degradation reaction order of total monomeric anthocyanins (*ANC*) content during thermal treatment (pasteurisation) from different blood orange juices was predicted using the general rate law in (Eq. (4)).

$$\frac{dC_{ANC}^{(t)}}{dt} = -k_{ANC}\left(C_{ANC}^{(t)}\right)^n \quad (4)$$

where k_{ANC} is reaction rate constant, n is the reaction order, $C_{ANC}^{(t)}$is *ANC* concentration (cyanidin-3-glucoside equivalents, mg L^{-1} of juice) at any given time (t), and t is the thermal treatment time (min). *ANC* thermal degradation was assumed to take place in a homogenous or nearly ideal system. Hence, the consideration of heat and mass transfer phenomena was ignored.

2.5.1. Effect of thermal treatment time on ANC degradation (Weibull distribution model)

The degradation reaction of ANC, as stated by Corradini and Peleg (2006), can be characterized as a cumulative representation of the temporal Weibull distribution, as depicted in Eq. (5).

$$F_{ANC}(t) = exp(-b_{ANC} \cdot t^n) \quad (5)$$

where $F_{ANC}(t)$ is the fraction of intact *ANC* from two blood orange juices after any thermal treatment time (t), n (shape factor) and b_{ANC} (its reciprocal is location factor) are the model constants. But since$F_{ANC}(t) = C_{ANC}^{(t)}/C_{ANC}^{(t=0)}$, Eq. (5) can be rewritten in the manner given in Eq.(6).

$$\frac{C_{ANC}^{(t)}}{C_{ANC}^{(t=0)}} = exp\left[-b_{ANC}(T)t^{n(T)}\right] \quad (6)$$

where $b_{ANC}(T)$ and $n(T)$ are temperature-dependent coefficients. Nevertheless, since $b_{ANC}(T)$ has the same unit $\left(\frac{1}{t}\right)$ as the reaction rate constant (k_{ANC}), Eq. (6) is considered as a kinetic model (Corradini & Peleg, 2004b; Corradini & Peleg, 2006). In many cases, the distribution's shape factor, $n(T)$ in Eq. (6), has only a weak temperature dependence and sometimes none at all (Van Boekel, 2002). Therefore, the model could be reduced to the Eq. (7).

$$\frac{C_{ANC}^{(t)}}{C_{ANC}^{(t=0)}} = exp[-b_{ANC}(T)t^n] \quad (7)$$

When $n > 1$, the isothermal degradation of the semi-logarithmic curve has a downward concavity and when $n < 1$, the curve shows an upper concavity. The first-order kinetics was in fact a special case when $n = 1$. Moreover, when $n \neq 1$, fractional values of *ANC* degradation kinetic order could perform a better fitting procedure (Derossi et al., 2010).

To obtain optimal values of the Weibullian model (i.e., Eq.(7)) fixed power n, a special MATLAB program was written to generate incremental values of n with a specified range. At each iteration (for each incremental n value), the isothermal $C_{ANC}^{(t)}$ data were fitted with the respect of the Eq.(7) and corresponding n value. The highest R_{adj}^2 and lowest *RMSE* for the tested n value was chosen as the representative power (optimal value).

2.5.2. Effect of pasteurisation temperature on ANC degradation rates (Log-Logistic model)

It has previously been experimentally demonstrated that the Weibullian 'rate parameter', $b_{ANC}(T)$, and $n(T)$ whenever it is not a constant, varied with the thermal temperature, T (°C), according to the Log-Logistic model, identified as the secondary model in Eq.(8), represents merely one of the manifold models that may be employed to elucidate the thermal temperature relationship of the Weibull parameters (Corradini & Peleg, 2004a; Corradini & Peleg, 2006; Corradini & Peleg, 2007; Peleg, Corradini & Normand, 2004).

$$b_{ANC}(T) = ln\{1 + exp[a_{ANC}(T - T_{cANC})]\} \quad (8)$$

where T_{cANC}indicates the thermal temperature level at which *ANC* degradation intensifies and a_{ANC} the process's acceleration at which $b_{ANC}(T)$climbs as the thermal temperature rises to a level well above T_{cANC}. According to this model, when $T \ll T_{cANC}$, $exp[a_{ANC}(T - T_{cANC})] \ll 1$ and $b_{ANC}(T) \simeq 0$ (i.e., there is no *ANC* thermal degradation at all). However, when $T \gg T_{cANC}$, $exp(T - T_{cANC}) \gg 1$ and thus $b_{ANC}(T) \simeq ln\{exp[a_{ANC}(T - T_{cANC})]\} = a_{ANC}(T - T_{cANC})$ (i.e., $b_{ANC}(T)$ increases linearly with thermal temperature) (Corradini & Peleg, 2004a; Peleg, 2006).

