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ORIGINAL ARTICLE

Effect of thermal and high pressure processing on stability of betalain extracted from red beet stalks

Cláudia Destro dos Santos[1] · Marliya Ismail[2] · Aline Schilling Cassini[1] · Ligia Damasceno Ferreira Marczak[1] · Isabel Cristina Tessaro[1] · Mohammed Farid[2]

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Abstract Red beet stalks are a potential source of betalain, but their pigments are not widely used because of their instability. In the present work, the applicability of high pressure processing (HPP) and high temperature short time (HTST) thermal treatment was investigated to improve betalain stability in extracts with low and high concentrations. The HPP was applied at 6000 bar for 10, 20 and 30 min and HTST treatment was applied at 75.7 °C for 80 s, 81.1 °C for 100 s and 85.7 °C for 120 s, HPP treatment did not show any improvement in the betalain stability. In turn, the degradation rate of the control and the HTST thermal treatment at 85.7 °C for 120 s of the sample with high initial betalain concentration were 1.2 and 0.4 mg of betanin/100 ml of extract per day respectively. Among the treatments studied, HTST was considered the most suitable to maintain betalain stability from red beet stalks.

✉ Cláudia Destro dos Santos
cacaud@enq.ufrgs.br

Marliya Ismail
m.ismail@auckland.ac.nz

Aline Schilling Cassini
alinesc@enq.ufrgs.br

Ligia Damasceno Ferreira Marczak
ligia@enq.ufrgs.br

Isabel Cristina Tessaro
isabel@enq.ufrgs.br

Mohammed Farid
m.farid@auckland.ac.nz

[1] Department of Chemical Engineering, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

[2] Department of Chemical and Materials Engineering, The University of Auckland, Auckland, New Zealand

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Introduction

Red beet (*Beta Vulgaris* L.) is the most common source of betalains containing of 40–200 mg of betanin/100 g, that belongs to betacyanin (Manchali et al. 2013; Stintzing and Carle 2008a).

Even being the abundant source of betalains, the red beet remained underutilized, since only the root, which is considered the main part of the plant, is used as food and as a natural source of pigments and enzymes (peroxidase) (Stintzing and Carle 2008a; Rudrappa et al. 2005). Betalains from the red beet root may have undesirable flavor and aroma, reminiscent of the smell of wet earth, due to the presence of pyrazine and geosmin compounds (Stintzing and Carle 2008a). Moreover, the large quantity of sugar in the root affects the tinctorial power, requiring the application of processes such as fermentation (Jackman and Smith 1996). In contrast, the stalks (stems or petioles) and leaves of the red beet are not widely used and are still considered an agricultural residue. When stalks are used as a source of natural pigments, they cease to be a residue and become by-products with added value. Therefore, the use of these by-products allows the enrichment of processed foods as well as ensure better use of natural resources. According to Koubaier et al. (2014), the extract from Tunisian red beet (*Conditiva* variety) stalks has considerable amounts of betalains, betacyanins and betaxanthins, and antioxidant capacity. Further, they reported that the extract from stalks has a higher total phenolic content than the root giving 10.4 ± 0.5 and 6.6 ± 0.7 mg of gallic acid equivalent per g of extract, respectively.

Although beetroot and stalks are a source of natural pigments, the low stability of betalains restricts its use. Earliest reports on stability suggest that betalains may undergo degradation due to external and internal factors. The external factors affecting betanin stability include oxygen, temperature, pH, light and food matrices. According to Jackman and Smith (1996), the betalains are more stable when pH of the food is between 3 and 7. Nevertheless, betanin from red beet showed the most stable form when it is in pH from 5.5 to 5.8 in the presence of oxygen and from 4.0 to 5.0 under anaerobic conditions (Huang and von Elbe 1987; Saguy 1979). With respect to temperature, studies suggest that the beet pigment degrades considerably when it is processed at temperatures between 50 and 120 °C (Güneşer 2016; Chandran et al. 2014; Saguy 1979).

The betalains degradation by thermal treatment is not only dependent on the temperature, but it also depends on the rate of heating, betalains concentration, light and presence of oxygen (Manchali et al. 2013; Herbach et al. 2006). In addition, betalains stability is directly influenced by the content of water/moisture in the extract (Damodaran et al. 2008; Herbach et al. 2006). Serris and Biliaderis (2001) observed that beetroot pigments encapsulated in maltodextrin matrix (Gluco Dry200) stored at 50 °C temperature and at different water activity levels, showed different degradation rates. The aforementioned authors observed a half-life period of 236.5 days for pigments having $a_w = 0.23$, while it was only 3.9 days at $a_w = 0.64$, at the same storage condition. Further, when these pigments were exposed to light, it causes an excitation of electrons of the betalains chromophore, affecting their stability (Herbach et al. 2006; Jackman and Smith 1996). Damodaran et al. (2008) showed that these natural pigments become more stable in the absence of oxygen; and Manchali et al. (2013) confirmed it further by mentioning that there is a linear decrease in stability with increasing concentration of oxygen.

