



Effect of microwave assisted blanching on the ascorbic acid oxidase inactivation and vitamin C degradation in frozen mangoes



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ABSTRACT

The effect of microwave assisted and conventional water blanching of mango (*Mangifera indica*) under two different blanching scenarios, High Temperature Short Time (HTST) and Low Temperature Long Time (LTLT) on ascorbic acid oxidase (AAO) inactivation and on vitamin C (L-ascorbic acid & dehydroascorbic acid) retention were comparatively studied. The impact of alternative blanching processes and subsequent frozen storage on enzymatic inactivation and vitamin C was kinetically modelled. Both water and microwave HTST as well as LTLT microwave treatments of mango pieces showed high degree of AAO inactivation. An approximately 30% residual AAO activity was observed and was described through a first order fractional conversion model. Microwave assisted blanching led to higher retention of total vitamin C in both LTLT and HTST treatments. In LTLT water blanching, vitamin C loss was mainly caused by mass transfer phenomena rather than temperature degradation, while after HTST treatments the decrease of total vitamin C content seemed to be mainly related to thermal degradation than due to the leakage of the nutrients in the blanching medium. Further inactivation of the thermostable fraction of AAO and degradation of total vitamin C were observed after frozen storage for 130 days at -18.63 ± 0.48 °C.

1. Introduction

Several preservation methods have been investigated, developed and exploited over the last years to preserve fruits and vegetables but freezing still remains one of the most popular among them offering fresh-like characteristics on the food matrix after long period of storage (Barba, Ahrné, Xanthakis, Landerslev, & Orlén, 2018; Dalvi-Isfahan, Hamdami, Xanthakis, & Le-Bail, 2017; Jha, Xanthakis, Jury, & Le-Bail, 2017). Blanching is a common mild thermal pretreatment prior to freezing aiming to inactivate deteriorative enzymes, decrease the microbial load and remove the air from the pores which subsequently can affect the nutritional characteristics of the fruits and vegetables upon storage (Munyaka, Makule, Oey, Van Loey, & Hendrickx, 2010; Xanthakis & Valdramidis, 2017).

Blanching is traditionally carried out by hot water or steam. Both techniques are highly effective in enzymatic inactivation but there are drawbacks regarding the degradation of the nutritional value (such as vitamin losses), high energy and water demands, as well as wastewater disposal. Recently, several novel alternative technologies have been

proposed such as high-pressure, ultrasound, microwave, ohmic, infrared, and radiofrequency heating for blanching of fruits and vegetables. Although these technologies are gaining momentum, there are still bottlenecks related to method simplicity, packaging materials and large-scale equipment that need to be solved and tangible advantages to be demonstrated before industrial implementation (Bernas & Jaworska, 2003; Jiang, Liu, Li, & Zhou, 2015; Lemmens et al., 2009; Xanthakis & Valdramidis, 2017; Xin, Zhang, Xu, Adhikari, & Sun, 2015).

Nutrient loss during blanching processes is strongly associated with the technology used and related heat and mass transfer phenomena. The basic mechanisms involved in blanching operations are simultaneous heat and mass transfer, which can result in temperature distribution and mass loss/gain. Depending on whether the product is emerged in water or not, heat can be transferred by water or electromagnetic energy. During water blanching the food matrix is interacting directly with the blanching medium and the physicochemical properties of the matrix such as porosity, nutrient solubility, composition, density and others are influencing the characteristics of the final product (Munyaka et al., 2010; Ramesh, Wolf, Tevini, & Bognár, 2002).

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Microwave blanching can be considered as a dry process since water is not required to increase product temperature and inactivate enzymes. Thus, leaching of water soluble compounds may be limited in comparison to water blanching. On the other side, microwave provides a faster but less homogeneous heating that may lead to non-uniform enzymatic inactivation of target enzymes such as oxidative enzymes including ascorbic acid oxidase, in the case of fruits and vegetables (Ramesh et al., 2002; Xanthakis & Valdramidis, 2017). Both water and microwave blanching are dynamic processes and time of treatment is an influential parameter that needs to be optimized not only in terms of enzymatic inactivation but also regarding the mass transfer and temperature distribution. Further to expected temperature gradients other important aspects should be taken into account during microwave thermal treatments, such as the penetration depth as well as the effect of the shape, size and dielectric properties of the samples on the electromagnetic field distribution (Ohlsson, Risman, & Rismanj, 1978). All these factors make a microwave blanching process difficult to be tuned and precisely controlled. Therefore when designing a fruit/vegetable microwave blanching process tailored conditions have to be selected based on all the above essential data and parameters.

Comparative studies reported in literature have not provided enough understanding about the underlying differences between the water and microwave blanching in terms of nutritional value of the frozen products (Bernas & Jaworska, 2003; Lin & Brewer, 2005; Tosun & Yucecan, 2008). Although there are available data in the literature regarding thermal inactivation kinetics of AAO, the residual activity of this enzyme after thermal treatment of broccoli, pumpkin (*Cucurbita maxima*), carrots (*Daucus carota* subsp. *sativus*) and others indicate that inactivation kinetics can be different with respect to the origin of the matrix (Leong & Oey, 2012; Munyaka et al., 2010; Porto et al., 2006). To our knowledge, there is not available information in literature regarding the thermal inactivation kinetics AAO present in mango. Furthermore, AAO inactivation has not been correlated with ascorbic acid concentration on frozen products and changes during storage. Thus, this study focus on mango and the retention of vitamin C after blanching and frozen storage, since this is one of the most attractive and highly perishables fruits of which around 30% is wasted (Baloch & Bibi, 2012). Ascorbic acid is usually used as nutritional quality indicator for evaluating the nutrient losses after blanching treatments due to its water solubility that makes it prone for leaching, its thermal lability, pH, metal, ion and light sensitivity as well as its oxidation catalyzed by AAO (Xiao et al., 2017).

The objective of this study was to use a kinetic approach to elucidate the differences between microwave and traditional water blanching of mango (*Mangifera indica*) prior to freezing in terms of vitamin C losses as well as in frozen mango during processing and storage. Loss of ascorbic acid due to thermal degradation, leaching and activity of AAO were determined which can be used for optimum design of mango processing or for further use in computer simulations of thermal processes.

2. Material and methods

2.1. Chemicals and reagents

Standards of L-ascorbic acid (A/8880/48) and L-dehydroascorbic acid (261556) were purchased from Fischer Scientific, Leics, UK and Sigma Aldrich, St Louis, MO, USA, respectively. Tris(2-carboxyethyl) phosphine hydrochloride (C4706-2G) and meta-Phosphoric acid (04103-250G) were purchased from Sigma Aldrich, Steinheim, Germany. Sodium phosphate monobasic monohydrate (480141) and sodium phosphate dibasic anhydrous (480087) were purchased from Carlo Erba Reagents SAS, Arese, Italy. Water gradients HPLC grade was purchased from Fischer Scientific, Leics, UK.