Unlike the Arrhenius equation, the Log-Logistic model does not require an assumption of a unique activation energy (Peleg, 2006). In other words, the Log-Logistic model does not implicitly assume that there is a universal similarity between the thermal degradation kinetics of complex molecules and basic chemical reactions, from which the Arrhenius model was originally derived (Corradini & Peleg, 2004a).

2.6. Statistical analysis and model evaluation

The least square method was used to estimate the model parameters via non-linear regression with the Levenberg-Marquardt iterative algorithm from the Curve Fitting Toolbox in Matlab ver. 9.42018a by The MathWorks Inc. in Natick, Massachusetts (USA). The evaluation of the model's appropriateness in fitting the experimental data was carried out via the assessment of the adjusted coefficients of determination (R^2_{adj}); confidence intervals (*Conf.I*) and root mean square error (*RMSE*, Eq. (9)). The superior R^2_{adj}values and the inferior *RMSE* values correspond to an enhanced fitting of the model to the experimental data.

$$RMSE = \sqrt{\frac{1}{(n-p)}\sum_{i=1}^{n}\left(C^{(t)}_{ANC}, Mod - C^{(t)}_{ANC}, Exp\right)^2} \quad (9)$$

Where, $C^{(t)}_{ANC}, Mod$ is the model predicted value, $C^{(t)}_{ANC}, Exp$ is the experimental value, n is the number of the data, and p is the number of parameters.

The analysis of variance (*ANOVA*) was conducted utilizing XLSTAT Release 10 (Addinsoft, Paris, France). To compare the means of the estimated thermal kinetic parameters, Tukey's multiple range test (HSD) was employed. The evaluations were carried out with a significance level of $p < 0.05$.

3. Results and discussion

3.1. Characteristics of the untreated blood orange juices (NFCJ vs. AAFJ-100)

The initial physicochemical attributes of the untreated blood orange juices, i.e., Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg L^{-1} (AAFJ-100, "0.01%"), are demonstrated in Table 1. As demonstrated, ascorbic acid fortification of the Moro blood orange juice has no significant difference ($p < 0.05$) in the physicochemical quality parameters in untreated AAFJ-100 "0.01%" as compared to untreated NFCJ (Table 1). The exception is logically made for ascorbic acid content. Generally, the former results for Moro orange juice are consistent with those reported in the literature (Kelebek, Canbas, & Selli, 2008; Kırca & Cemeroğlu, 2003), except for a higher tint value and slightly lower concentrations of anthocyanins and ascorbic acid. These variations can be related to the Moro blood orange Bejaia's growing, climatic and varietal conditions.

Furthermore, experiments conducted separately on freshly pressed juice and juice subjected to the freezing-thawing process revealed no substantial alterations in composition following the freeze-thaw process (data not shown).

Table 1
Main characteristics of the blood orange juices: Squeezed Juice (Non-Fortified Control Juice, NFCJ) and, Ascorbic Acid Fortified Juice with 100 mg L^{-1} (AAFJ-100).

Analysis	NFCJ	AAFJ-100	Kelebek et al. (2008)
Juice yield (%)	30.1 ± 3.0 [a]	30.1 ± 3.0 [a]	35.1 ± 3.25
pH	3.29 ± 0.04 [a]	3.22 ± 0.20 [a]	3.35 ± 0.02
Total soluble solids (°Brix)	13.0 ± 1.7 [a]	12.8 ± 1.0 [a]	12.0 ± 0.2
Titratable acidity * (g L^{-1})	13.0 ± 0.3 [a]	13.7 ± 0.9 [a]	11.3 ± 1.1
Dissolved oxygen (mg L^{-1})	6.3 ± 0.9 [a]	6.0 ± 0.7 [a]	–
Total dry matter (%)	11.6 ± 1.0 [a]	12.6 ± 1.5 [a]	–
Colour intensity	3.74 ± 0.20 [a]	3.95 ± 0.10 [a]	1.40 ± 0.12
Tint	0.94 ± 0.01 [a]	0.99 ± 0.03 [a]	0.59 ± 0.01
% Yellow	41.2 ± 2.1 [a]	39.4 ± 1.5 [a]	55.0 ± 0.16
% Red	20.4 ± 4.1 [a]	25.6 ± 2.0 [a]	12.4 ± 0.04
% Blue	38.7 ± 2.0 [a]	37.8 ± 1.9 [a]	32.6 ± 0.04
Anthocyanin ** (mg L^{-1})	129.5 ± 7.9 [a]	134.4 ± 5.6 [a]	291.3 ± –
Ascorbic acid (mg L^{-1})	426.0 ± 5.9 [b]	510.6 ± 3.4 [a]	506.5 ± 21.3