On the other hand, the internal factors that affect betalain stability are mainly enzymes: *b*-glucosidases, polyphenol oxidases (PPOs) and peroxidases (PODs). PODs are present mainly in the central vacuoles of plant cells (Yong 2014; Neelwarne and Rudrappa 2013) and when POD is out of a "plant system", it can catalyze the dehydrogenation of a wide range of phenolic, aromatic amines and hydroquinone compounds. Although, not all peroxidase functions are completely understood, it has been confirmed that this enzyme can degrade natural pigments present in the form of carotenoids (Huang et al. 2013), anthocyanins (Jackman and Smith 1996) and notably betalains from beetroot extracts (Damodaran et al. 2008). The red beet is rich in POD; consequently, it is important to control POD activity to retain betalains in beetroot extract. It has been understood that POD activity is mainly affected by temperature, pH, water activity and light (Damodaran et al. 2008).

Liu et al. (2008) studied the POD inactivation of the beetroot extract at varying pH and temperature. They observed a POD residual activity (RA) of 78.3% in red beetroot extract (pH 5.83) after a thermal treatment at 55 °C for 60 min. Prior to this, a similar result was observed by Rudrappa et al. (2005), who investigated the thermal stability of red beetroot extract at different pHs (4, 6, 7 and 9) and temperatures (50, 60 and 70 °C). The aforementioned authors obtained a reduction of enzymatic activity less than 40% when a beetroot extract at pH 6 was thermally treated at 50 and 60 °C for 40 min. These studies show that moderate thermal treatment (50–60 °C) is not sufficient for POD inactivation of red beet when it is applied for short time. This result was further confirmed by Liu et al. (2008), who estimated a decimal reduction time (*D* value) of POD inactivation equal to 555.56 min, at 55 °C, i.e., a thermal treatment for more than 550 min at 55 °C is required to obtain a 90% reduction of RA of POD. However, long processing times could cause extensive degradation of the betalains. On the other hand, Rudrappa et al. (2005) observed an increase in POD inactivation when the temperature was increased to 70 °C, for 10 min. This result is in accordance with Yong (2014) who mentioned that enzymes lose their activity at temperatures above 60 °C.

Therefore, high temperature short time (HTST) thermal treatment could be an alternative for treatment of betalains extract since it allows the application of heat in a short period of time, resulting in less change in the natural characteristics of the products, when compared to conventional thermal treatment. Furthermore, the use of betalains as food colorings must be evaluated not only when they are being processed, but also during the storage period to ensure their stability. However, to the best of our knowledge, there are no studies on long term stability of betalain using this technology neither from extracts of red beetroot nor from red beet stalks.

Alternatively, some other technologies are being studied in order to promote the betalain stability while keeping the pigment content as high as possible. In the study of Liu et al. (2008), high pressure carbon dioxide (HPCD) at 37.5 MPa and 55 °C was applied to red beet extract for the inactivation of PODs and PPOs. They obtained a *D* value of POD inactivation after a long processing time of 74.6 min. Moreover, Santos et al. (2016), used microfiltration combined with ultrafiltration to reduce the POD activity by 99.5%. However, the authors reported that there was significant reduction in the concentration of betalains due to permeate flux reduction and fouling phenomenon during the membrane separation process.

High pressure processing (HPP) is a promising technology, which allows the development of new food products with sensory and nutritional quality and for shelf life extension (Barbosa-Cánovas et al. 2005). The literature reveals that this technology has been used to inactivate enzymes such as polyphenol oxidase and peroxidase, and to inactivate undesirable microorganisms such as aerobic mesophiles, yeast and molds (Hulle and Rao 2016; Briones-Labarca et al. 2015). Nevertheless, there is no study related to betalain extraction from red beet stalks and evaluation of HPP on betalain stability.

In this context, the main objective of this study was to investigate, the applicability of two distinct technologies, HPP and HTST thermal treatment, on POD inactivation and stability of betalain from red beet stalks extract.

Materials and methods

The red beet (*Beta vulgaris* L.) stalks of Detroit Dark Red variety, produced in Clevedon, New Zealand was used in this study and all tests were carried out in duplicate.

Extract preparation

The extract from red beet stalks was obtained by crushing (Juicer, Breville model BJE200C, Australia and China) and subsequent solid–liquid extraction process (domestic blender, Breville model BBL420, Australia and China).