2.2. Sample preparation

A sufficient batch of medium ripe mangoes (*Mangifera indica*, cv. “Kent” originated from Sénégal, harvested the same day from the same area), for carrying out all the experiments of the present study, was purchased from a local supermarket (Willys, Sijön, Göteborg, Sweden) on the first day of distribution. Temperature during transportation and storage of mangoes was at 4 °C avoiding temperature fluctuations. Cylindrical pieces of mangoes (25.0 mm diameter and 17.0 mm height) were carefully cut. The weight of each sample was measured giving an average mass equal to 9.48 ± 0.26 g. During microwave blanching treatments, each sample was installed into the cylindrical cavity of a sample holder made by polyoxymethylene (POM) while the extremity of the optical fiber was centered in the cylinder at midheight. The fiber optic probe was inserted to a predetermined length to locate the tip at the exact place of temperature measurement for each sample. For the water blanching treatments unpacked or vacuum packed in plastic pouches samples were prepared with the aforementioned dimensions. Vacuum was gently applied during packaging so as to avoid any possible leaching. Samples (both packed and unpacked) were prepared in triplicates so as to facilitate all experimental conditions, both conventional (water) and microwave blanching (described in the following section). A second series of identical samples were also prepared and used for temperature recording during all blanching conditions investigated. After all blanching conditions samples were cooled down and stored at -80 °C before further analysis.

2.3. Blanching and enzyme inactivation kinetics procedures

Packed mango samples were conventionally blanched in a water bath (SW23 – Julabo USA Inc.) containing 10 L of water for the needs of ascorbic acid oxidase inactivation and vitamin C thermal degradation kinetic study. For both the kinetic studies, the water bath was preheated at 80 °C, 90 °C and 95 °C and samples were submerged in the water for different time intervals.

Microwave (MW) blanching treatments carried out by means of a tailored prototype experimental set-up built for the needs of this study. The prototype equipment, it consisted of a treatment cavity connected, through a coaxial cable, to a microwave solid-state generator supported by an automatic pulse generator (200 W, SAIREM, NEYRON, France), which could emit constant or pulsed power microwave radiation. MW generator could be manually tuned to transmit pulsed electromagnetic radiation. The optical fiber temperature sensors were linked to a data logger, which was connected to a computer in order to follow and record the temperature profile of the samples and cavity using compatible software (Optilink).

In order to compare the different blanching treatments packed and unpacked samples were tested for water blanching while only unpacked samples were tested for microwave blanching. Two different blanching scenarios were selected in both cases which were high temperature short time (HTST) and low temperature long time (LTLT). The time – temperature blanching cases were submerged for 5 min in water bath thermostatted at 90 °C and for 12 min in water bath thermostatted at 70 °C for HTST and LTLT respectively. During the MW treatments the temperature was followed by an optical fiber temperature sensor inserted in the geometrical center of each sample. The incident power was 120 W and 100 W for HTST and LTLT respectively and remained constant till the target temperature was reached. The nominal power densities during MW radiation were 14.39 W/cm^3 (HTST) and 11.99 W/cm^3 (LTLT). Once the target temperatures were reached in the geometrical center of the samples, the temperatures remained constant by irradiating the samples with a sequence of microwave pulses for the remaining time periods in each case. The total time periods of MW irradiation of the samples were 5 min and 12 min for HTST and LTLT, respectively.

2.4. Ascorbic acid oxidase extraction and enzymatic activity determination

AAO enzymatic activity was determined according to Oberbacher and Vines (1963) with some modifications. 3.0 g of mango tissue was mixed with 6.0 mL of phosphate buffer (0.1 M, pH 5.6, 0.5 mM EDTA) and homogenized for 30 s. The homogenate was then centrifuged at 13400g for 5 min in a thermostat centrifuge at 4 °C. AAO enzyme activity was determined in the supernatant enzyme extract. AAO activity was determined by a spectrophotometer (Unicam Helios, Spectronic Unicam EMEA, Cambridge, UK) measuring the absorbance decrease at 265 nm (ambient temperature) in the reaction assay mixture for 5 min. The reaction assay mixture was consisting of 2.85 mL phosphate buffer (0.1 M, pH 5.6, 0.5 mM EDTA), 100 μ L substrate and 50 μ L enzyme extract while the blank sample was consisting of phosphate buffer (0.1 M, pH 5.6, 0.5 mM EDTA). The remaining enzyme activity of AAO was expressed as the percentage ratio of the absorbance decrease rates between treated (treated) and corresponding untreated (fresh) mango samples.

2.5. L-Ascorbic acid and L-dehydroascorbic extraction and quantification

L-Ascorbic acid (L-AA) content was determined according to Giannakourou and Taoukis (2003), 3.0 g of mango tissue was mechanically stirred in 9.0 mL of a 4.5% (w/v) solution of meta-phosphoric acid for 15 min in ice-water bath. The resulting extract was vacuum-filtered and the resulting aliquot was filtered through a Millipore filter (0.45-mm) prior to injection into the chromatographic column. The HP instrumentation details are: HP Series 1100 (quaternary pump, vacuum degasser, a Rheodyne 20-mL injection loop and a Diode-Array Detector, controlled by HPChemStation software); Hypersil ODS column (2504.6 mm) of particle size 5 mm; mobile phase: HPLC grade water with metaphosphoric acid to pH 2.2; detection at 245 nm; calibrated by external standard method [by standard calibration curve using L-ascorbic acid (Sigma-Aldrich)]. L-Ascorbic acid content was expressed as milligrams per 1 g of dried weight of mango tissue.

L-Dehydroascorbic acid (L-DHAA) was determined according to Lykkesfeldt (2000) with slide modifications. 100 μ L of L-AA extract was subjected to reduction with the addition of 50 μ L 2 μ M of Tris(2-carboxyethyl)phosphine hydrochloride. After vortex mixing, sample was kept light protected at 35 °C min for 10 min before filtered through a 0.45-mm Millipore filter prior to injection into the chromatographic column following the same HPLC protocol applied for L-AA content determination. Total L-AA content was expressed as milligrams per 1 g of dried weight of mango tissue. The concentration of L-DHAA was determined by subtracting the two chromatographs (L-AA and Total L-AA) and by external standard method [by standard calibration curve using L-DHAA (Sigma-Aldrich)]. L-DHAA content was expressed as milligrams per 1 g of dried weight of mango tissue.

2.6. Frozen storage

Mango samples treated with all the blanching treatments were stored at a low-temperature incubator (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan) for a total storage period of 130 days (4.3 months). Frozen storage duration was selected to simulate the time that frozen fruits/vegetables are expected to enter the stage of retail display. According to data records retrieved from the Cold Chain Database (Gogou, Katsaros, Derens, Alvarez, & Taoukis, 2015) the time that frozen fruits/vegetables enter the stage of retail display in Europe can vary between 1 and 4 months, depending on the logistics/supply chain model adopted by the collaborating fruits/vegetables producer and retailers. During storage, temperature was constantly monitored and confirmed with electronic, programmable miniature dataloggers (COX TRACER®, Belmont, NC). Mango samples storage temperature during the time period of 130 days was -18.63 ± 0.48 °C.

2.7. Data analysis and statistical analysis

All blanching experiments were performed in triplicate while all analytical methods were performed in three repetitions for each replicate; presented results are mean of experimental values. The development of AAO inactivation and vitamin C degradation kinetic mathematical models was performed with least square exponential fit obtained by non-linear regression with Sigmaplot 10.0.