Values are the means of three determinations ± standard deviation.
Values with different letters (*a-b*) were significantly different (Tukey, $p < 0.05$) for the two types of juice.
* As citric acid.
** As cyanidin-*3*-glucoside.

3.2. Pasteurisation severity parameter of blood orange juice

The severity parameter of thermal treatment (Pasteurisation Value, PV or $F^{10°C}_{70°C}$) at different set-up temperatures; based on the juice temperature variations (*T (t)*; Fig. 1) of the cold spot and calculated by the Biglow equation (Eq. (2)) during the stationary stage of the thermal history; for NFCJ and AAFJ-100 "0.01%" were shown in Table S1. The longer processing time and higher thermal temperature provide the highest pasteurisation values; which in fact result in more nutrient depletion from thermally pasteurised blood orange juice.

Fruit beverages with Pasteurisation Values ($F^{10°C}_{70°C}$) of 100 to 200 min (Table S1) were considered a typical and safe thermal treatment for achieving a "logarithmic reduction" in the number of viable microorganisms, reducing their number. Hence, they are unlikely to cause disease (Cisse et al., 2009). Hence, blood orange juices thermally treated for/or >90, 10 and 5 min at 70, 80 and 90 °C, respectively, reached the recognised $F^{10°C}_{70°C}$values. The Pasteurisation Values of the former time-temperature profiles ranged from 118 to 37,573 min corresponding to the juices treated at 70 °C for 90 min and 90 °C for 180 min, respectively. The calculation of pasteurisation severity parameters will provide a safe range of time-temperature profiles that correspond to the needed level of microbiological quality while preserving the nutritional and flavour juice properties.

3.3. Changes in physicochemical quality during pasteurisation

The supplementary materials (from Fig. S1 to S4) display how isothermal processing, notably pasteurisation, affects the change among several physicochemical characteristics in both Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with a concentration of 100 mg L^{-1} (AAFJ-100, "0.01%") at different durations and temperatures ranging from 60 to 90 °C.

a) *Titratable acidity and pH*

The changes in pH and titratable acidity, respectively, from Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg L^{-1} (AAFJ-100, "0.01%") during thermal treatment (pasteurisation) at 60 °C (A), 70 °C (B), 80 °C (C) and 90 (D) is depicted in Figs. S1 and S2.

The occurrence of organic acids is essential in shaping the distinct

flavour and desirability of orange juice. The buffer capacity of orange juices is predominantly controlled by these organic acids and their inorganic salts, which serve as protection against the growth of pathogens (Kaddumukasa, Imathiu, Mathara, & Nakavuma, 2017). The least common acid discovered in orange juice is citric acid, pursued by malic acid, with both primarily existing in the form of free acids (Dahdouh et al., 2015). Several non-volatile free acids (including oxalic, tartaric, galacturonic, quinic, among others) are present in significantly lower concentrations (Esteve, Frígola, Rodrigo, & Rodrigo, 2005).

Following the application of the different time and temperature thermal processes, a small pH change was noticed in the two blood orange juices (Fig. S2.). However, total titratable acidity values (Fig. S3.) showed a significant increase ($p < 0.05$) from AAFJ-100 "0.01%" at most intensive heat-treated conditions (90 °C), but in all cases, it was within the recommended values of thermal-treated juices (6 to 16 g L^{-1}) (Redd, Hendrix, & Hendrix, 1986). Velázquez-Estrada, Hernández-Herrero, Guamis-López, and Roig-Saguès (2019) have also observed an increase in the total titratable acidity after orange juice pasteurisation.

Moreover, as found by Dahdouh et al. (2015), no definite relationship was identified between pH and total titratable acidity. Larsen and Nyvad (1999) explained this finding by the short ranges differences in acid concentration in the two studied juices; which contribute to the orange juice buffer capacity depending upon the kinds and amounts of buffer salts present. Therefore, two samples of orange juice having rather large differences in total acidity may have the same pH value.

b) *Total soluble solids (TSS) and total dry matter (TDM)*

Changes in total soluble solids (TSS) and total dry matter (TDM), respectively, from Non-Fortified Control Juice (NFCJ) and Ascorbic Acid Fortified Juice with 100 mg L^{-1} (AAFJ-100 "0.01%") during isothermal treatment (pasteurisation) at 60 °C (A), 70 °C (B), 80 °C (C) and 90 °C were depicted in Fig. S3. and Fig. S4.

In citrus juices, °Brix denotes the proportion of soluble solids, it is an important factor for grading the quality of a citrus juice. Generally, the total soluble solids (Fig. S3) and total dry matter (Fig. S4.) in the two-blood orange juice upon the application of the various temperature and time thermal processes were not significantly affected during isothermal treatment (pasteurisation).

3.4. Degradation kinetic of total monomeric anthocyanins (ANC) during pasteurisation

Fig. 2 shows the change in ANC change in different blood orange juices throughout the isothermal processing (pasteurisation) at 60, 70, 80, and 90 °C, for the Weibull model.

3.4.1. Effect of thermal treatment time on ANC degradation (Weibull distribution model)

The Weibull model (Fig. 2) with variable *n* (Fig. 2A) and fixed *n* (Fig. 2B) depicts that the change of *ANC* content decreased according to time and thermal temperature for the various juices, except for the Weibull model with variable *n* from NFCJ (Fig. 2A). The *ANC* degradation becomes more pronounced as the duration of time and the level of thermal temperature intensify. Although the fitted curves from AAFJ-100 "0.01%" in Fig. 2A and Fig. 2B are very similar, but not identical.

An evident discrepancy was observed in the fitted *ANC* data by the Weibull model (Fig. 2) with variable *n* (Fig. 2A) and fixed *n* (Fig. 2B) for all thermal temperatures from NFCJ. However, the *ANC* data fitted by the Weibull model with fixed *n* (Fig. 2B) show clearly that *ANC* retention is better from AA-fortified juice.

The estimated kinetic constants ($b_{ANC}(T)$) along with the R^2_{adj}, RMSE for the Weibull distribution model are displayed in Table 2, obtained through a least square fitting procedure of the *ANC* thermal degradation kinetics. As illustrated in Table 2, the Weibull distribution model

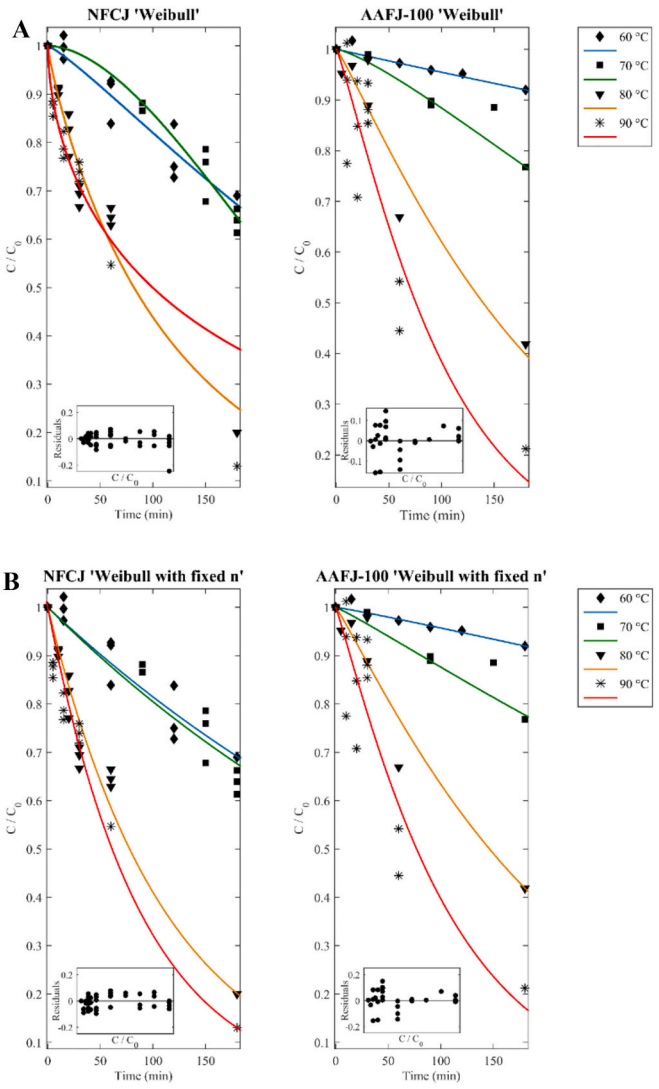

Fig. 2. Anthocyanins (*ANC*) degradation kinetics from two blood orange juices: control (NFCJ), AA-fortified (AAFJ-100); and the residuals plot of *Ct/C0* experimental data for Weibull model (Eq. (7)) with variable *n* (A) and fixed *n* (B) during thermal treatment at temperature 60, 70, 80, and 90 °C.