Statistical analysis was carried out using software STATISTICA 7 (Stat soft. Inc., 1999). The analysis of variance technique and Tukey's multiple range tests were used to determine the significant difference in L-ascorbic acid and L-dehydroascorbic content, in AAO remaining enzyme activity, in °Brix and water/solid loss, between the two blanching treatments (microwave assisted and conventional water blanching) at 95% confidence level ($P < 0.05$).

3. Results and discussion

3.1. Mapping temperature evolution during microwave and water blanching of mango

HTST (target temperature: 90 °C, total time: 5 min) and LTLT (target temperature 70 °C, total time: 12 min) blanching treatments using microwave or conventional hot water were performed. Fig. 1 shows representative examples of the real time - temperature histories in the geometrical center of mango cylinders for each of the blanching processes performed.

During microwave blanching the incident power remained constant at 100 W till mango reached the target temperatures of 70 °C and 90 °C for HTST and LTLT treatments and then short pulses of the same power were used to keep the temperature constant as shown in Fig. 1. The total irradiation time periods were 5 min for HTST and 12 min for LTLT microwave treatments. Temperature profiles between the two tested technologies differ due to their different mechanisms of heat transfer. Infrared thermal images of mango cross sections illustrating the differences in temperature distribution during warm water and MW treatments respectively are presented in the Fig. 2. In the case of water blanching the main mechanism of action is based in heat transfer by convection from warm water and conduction inside the product. This typical mechanism leads to an initial warming of the surface while the temperature is progressively increasing to the inner part (Fig. 2a). In case of microwave blanching of food stuff, the heat distribution within the matrix is a more complex phenomenon which can be influenced from the frequency of the electromagnetic energy, the cavity of treatment dimensions, the position of the sample in the cavity, the shape, size, composition and properties of the product (Ohlsson et al., 1978). In our case as illustrated in the cross-section of the cylindrical sample at the infrared thermal image in Fig. 2b, the mango sample is initially heated in the central domain while in the outer areas the temperature is colder.

The two different heating mechanisms and the temperature profiles acquired, indicated that although the temperature evolution seemed in both HTST and LTLT more intensive in the case of microwave treatments, in reality the overall heating can be comparable since the central location of the conventionally water blanched samples is the eschaton area that it is being heated.

Blanching conditions have been reported to have a significant impact on the final quality attributes of a food product (Ahrné, Gonzalez-Martinez, Sjöholm, & Nilsson, 2003; Oguntowo, Obadina, Sobukola, & Adegunwa, 2016). Blanching temperatures and times depend on the blanching method, the initial properties of the matrix, the desired characteristics of the final product and the subsequent preservation method. Optimum blanching conditions can vary between the different fruits and vegetable kind but even between the different cultivars of the same kind (Ngobese, Workneh, & Siwela, 2017). Since there is no general rule for the optimum blanching time - temperature

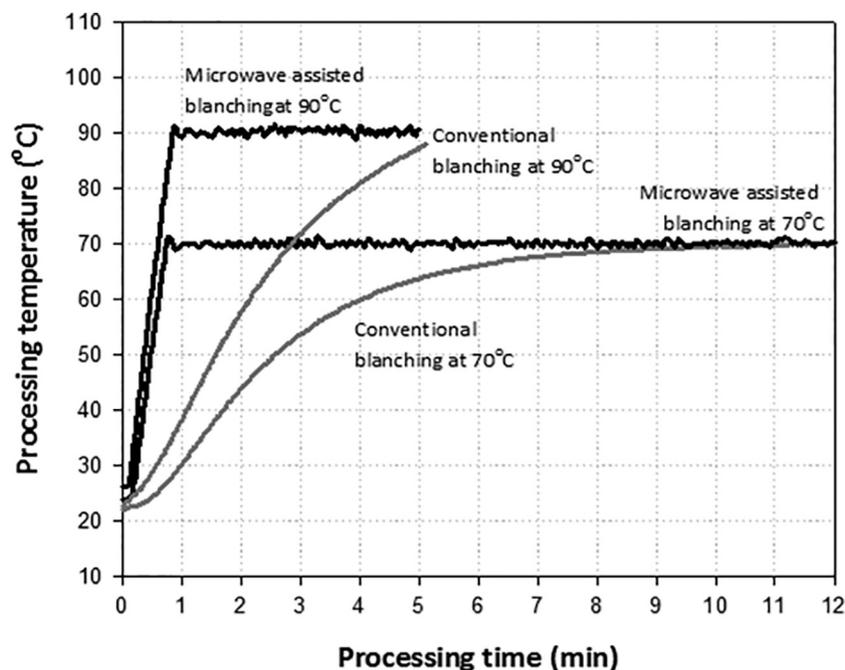


Fig. 1. Temperature evolution during the HTST (90 °C) and LTLT (70 °C) treatments under conventional-water (–) and microwave blanching (·).

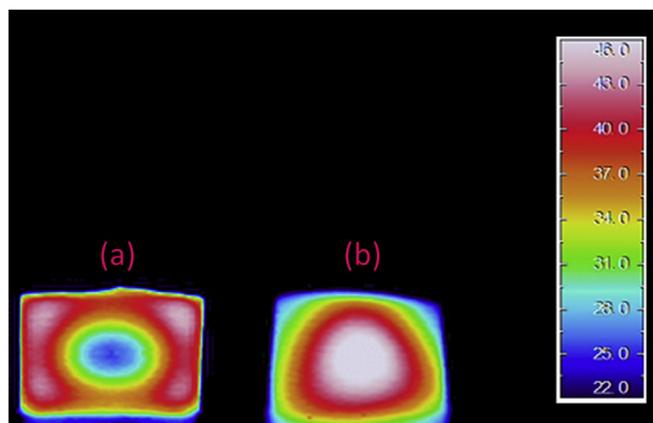


Fig. 2. Infrared thermal image of mango cross section sample illustrating the temperature distribution at 30 s of (a) water and (b) microwave blanching treatments.

combinations as they can influence nutrients content, texture, color, shelf-life and other characteristics differently for each matrix, the two aforementioned combinations were selected in this study (Beveridge & Weintraub, 1995; Olivera et al., 2008; Patras, Tiwari, & Brunton, 2011) because HTST is a traditional process, while LTLT processes have been associated with improvement of texture due to activation of enzymes like pectin methylesterase (PME), or polygalacturonase (PG) (Abu-Ghannam & Crowley, 2006; Anthon & Barrett, 2006; Ni, Lin, & Barrett, 2005). Mango structure is very sensitive and in addition to significant losses of nutrients due to leaching, a longer blanching time may also promote textural degradation.

3.2. Impact of conventional and MW blanching of physical and chemical properties of mango

Fig. 3 shows the impact of the two different blanching scenarios (LTLT & HTST) through water and microwave assisted blanching on total soluble solids (°Brix), total mass losses, remaining activity of AAO and total vitamin C of the matrix. Regarding the total soluble solids

content, which in the case of fruit matrices are strongly related to sugar moieties, it can be seen that both the water and the microwave assisted blanching are followed by higher soluble solids losses in the case of low temperature treatments (Fig. 3a). Another point that can be derived is that both MW treatments retain slightly higher soluble solids content than water blanching, however no statistical ($P > 0.05$) difference was observed between microwave and water blanching in the case of LTLT treatments except for MW-HTST treatment that was statistically different ($P < 0.05$) compared to the rest of their treatments in terms of soluble solids content. Those results can be related to the total mass changes occurring during the different blanching treatments (Fig. 3b). This graph is based on the dry matter and total water content measurements of the samples after the blanching treatments. A main difference that can be derived is that in both the LTLT and HTST water treatments the samples had a higher increase in water content than the total solid losses (no statistical difference observed, $P > 0.05$) which in turn led to total mass increase of the samples after the process. MW and water blanched samples total solids loss showed no statistical difference ($P > 0.05$) in both the LTLT and HTST treatments. LTLT treatments (both MW and water) showed slightly higher losses in total water content that could be attributed mainly to evaporation during longer blanching period.

Regarding the enzymatic inactivation efficiency of the selected processes, conventional water LTLT blanching resulted in statistically different ($P < 0.05$) inactivation of AAO leading to $> 65\%$ remaining activity (Fig. 3c). Conventional water HTST as well as both MW blanching methods had no statistical difference ($P > 0.05$) in AAO remaining activity. It is worth noting that remaining activity lower than 30% of the initial was not achieved in any of the tested blanching methods. AAO inactivation is an important indicator since it is related to the oxidation of vitamin C in fruits during thawing and storage. Another critical factor that was taken into account for the evaluation of the blanching treatments is the direct effect of each process on the native vitamin C degradation. Fig. 3d shows total vitamin C content as well as the dehydroascorbic acid and L-ascorbic acid fractions in the initial fresh samples and the corresponding treated ones. The results acquired regarding total vitamin C indicated that there was a loss due to treatment in all cases, with no statistical ($P > 0.05$) difference observed between MW and water blanching. After conventional water

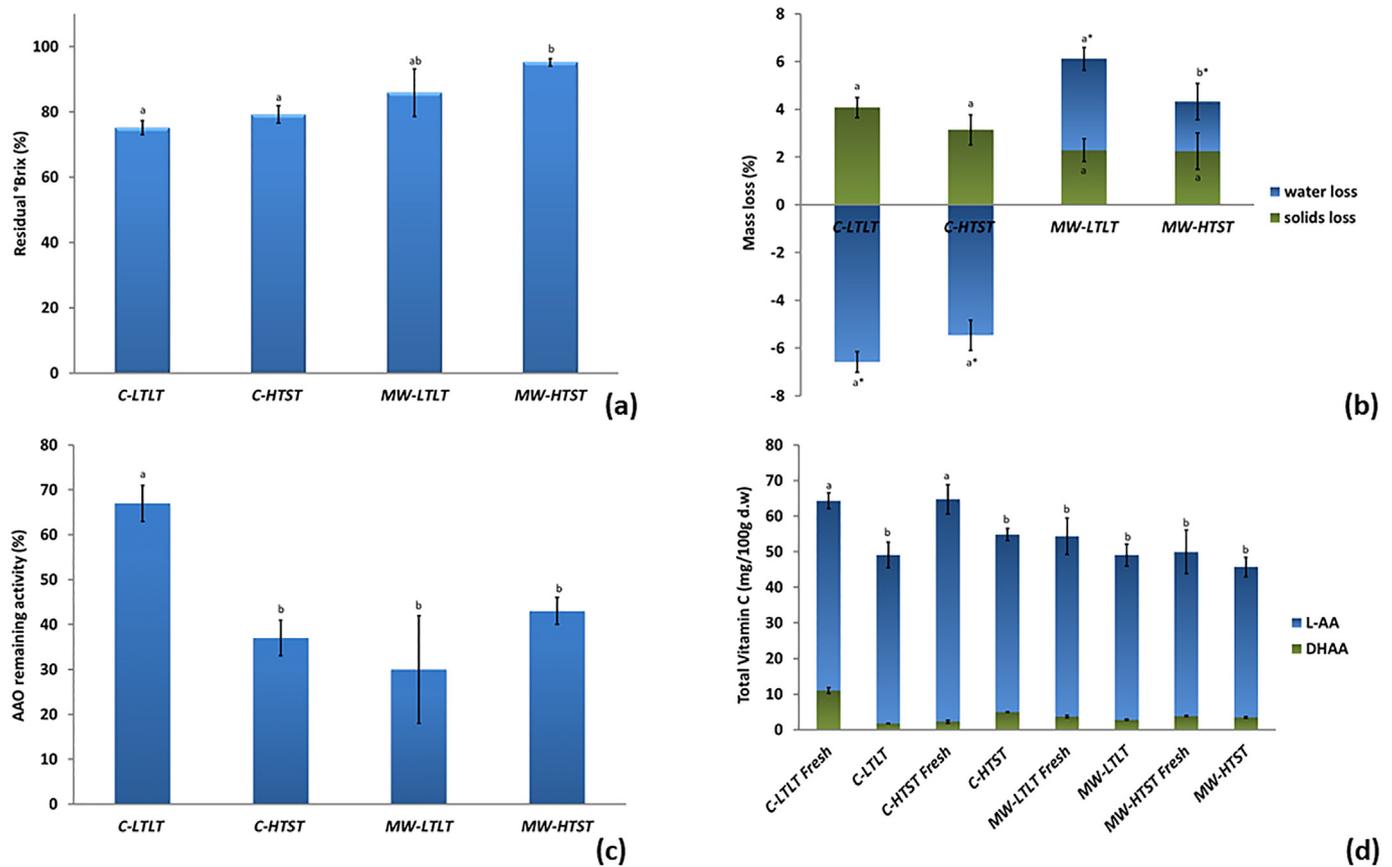


Fig. 3. Comparison of LTLT and HTST water (C) and microwave (MW) blanched samples of mango in terms of (a) Residual °Brix, (b) Mass losses, (c) AAO remaining activity and (d) L-AA and DHAA fractions of total vitamin C. Values with different superscripts differ significantly ($P < 0.05$) ($a < b$).

blanching the remaining total vitamin C was $76.4 \pm 2.8\%$ and $84.7 \pm 2.9\%$ for LTLT and HTST treatments respectively. While remaining vitamin C was $90.3 \pm 4.1\%$ and $91.5 \pm 4.4\%$ after LTLT and HTST MW blanching, respectively. Concerning the fractions of DHAA and L-ascorbic acid it can be mentioned that LTLT water blanching was followed by 85% decrease of DHAA while on the contrary HTST water blanching followed by almost 100% increase. Both the MW treatments resulted in a decrease of the DHAA since the remaining DHAA was $75.9 \pm 4.1\%$ and $89.6 \pm 3.3\%$ for LTLT and HTST respectively. Regarding the L-AA it can be mentioned that conventional water LTLT treatment followed by higher remaining L-AA ($88.9 \pm 2.4\%$) than the HTST treatment ($79.8 \pm 3.1\%$) although that the remaining total vitamin C content was higher in the case of HTST. From these results it seems that HTST water blanching induced a limited conversion of L-AA to DHAA form. The aforementioned results indicated that microwave assisted blanching led to higher retention of total vitamin C in both cases of LTLT and HTST treatments. This is in accordance with available literature where it has been reported that microwave blanching followed by higher retention of vitamin C when compared to conventional hot water blanching in various fruits/vegetables such as spinach, carrots (Ramesh et al., 2002; Xiao et al., 2017). Agüero, Ansorena, Roura, and del Valle (2008) investigated hot water blanching of squash slices under different time temperature settings and concluded that longer treatments at lower temperatures resulted in higher losses in ascorbic acid. Our results were in agreement with the results acquired by Agüero et al. for conventional blanching. Similar trend was also observed for the retention of vitamin C of microwave assisted blanched samples. The chemical degradation further than the primary oxidation of L-AA to DHAA, is followed by irreversible hydrolysis of DHAA to 2,3-diketogulonic acid which is favored during the conventional water blanching (Fennema, 1996). Although DHAA exhibits the same vitamin activity as

L-AA, because it is almost completely reduced to L-AA in the body, 2,3-diketogulonic acid is responsible for loss of vitamin C activity since it is a nutritionally inactive product (Fennema, 1996). In paragraph 3.5 a detailed kinetic study regarding the L-ascorbic acid thermal degradation is further discussed.