exhibited a satisfactory fit to the experimental data, regardless of the pasteurisation temperature and juice formulation ($0.808 \leq R^2_{adj} \leq 0.970$ and $0.01 \leq RMSE \leq 0.11$ for variable *n;* $0.824 \leq R^2_{adj} \leq 0.988$ and $0.01 \leq RMSE \leq 0.10$ for fixed *n*). Furthermore, as shown in Table 2, the Weibull model exhibited suboptimal performance in terms of curve fitting (with $R^2_{adj} < 0.900$) at temperatures of 60 °C (from NFCJ) and 90 °C (from AAFJ-100, "0.01%") with both variable n values and the fixed n values (optimal value of n). Nevertheless, a slight improvement in the goodness of fit for the Weibull model with fixed *n* (Table 2) was observed due to optimized typical values for shape factor *n,* which allowed to obtain the highest R^2_{adj} and lowest *RMSE* (*n* optimal values were 1.00 and 1.10 for NFCJ and AAFJ-100 "0.01%", respectively). This finding is also corroborated by the residual plot analysis (Fig. 2), which demonstrates that the Weibull model fitting with fixed *n* was closer to zero than those with variable values of *n* (mainly for NFCJ). This demonstrates, in compliance with the observations of Van Boekel (2002), Corradini and Peleg (2004b) and earlier reports, that the Weibull model with fixed *n* can be an efficient and economical tool to describe *ANC* thermal degradation.

The estimated kinetic constants ($b_{ANC}(T)$) with the Weibullian

Table 2
Kinetic parameters of the Weibull model (with variable and fixed n) of different blood orange juices for anthocyanins (*ANC*) degradation during pasteurisation at temperatures 60, 70, 80 and 90 °C.

	Weibull model with variable n				
Juice type	T (°C)	$b_{ANC}(T)$ ($\times 10^{-3}$ min^{-1})	variable n (–)	R^2_{adj}	*RMSE
NFCJ	60	0.85 (2.12)	1.19 (0.52)	0.886	0.04
	70	0.04 (0.15)	1.81 (0.82)	0.916	0.04
	80	14.68 (10.11)	0.87 (0.17)	0.938	0.05
	90	46.54 (20.28)	0.59 (0.11)	0.970	0.04
AAFJ-100	60	0.46 (1.16)	1.00 (0.51)	0.958	0.01
	70	0.31 (1.00)	1.30 (0.64)	0.942	0.02
	80	2.77 (6.70)	1.12 (0.51)	0.915	0.06
	90	4.79 (9.08)	1.15 (0.47)	0.808	0.11
	Weibull model with fixed n				
Juice type	T (°C)	$b_{ANC}(T)$ ($\times 10^{-3}$ min^{-1})	Fixed n (–)	R^2_{adj}	*RMSE
NFCJ	60	2.0 (0.4)	1.00	0.887	0.04
	70	2.2 (0.4)		0.865	0.05
	80	8.9 (1.3)		0.931	0.05
	90	11.3 (2.0)		0.944	0.05
AAFJ-100	60	0.3 (0.0)	1.10	0.988	0.01
	70	0.8 (0.1)		0.952	0.02
	80	2.9 (0.9)		0.931	0.06
	90	5.8 (1.9)		0.824	0.10

T: pasteurisation temperature (°C).
b_{ANC} *(T)*: rate constant of anthocyanin degradation with the Weibullian model's optimal n values at any pasteurisation temperature ($\times 10^{-3}$ min^{-1}).
Values between brackets (for b_{ANC} (T) and n) are confidence intervals (estimated with 95% of probability);
R^2_{adj}: adjusted R^2; *RMSE*: root mean square error between experimental and predicted data.

model's optimal n values not only increased systematically the goodness of fit at all temperatures for both types of juice (Table 2); but also showed logical kinetic values compared to those generated by the Weibull model with variable n, varying extremely erratically. Especially when the $b_{ANC}(T)$ values from both types of juice at 70 °C (0.04 and 0.31 $\times$ 10^{-3} min^{-1} for NFCJ and AAFJ-100 "0.01%", respectively) were found to be lower compared to those at 60 °C (0.85 and 0.46 $\times$ 10^{-3} min^{-1} for NFCJ and AAFJ-100, respectively).

The Weibull model (Table 2) in the primary modelling of *ANC* data (effect of pasteurisation time) improves the goodness of fit quality (R^2_{adj}, RMSE, confidence interval). Nevertheless, when it comes to the kinetic constant values, this improvement was not consistent among the different temperatures in the same type of juice (for variable values of n) and even between the two types of juice (for optimal n values). In alternative terms, the Weibull model produced a wide range of n values (representing a broad spectrum) for the different pasteurisation temperatures in the same type of juice (considering fluctuating n values) and for various types of juice (considering optimal n values). Almost, the same kinetics constants values (also the same goodness of fit quality) were obtained for the NFCJ fitted data by the Weibull model with optimal n values and otherwise by the first-order non-linear model (a special case of the Weibull model when the shape parameter is equal to 1) over the studied thermal temperatures (60–90 °C). The obtained optimal n value ($n = 1$) of the Weibull model from NFCJ confirms first-order kinetic.

The huge variability in n values (from 0.59 to 1.81) from NFCJ, over the studied thermal temperatures, has a substantial influence on the obtained values of Weibullian kinetic constants ($b_{ANC}(T)$). This can be explained by the various reaction mechanisms of *ANC* degradation from NFCJ at 60 to 90 °C, which is related to variability in (n) values (Manso, Oliveira, Oliveira, & Frías, 2001; Van Boekel, 2002). The thermal degradation mechanisms of monomeric ANC might be influenced by heat and pH levels, involving the opening of the heterocycle and potential formation of chalcone form, with the process following first-order reaction kinetics; reducing pH can mitigate this degradation (Remini et al., 2018). Another mechanism suggests that heat and pH levels (2–4) lead to hydrolysis of the glycosidic bond, conversion of the aglycone to chalcone, and then to degradation products (Levy, Okun, & Shpigelman, 2019). In an aqueous solution at pH 2–4, temperature elevation results in the loss of sugar moieties of the anthocyanins, leading to further colour loss. The presence of the sugar group increases water solubility and stability, with the number of sugar rings also influencing stability (Remini et al., 2018).

A link can be made between the obtained (n) values with *ANC* degradation as follows; The optimal n value = 1 from NFCJ, thermally treated between 60 and 90 °C, could demonstrate that the probability of *ANC* degradation does not depend on time. In other terms, each *ANC* molecule is equally susceptible to degradation, no matter how long the thermal treatment lasts (Van Boekel, 2002). Whereas, the optimal n values >1 from AA-fortified juice, thermally treated between 60 and 90 °C, depict that the remaining *ANC* were increasingly destroyed and more sensitive to heat over time (Van Boekel, 2002).

The former findings (using the Weibull distribution model) contradict the previous research on the combined effects of flavonoids, ascorbic acid, and sugars on the *ANC* thermal degradation in a model system, at 70–90 °C, from the Chinese blood orange juice (Cao et al., 2009). The same study also showed that AA-fortification significantly accelerated *ANC* degradation, they found that the flavonoid protective effect was more relevant in *ANC* degradation than the negative effect of AA (Cao et al., 2009). Moreover, they indicated that thermal temperature and AA did not synergistically affect *ANC* degradation. So, the effects of ascorbic acid fortification on anthocyanins' kinetic behaviour in juices during thermal treatment are controversial and depend on various factors (juice type; processing conditions "temperature, duration of heating, and presence of oxygen", the amount of ascorbic acid added) (Remini et al., 2018).

As observed for the ANC data fitted by the Weibull model with fixed n (Fig. 2B), it showed clearly that ANC retention is better from AA-fortified juice with 100 mg L^{-1} (AAFJ-100, "0.01%"). The concentration of ascorbic acid fortification (low or high) can tip the scales towards protection or degradation (Remini et al., 2018). At lower levels (< 0.1% of AA fortification), anthocyanins have the potential to be protected from degradation caused by heat and oxygen by competing with flavanols or flavonols to interact with anthocyanins, rather than with ascorbic acid; which might have beneficial effect on colour stability (González-Molina, Moreno, & García-Viguera, 2009; Shrikhande & Francis, 1974). At higher concentration levels (≥ 0.1% of AA-fortification), ascorbic acid itself can become a pro-oxidant during thermal treatment, promoting anthocyanin breakdown through mechanisms; like the increased generation of free radicals through interaction with oxygen; the depletion of the more stable polymeric forms of anthocyanins and loss of colour in anthocyanin-coloured solutions (Levy et al., 2019).