3.3. Effect of frozen storage on AAO and vitamin C

The differently blanched samples were stored at -18°C for a period of 18 weeks and were analyzed for total vitamin C content and AAO activity (Table 1). Total soluble solid content, (°Brix), remained unchanged in all stored samples as expected, since no mass transfer phenomena in the form of leaching and/or evaporation occurred during the storing period in the packed samples. Regarding the total vitamin C, further loss during frozen storage was measured, mainly due to a decrease of L-AA. Although L-AA decreased by almost 40% in all cases, DHAA did not show any increase during frozen storage. This indicates that all of the intermediate DHAA production through the primary oxidation of L-AA, was followed by irreversible hydrolysis of DHAA to the nutritionally inactive 2,3-diketogulonic acid during the frozen storage. Rickman, Barrett, and Bruhn (2007), published a review article comparing the vitamin C losses of several fruits and vegetables after freezing and frozen storage. In this study it was indicated that the losses in vitamin C are not only related with each step of the process but they also differ between the different species and cultivars when processed under the same conditions. Blanching, freezing and frozen storage can cause from 10 to 100% of vitamin C degradation depending on the vegetable matrix. Our study showed, that in the case of mango the major part of vitamin C degradation was related with freezing and freezing storage rather than the blanching pretreatment. A recent study by Zhang et al. (2017) regarding the impact of the temperature

Table 1

Comparison of LTLT and HTST water and microwave blanched samples of mango in terms of AAO remaining activity and L-AA and DHAA fractions of total vitamin C, directly after blanching and after 18 weeks of frozen storage.

	Water blanching				Microwave blanching			
	HTST		LTLT		HTST		LTLT	
	After blanching	Frozen storage 18 weeks						
L-AA (mg/100 g d.w.)	49.8 ± 1.6 ^a	24.8 ± 2.6 ^b	47.4 ± 3.6 ^a	27.2 ± 2.7 ^b	42.2 ± 2.7 ^a	25.3 ± 1.1 ^b	46.2 ± 3.0 ^a	26.3 ± 1.4 ^b
DHAA (mg/100 g d.w.)	5.0 ± 0.1 ^a	4.4 ± 0.5 ^{ab}	1.8 ± 0.1 ^c	1.4 ± 0.1 ^c	3.5 ± 0.2 ^b	1.8 ± 0.4 ^c	2.8 ± 0.2 ^{bc}	2.5 ± 0.2 ^{bc}
% AAO remaining activity	37 ± 4 ^a	13 ± 3 ^b	67 ± 4 ^a	56 ± 15 ^a	43 ± 3 ^a	33 ± 12 ^a	30 ± 12 ^a	20 ± 9 ^b

Values with different superscripts within the same row differ significantly ($P < 0.05$).

oscillations during frozen storage on the ascorbic acid of mangoes showed that even when the mangoes were stored below the glass transition temperature without oscillation a ca.30% degradation of ascorbic acid occurred after 4 weeks. The vitamin C degradation in the case of constant temperature stored in a glassy state vitamin C degradation was attributed to the possible occurrence of enthalpy relaxation during storage (Zhang et al., 2017). Moreover, it has been reported that during the first two weeks of frozen storage there is a change in the freezable water content in mango matrices (Zhang et al., 2018) due to the fact that even limited temperature oscillations can make part of bound water in the cell walls to be released and become available for phase transitions. Glass stage storage is not in general cost efficient and sustainable and industrial, retail and distribution storage commonly occurs in the temperature range applied in our study. Since the mobility of water molecules seems to be important for the degradation of vitamin C during frozen storage, it would have been expected that the different dry and water blanching could result in different amounts of remaining vitamin C. As shown in Fig. 2b, microwave blanching treatments led to loss of water while after water blanching the samples absorbed water from the blanching medium leading to total weight increase. However, only in case of HTST microwave blanched samples showed higher vitamin C retention ($59.3 \pm 1.9\%$) compared to water blanched ($53.2 \pm 1.8\%$). In the case of LTLT no difference was observed between the two methodologies in the remaining vitamin C (58.1 ± 1.9 ; $58.8 \pm 1.7\%$ for water and MW respectively).

Further than the impact on the remaining vitamin C, the effect of frozen storage on AAO activity was investigated. As it can be seen in Table 1 for all different blanching treatments tested there was always a fraction, higher than 30%, of AAO remaining activity, further inactivation of the enzyme occurred after 18 weeks of frozen storage no matter what the blanching pretreatment was. Higher inactivation of AAO ($65 \pm 2\%$) was observed for the HTST water blanched samples during freezing storage, while in the case of LTLT water blanching the lowest further inactivation ($16 \pm 4\%$) occurred. In the cases of microwave blanched samples the frozen storage resulted in further inactivation of $23 \pm 4\%$ and $33 \pm 5\%$ for HTST and LTLT respectively. These results indicate that AAO contained in blanched mango pieces is susceptible to denaturation during frozen storage, and hence, loss of activity. It can be mentioned that in the cases where more gentle treatment of AAO carried out during blanching (such as water LTLT and MW HTST) the impact of frozen storage on its further inactivation was less pronounced. Freeze-induced protein denaturation has been in general attributed to the formation of ice crystals, dehydration and concentration of solutes in a biological tissue (Xiong, 1997). Water molecules are essential for the retention of the active conformation of the proteins. In biological tissues such as mangoes where water is the major component in the cells, proteins exposed to the aqueous environment have a hydrophobic interior and a polar surface where water molecules are bound (Xiong, 1997). As it was already discussed, during

the frozen storage there is an increased mobility of water through recrystallization phenomena due to temperature oscillations. As it has been reported, during frozen storage there is a gradual increase of the freezable water content in mango matrices (Zhang et al., 2018). An increase in freezable water can be related to a decrease of protein bound water which in turn can result in enzymatic inactivation (Xiong, 1997). Therefore, the combination of blanching and freezing may lead to higher inactivation of AAO than the single blanching pretreatment.

3.4. Leaching and thermal effects on AAO and vitamin C

In order to explain the phenomena which lie behind the previously described results of this study, two different types of conventional water blanching, such as blanching of samples packed in plastic pouches and unpacked samples were carried out. Temperature and treatment time conditions remained the same for both the LTLT and HTST treatments. Fig. 4a indicates that total remaining soluble solids content was higher for both blanching temperatures in packed samples that was statistically different with unpacked samples ($P < 0.05$). Those differences can be attributed to the packaging protection layer from the interaction of the sample with the warm water medium. As it can be seen in Fig. 4b, further to the inhibition of the dilution and dispersion of solids in the water medium in packed samples, there was also a significant increase of total water content and total mass in both cases of unpacked samples, which can be related to diffusion of water from the blanching medium in the samples. Regarding AAO inactivation, it can be assumed that packaging improved the efficiency of LTLT blanching while in the case of HTST packed samples the remaining activity was not statistically different ($P > 0.05$) with the unpacked samples. Those results seem to show that the reduction of AAO activity in the unpacked samples was not related to the leaching of proteins in the blanching medium but mainly due to thermal denaturation.

Vitamin C in both Figs. 3 and 4 has been compared to the content of the corresponding fresh sample since the initial content was different for each sample. Among the many pre- and postharvest factors that can influence vitamin C content of horticultural crops is the maturity stage (Lee & Kader, 2000). In the case of remaining total vitamin C it can be seen that in packed LTLT treated samples there was no statistical difference compared to the corresponding fresh sample, while in the case of the unpacked LTLT treatment was followed by significant losses of total vitamin C (Fig. 4d). This fact indicates that vitamin C may have been leached during blanching since as it has been reported the higher the blanching time, the more intense the leaching (Reis, 2017). After HTST treatments both packed and unpacked samples showed similar level of total vitamin C loss. Therefore, it can be concluded that vitamin C loss in the case of LTLT treatment is mainly related to the mass transfer phenomena rather than to temperature degradation, while after HTST treatments the decrease of total vitamin C content seemed to be mainly related to thermal degradation than due to the leakage of the

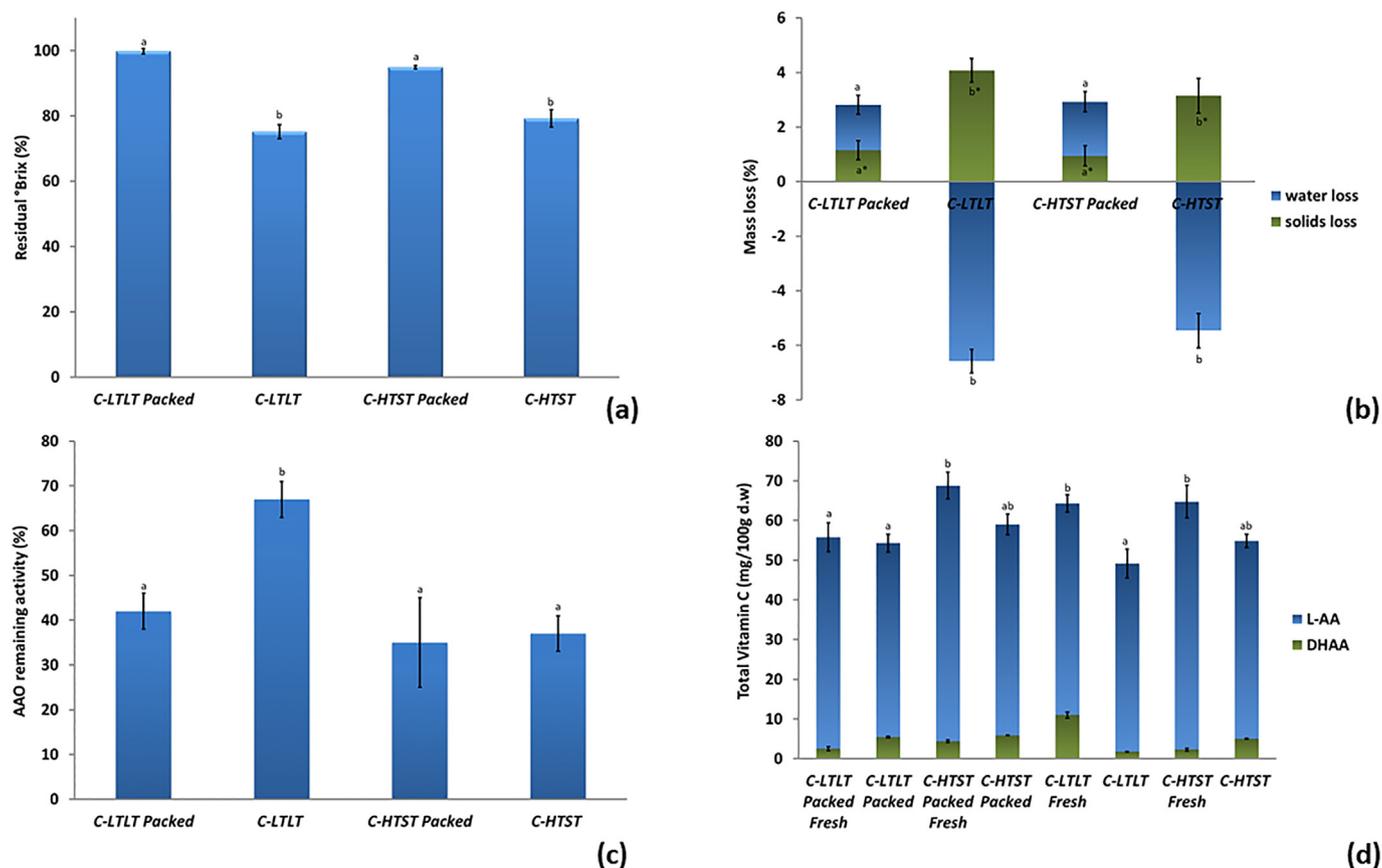


Fig. 4. Comparison of packed and unpacked samples of mango after LTLT and HTST water blanching in terms of (a) Residual °Brix, (b) Mass losses, (c) AAO remaining activity and (d) Total vitamin C. Values with different superscripts differ significantly ($P < 0.05$) (a < b).

nutrients in the blanching medium.

From the overall comparison of the differently blanched samples it can be concluded that the LTLT conventional hot water blanching did not seem to be suitable process since the ascorbic acid oxidase activity and the losses in vitamin C were higher after processing. Water LTLT treatment of packed samples was found to be more efficient.

3.5. Ascorbic acid oxidase thermal inactivation and L-ascorbic acid thermal degradation kinetics

Ascorbic acid oxidase thermal inactivation and L-ascorbic acid thermal degradation kinetic study was performed in the temperature range of 80–95 °C. A residual AAO activity (approximately 30% of the activity in the untreated sample), was found after each blanching treatment. Inactivation of AAO activity was therefore described by a first order fractional conversion model (Eq. (1)).

$$\frac{A}{A_{\text{untreated}}} = \frac{A_{rf}}{A_{\text{untreated}}} + \frac{A_{\text{untreated}} - A_{rf}}{A_{\text{untreated}}} \cdot e^{-k_T \cdot t} \quad (1)$$

where, $\left(\frac{A}{A_{\text{untreated}}}\right)$ is the remaining ascorbic acid oxidase expressed as the percentage ratio between treated and corresponding untreated (fresh) mango samples; A_{rf} is the resistant enzyme fraction (accounts for the non-zero AAO activity after prolonged heating), k_T is the inactivation rate constant at a constant temperature, T , (min^{-1}) and t is the blanching time (min).