The mechanism of this interaction is not fully understood, but there are three main hypotheses for the mechanisms involved in this degradation: (i) The condensation of ascorbic acid at Carbon-4 (C_4) of the anthocyanin, which is the most susceptible site to electrophilic attack (Jurd, 1972; Poei-Langston & Wrolstad, 1981; Skrede & Wrolstad, 2002); (ii) the degradation of the anthocyanin preventing the oxidation of ascorbic acid (García-Viguera & Bridle, 1999; Pang, Zhang, Duan, & Ji, 2001) and (iii) the degradation of anthocyanins by the hydrogen peroxide generated from the ascorbic acid oxidation (Jackman & Smith, 1996).

Overall, the impact of ascorbic acid fortification on anthocyanins in juices during thermal treatment depends on various factors. More research is needed to fully understand the interplay between these elements.

3.4.2. Effect of pasteurisation temperature on ANC degradation rates

a) *Temperature dependence of the $b_{ANC}(T)$*

The Log-Logistic model (empirical model) was argued as the secondary model for the temperature dependence description of Weibull model rate parameters, $b(T)$, of nutrient in food science (Corradini & Peleg, 2004a; Corradini & Peleg, 2004b; Derossi et al., 2010; Yu, Wu, Hu, Cui, & Yu, 2011).

The temperature dependence of the *ANC* rate parameters ($b_{ANC}(T)$), for the two types of blood orange juice, as fitted by the Log-Logistic model (plot of $b_{ANC}(T)$vs. temperature relationship 'Weibull model with fixed n') from Eq. (8) is depicted in Fig. 3. The corresponding regression and fit parameters are presented in Table 3. As illustrated in Fig. 3, the Log-Logistic model was observed to give a good fit with the temperature dependence of $b_{ANC}(T)$ for both types of juice ($0.828 \leq R^2_{adj} \leq 0.991$ and $0.0002 \leq RMSE \leq 0.0019$). This goodness of fit quality stated the accuracy and ability to use the Log-Logistic model as a secondary model.

The Log-Logistic model parameters (n, $a_{ANC}$and T_{cANC}), which describe *ANC* sensitivity towards the different thermal temperatures, were found to vary with the juice type (Table 3)., the narrow range values of a_{ANC} (0.06 and 0.09 °C^{-1} from NFCJ and AAFJ-100, respectively) and T_{cANC} (168 and 143 °C from NFCJ and AAFJ-100, respectively) for both juices can explain similar temperature dependence of $b_{ANC}(T)$. Nevertheless, the AAFJ-100 "0.01%" had the lowest values of T_{cANC} (T_{cANC}= 143 °C), which points out that $b_{ANC}(T)$ from AAFJ-100 "0.01%" was more impacted by temperature, over the range of 60–90 °C, than NFCJ (Table 3). In other terms, *ANC* thermal degradation in AAFJ-100 "0.01%" intensifies at a lower temperature compared to NFCJ. However, almost the same acceleration of *ANC* thermal degradation (a_{ANC}); at which $b_{ANC}(T)$climbs as the temperature rises to a level well above T_{cANC}; was found for both types of juice.

4. Conclusion

The investigation explored the impact of adding 100 mg L^{-1} "0.01%" of ascorbic acid (AA-fortification) and pasteurisation processing at temperatures ranging from 60 to 90 °C on the anthocyanin (ANC) degradation kinetics, as well as the changes in physicochemical parameters (such as pH, titratable acidity, and soluble solid content) of Moro blood orange juice. A novel approach known as the Weibull-Log-Logistic 'WeLL' combination was employed to model the kinetics of anthocyanin degradation. This approach accurately predicts the temperature-dependent rate constants based on fixed n values of the Weibull model. The findings of this research show that the temperature at which pasteurisation takes place has a considerable effect on the thermal degradation of *ANC*. The AA-fortification low concentration (0.01%) enhances anthocyanin retention but also increases its thermal sensitivity over time. Consequently, AA-fortification can be considered for blood orange juice processed using high-temperature-short-time (HTST) to effectively preserve *ANC* and prevent thermal degradation. This study offers valuable insights into the production of authentic and unadulterated pure natural juice, employing advanced 'WeLL' modelling and AA-fortification techniques. Nevertheless, further investigation is required to fully comprehend the mechanisms of degradation that occur throughout thermal processing and to determine and scrutinize the ensuing deterioration by-products.

Table 3
Kinetic parameters of anthocyanins (*ANC*) degradation from two blood orange juices during pasteurisation temperatures of 60, 70, 80 and 90 °C following Log-Logistic model.

Juice type	Fixed n (–)	a_{ANC} (°C^{-1})	T_{cANC} (°C)	R^2_{adj}	$RMSE^*$
NFCJ	1.00	0.06 ± 0.08 [a]	168 ± 118 [a]	0.828	0.0019
AAFJ-100	1.10	0.09 ± 0.04 [a]	143 ± 21 [a]	0.991	0.0002

Values are mean ± 95% confidence interval.
Values with different letters (*a-b*) were significantly different (Tukey, $p < 0.05$) for the two types of juice.
a_{ANC}: acceleration process at which climbs as the thermal temperature rises to a level well above $T_{c\ ANC}$.
$T_{c\ ANC}$: thermal temperature level at which the anthocyanin degradation intensifies.
R^2_{adj}: adjusted R^2.
* *RMSE*: root mean square error between experimental and predicted data.

CRediT authorship contribution statement

Yasmine Remini-Sahraoui: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation. **Hocine Remini:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Farid Dahmoune:** Validation, Software, Formal analysis, Data curation. **Sofiane Dairi:** Validation, Software, Formal analysis, Data curation. **Omar Aoun:** Writing – review & editing, Writing – original draft, Resources, Investigation. **Amine Belbahi:**

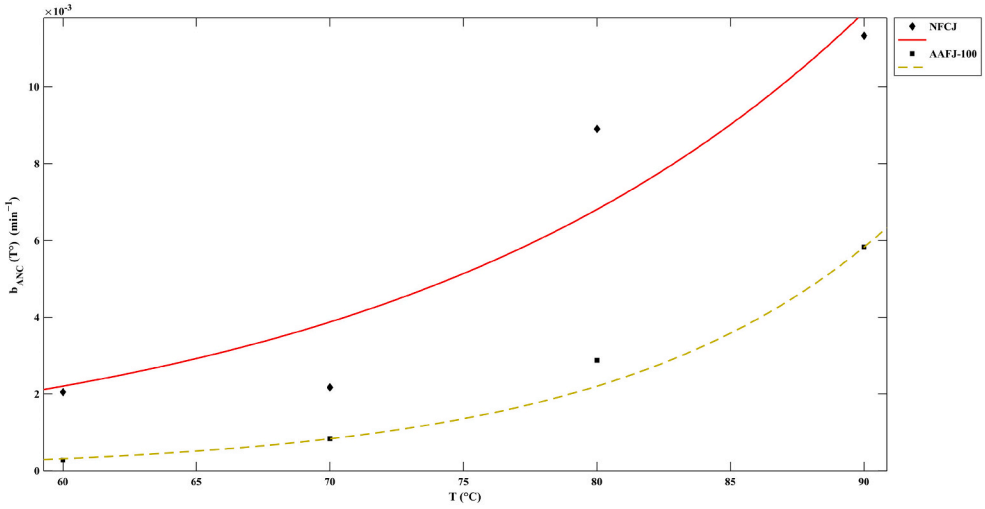

Fig. 3. Effect of the pasteurisation temperature on *ANC* degradation following Log-Logistic model based on *bANC (T)* of Weibull model from two blood orange juices: control (NFCJ), AA- fortified (AAFJ-100); during thermal treatment at temperatures 60, 70, 80 and 90 °C.

Writing – review & editing, Writing – original draft, Resources, Investigation. **Sonia Oukhmanou-Bensidhoum:** Writing – original draft, Resources, Investigation. **Nabil Kadri:** Writing – original draft, Resources, Investigation. **Khodir Madani:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

There are no conflicts of interest between the authors that may undermine the credibility or reliability of this study. The research received no support, from any industry or organization with a vested interest in the topic or materials under consideration. The authors have no associations that could compromise their integrity. Additionally, there are no affiliations with any entities or organizations that are interested in the results or publication of this manuscript.

Data availability

No data was used for the research described in the article.

Acknowledgements

The authors gratefully acknowledge the Algerian Ministry of Higher Education and Scientific Research for funding the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2024.103724.

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