The developed fractional model is adequately describing the existence of two AAO fractions in mango; a heat labile AAO fraction (A) that is following first order inactivation kinetics and a second fraction (A_{rf}) that shows a resistance in heat and is hardly inactivated in the studied blanching temperature domain (80–95 °C). Similar fractional conversion kinetic models have been reported in avocado

polyphenoloxidase inactivation under high pressure treatment (Weemaes, Ludikhuyze, Broeck, & Hendrickx, 1999), pectin methyl esterase in high pressure treated orange juice (Polydera, Stoforos, & Taoukis, 2005; Sampedro, Rodrigo, & Hendrickx, 2008) and carrots (Ly-Nguyen et al., 2003). The fractional-conversion models is usually applied when a fraction is inactivated and another fraction remains constant and A_{∞} , even after prolonged process treatment time. The inactivation rate constant (k_T) in Eq. (1) only relates to the temperature labile fraction of AAO. Since, k_T values derived from the fractional conversion analysis the AAO inactivation kinetic model reflect the effects of blanching temperature and time on the temperature sensitive enzyme fraction. Temperature dependence of the temperature sensitive AAO fraction was adequately expressed by the Arrhenius kinetic approach equation as depicted in Fig. 5. Nonlinear regression analysis on the AAO inactivation data was used to estimate activation energy of AAO inactivation kinetics. Activation energy was equal to $81.3 \pm 10.9 \text{ kJ/mol}$ while inactivation rate constant at reference blanching temperature of 92 °C was found to be equal to $1.1720 \pm 0.0914 \text{ min}^{-1}$.

As reported, AAO present in fruits is detected in the cell wall, in the extracellular matrix and in the vacuole and therefore intact cell structure protects AAO from thermal inactivation and leaching (Munyaka et al., 2010). In a recent study regarding mango matrices it was also observed that AAO in whole pieces of mango was in some extent heat resistant and a part of remaining activity was observed after processing (Guiamba, 2016). Cardello, Moraes, and Cardello (1993/1994) also observed that the whole samples which were blanched followed by remaining activity while in the case of mango puree AAO was almost completely inactivated in the same time-temperature conditions. Therefore cell structure and thermosensitivity of the species or cultivars can influence the remaining activity of AAO after each treatment.

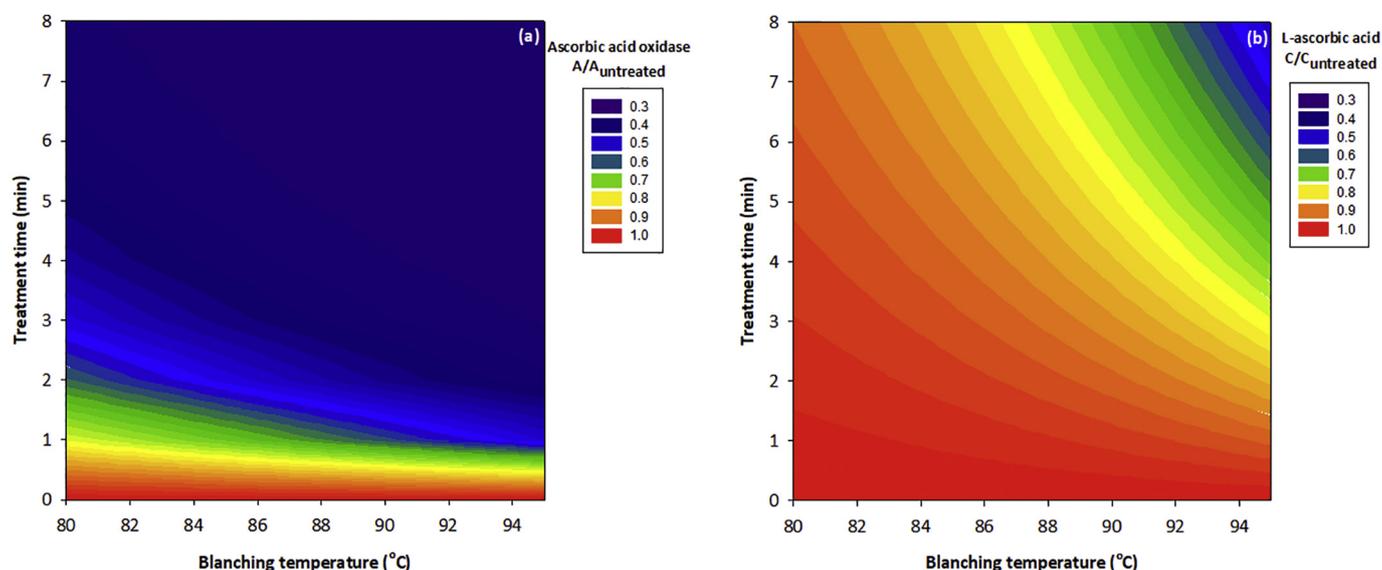


Fig. 5. Simulated isorate contour plot of (a) ascorbic acid oxidase (AAO) inactivation and (b) L-ascorbic acid (L-AA) degradation during blanching at the temperature range of 80–95 °C.

Thermal degradation of L-ascorbic acid in mango was adequately described by first order kinetics (Eq. (2)).

$$\frac{C}{C_{untreated}} = \exp(-k_T \cdot t) \quad (2)$$

where, $\left(\frac{C}{C_{untreated}}\right)$ is the remaining L-ascorbic acid content expressed as the percentage ratio of the L-ascorbic acid content between treated and corresponding untreated (fresh) mango samples; k_T is the inactivation rate constant at a constant temperature, T , (min^{-1}) and t is the blanching time (min).

L-Ascorbic acid degradation rate constant was found to follow the Arrhenius kinetic model with an activation energy of 138.8 ± 8.2 kJ/mol and degradation rate constant of $0.0622 \pm 0.0123 \text{ min}^{-1}$ at blanching reference temperature of 92 °C.

Comparison between the observed AAO and L-AA inactivation/degradation rate constant value reveals that AAO is more heat sensitive than L-AA; as depicted in Table 2 magnitude order for AAO is more than twice compared to L-AA degradation rate constant values. On the other hand L-AA was found to be more sensitive to temperature increments as the activation energy was as high as 138.8 kJ/mol, while AAO degradation showed a lower value of activation energy (81.3 kJ/mol). This specific findings can be used as process design and optimization tools to select optimum blanching temperature that can effectively inactivate AAO while retaining L-AA as much as possible. However, in pursue of optimal blanching operational parameters of mango other aspects should be taken into account as well, including temperature induced texture breakdown, color degradation and overall sensory

Table 2

Kinetic parameters for thermal inactivation of ascorbic acid oxidase (AAO) as described in Eq. (1) and L-ascorbic acid (L-AA) thermal degradation as described in Eq. (2) in mango at blanching temperatures of 80–95 °C.

Temperature (°C)	AAO inactivation rate constant, k (min^{-1})	L-AA degradation rate constant, k (min^{-1})
80	0.5477 ± 0.0792	0.0261 ± 0.0044
90	0.9023 ± 0.1629	0.0374 ± 0.0023
95	1.5088 ± 0.2273	0.0944 ± 0.0088
Activation energy, E_a (kJ/mol)	81.3 ± 10.9	138.8 ± 8.2
k_{ref} (min^{-1}) $T_{ref} = 92$ °C	1.1720 ± 0.0914	0.0622 ± 0.0123

quality deterioration. The developed kinetic model on L-AA degradation can be used as a mathematical tool to predict L-AA loss in a given mango blanching process and thus evaluate nutrients loss during blanching. In general, in the case of fruits/vegetables blanching operations a mathematical correlation between adequate enzymatic treatment and nutrients loss can be essential in terms of process evaluation and optimization. In the present study the concept of correlating AAO (serving as mango blanching target) and L-AA (serving as nutrient/quality indicator) was visualized as a comparison between the respective isorate contour plots. Isorate contour plot graphs are often used to enable a graphical representation of the combined process parameters dependency for a given quality/process indicator (Fachin, Van Loey, Oey, Ludikhuyze, & Hendrickx, 2002; Ly-Nguyen et al., 2003). In the present study isorate contour plots were used to graphically depict blanching temperature-time dependency of AAO inactivation vs. L-AA retention.

Isorate contour plots were constructed based on the developed mathematical kinetic models and the kinetic parameters estimation (Table 3), for AAO inactivation and L-AA degradation in blanching processes, respectively. The isokinetic diagram of AAO and L-AA is illustrated in Fig. 5; from the comparison between the two isorate contour plots it is clear that AAO is more heat sensitive than L-AA. Similar trend has been identified regarding the AAO and L-AA in the case of whole broccoli florets blanching, while in the case of crashed florets treatment L-AA found to be also thermosensitive (Munyaka et al., 2010). This can be considered as a key tool to select blanching conditions, optimum blanching processing of mango would require the highest possible inactivation of AAO while maintaining the maximum possible L-AA content.

3.6. Comparison of the two blanching processes based on kinetics

The developed kinetic models of AAO inactivation and L-AA degradation were used to predict the remaining content of AAO and L-AA of the selected blanching processes under investigation i.e. water and microwave blanching at 70 °C for 12 min (LTLT) and at 90 °C for 5 min (HTST). Data retrieved by the actual time-temperature profiles of the performed blanching processes (Fig. 1) were integrated into the developed kinetic models (Eqs. (1) and (2)) to estimate the predicted values of remaining AAO activity and L-AA concentration after blanching. Predicted values were compared to the experimentally observed ones as determined by means of AAO and L-AA analytical

Table 3

Evaluation of mango AAO inactivation and L-AA degradation kinetic models efficiency to predict the % remaining AAO and L-AA content after different blanching processes based on the $F_{92^{\circ}\text{C}}$ -value of the processes under investigation.

	$F_{92^{\circ}\text{C}}$ (min)	Predicted % AAO	Experimental % AAO	% Error of prediction	Predicted % L-AA	Experimental % L-AA	% Error of prediction
Water blanching 70 °C, 12 min (LTLT)	1.9	42	67 ± 14	−60	89	89 ± 7	0
Water blanching 90 °C, 5 min (HTST)	1.3	49	37 ± 6	24	92	80 ± 2	15
Microwave blanching 70 °C, 12 min (LTLT)	2.8	37	30 ± 12	19	84	92 ± 5	−9
Microwave blanching 90 °C, 5 min (HTST)	3.9	36	43 ± 3	−19	78	90 ± 6	−13
Microwave blanching 70 °C, 8.8 min (LTLT)	2.0	41	n.a	n.a	88	n.a	n.a
Microwave blanching 90 °C, 2.9 min (HTST)	2.0	41	n.a	n.a	88	n.a	n.a

methods in mango samples before and after the performed blanching treatments. The efficiency and validation of the developed kinetic models were performed in terms of prediction error percentage (Eq. (3)), given in Table 3.

$$\% \text{ Error of prediction} = \frac{\text{Value}_{\text{predicted}} - \text{Value}_{\text{observed}}}{\text{Value}_{\text{predicted}}} \% \quad (3)$$

It should be noted that the calculated error of prediction includes two sources of error; (i) the accuracy in measuring enzyme activity and L-AA concentration and (ii) the kinetic models prediction error. As summarized in Table 3, predictive performance of the developed kinetic models can be characterized as acceptable with the error value ranging from −12 to 15%. In order to further facilitate a comparison between the different blanching processes (microwave vs. water blanching) in terms of process impact, the F-value was estimated. F-value was calculated by integrating the actual blanching processes time-temperature profiles using the AAO inactivation kinetic model (i.e. AAO served as the blanching target attribute) from Eq. (4):

$$F_{92^{\circ}\text{C}} = \int_0^t \frac{k_T}{k_{92^{\circ}\text{C}}} \cdot dt \quad (4)$$

As given in Table 3, the performed microwave blanching treatments were described by much higher F-values than the performed water blanching treatments. Based on this observation and in order to compare microwave and water blanching in terms of remaining AAO and L-AA, two new case studies of microwave blanching were considered. The two case studies were selected to have an F-value of 2 min, one characterized as LTLT (low temperature, long time) and the second as HTST (high temperature, short time). Based on the developed AAO inactivation kinetic model LTLT and HTST microwave blanching with an F-value of 2 min would require blanching at 70 °C for 8.8 min and at 90 °C for 2.9 min, respectively.

Overall, estimated F-values of the performed microwave blanching treatments were much higher than those in water blanching treatments. This was expected as target temperature in microwave blanching is rapidly achieved in the geometrical centre of food samples due to the underlying mechanism of heat transfer in microwave heating. However, it must be noted that in the present study F-value estimation was based on integrating time-temperature data recorded in the geometrical centre of mango cylinder samples without taking into account temperature gradients as depicted in Fig. 2. Thus, in the case of microwave assisted blanching, F-values estimation gave an over prediction of the cumulative effect of temperature. When designing a microwave blanching process to be equivalent to a water blanching processes (in terms of required F-value) difference in heat transfer and temperature gradients should be taken into account using mathematical tools such as computational modelling.

4. Conclusions

The results of this study showed that microwave HTST as well as LTLT microwave treatments of mango pieces showed higher degree of AAO inactivation compared to the conventional water blanching. A residual AAO activity ca. 30% was observed and it was confirmed through a first order fractional conversion model acquired from the kinetic study. Freezing and frozen storage of the mango samples were followed by further inactivation of the thermostable fraction of AAO remaining after blanching. Microwave assisted blanching led to an overall higher retention of total vitamin C in both cases of LTLT and HTST treatments. In water blanching, vitamin C loss in the case of LTLT treatment was mainly related to the mass transfer phenomena (leaching) rather than to temperature degradation, while in HTST treatments the decrease of total vitamin C content seemed to be mainly related to thermal degradation than due to the leaching of the nutrients in the blanching medium. Moreover, our study showed that in the case of mango the major part of vitamin C degradation was attributed to freezing and frozen storage rather than the blanching pretreatment.

The results presented, indicated the differences of AAO and vitamin C behavior contained in mango pieces compared to other fruits and vegetables. The relation of thermostability, mass transfer and cryo-sensitivity as well as the kinetic models of AAO and vitamin C thermal degradation given in the present study can be useful for the optimal industrial design of frozen mango products.

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