There is a natural variation in the pigment content in red beets, and the initial concentration of betalains could be one of the main factors that affect the stability of the extract. Therefore, this study was carried out using extracts with low and high initial betalain concentrations [with significant difference ($p < 0.05$) according to the Tukey test].

After the HPP or HTST treatments, the extracts were centrifuged (2900 G for 10 min at 4 °C), filtered and stored in sealed tubes protected from light under refrigerated conditions. Prior to treatment, 20 ml each of extracts were transferred to plastic pouches and were sealed.

High pressure processing

The HPP tests were performed using Avure 2L-700 HPP Laboratory Food Processing System (Serial No. 101130, USA), at room or moderate (around 40 °C) temperatures under 4000 or 6000 bar of pressure. The HPP machine use distilled water as the pressure-transferring medium in the chamber and the temperature is registered by a thermocouple. After the treatment, the chamber was instantaneously decompressed.

Preliminary tests

Processing time Preliminary tests were carried out to evaluate the content of betalain in the extracts as a function of time during HPP processing. The HPP treatment was applied for the extract in sealed pouches for 10, 20, 30, 40 and 50 min, at moderate temperature and under 4000 bar of pressure. Subsequently, the analysis of betalain concentrations were carried out in treated and untreated (control) samples. Control samples were prepared in the same way as the treated samples in order to eliminate the effect of degradation due to the sample preparation.

Processing pressure In addition, a preliminary test was applied to evaluate the impact of pressure on the content of betalain in the extracts subjected to HPP. Packed extract were treated at 4000 and 6000 bar, during 20, 30 and 40 min, at moderate temperature. After the test, the betalain concentration of each sample was estimated as given in "Betalain concentration".

Betalain stability treated by HPP

Investigations on the stability of betalain were carried out with the most suitable operating conditions obtained from the preliminary study. Therefore, extracts containing 12.0 ± 0.2 mg of betanin/100 ml and 17.3 ± 0.3 mg of betanin/100 ml were treated for 10, 20 and 30 min, at room temperature and 6000 bar of pressure. The betalain concentration was analyzed on the first day of treatment (1st day), on the 2nd and 4th day and the POD activity was estimated on the 2nd day.

HTST thermal treatment

Determination of effective treatment temperature (T_{eff})

In thermal treatments, it is common that the authors refer to the average or final temperature as the treatment temperature. However, this may not be the accurate temperature used in the treatment, since degradation kinetics could be affected by the non-isothermal behavior of systems during thermal treatment. Therefore, it is necessary to calculate the effective temperature as suggested by Farid and Alkhafaji (2012). The authors defined the effective temperature as the temperature in which the reaction rate constant is equal to its integral value within the temperature change as given by Eq. (1):

$$T_{eff} = \frac{-\frac{E}{R}}{\ln\left(\frac{\sum_{0}^{n} e^{-E/RT}}{n-1}\right)} \quad (1)$$

where E is the activation energy of betanin [82,061.3 J mol^{-1} (Saguy 1979)] at pH close of the red

beet stalk extract (pH 5.8), R is the gas constant (8.314 J mol^{-1} K^{-1}), T is the absolute temperature (K) and n is the number of measuring points during the thermal treatment (the temperature was measured every 10 s).

Betalain stability treated by HTST thermal treatment

In the thermal treatment, 20 ml each samples of 12.2 ± 0.1 mg of betanin/100 ml of extract and 17.3 ± 0.3 mg of betanin/100 ml of extract, that were in sealed pouches were placed in an oil bath preheated at 120 °C, and were treated for 80, 100 or 120 s. After these periods, the samples were rapidly cooled in ice water bath. The cold samples were filtered and stored in Falcon tubes, under refrigerated conditions and protected from light for further analyses. The betalain concentration was checked on the first day and several days after the treatment (2nd, 4th and 8th day) and the POD activity was observed on the 2nd day.

Analyses of extracts

Betalain concentration

The betalains concentrations were determined as a function of betanin concentration in the extracts (mg of betanin/100 ml of extract), because betanin is the major betalain component in red beet. In this analysis, the Nilsson method was applied to quantify betanin concentration by using a UV/VIS spectrophotometer (Agilent 8453) as described by Stintzing and Carle (2008b). The assay was performed in duplicate and the betalain concentration is calculated using Eq. (2):

$$Betalain\ content = A \times DF \times MW \times \frac{1000}{\varepsilon \times L} \quad (2)$$

where A is the absorption λ maxim at 536 nm corrected by absorption at 600 nm; MW represents the molar mass of betanin (g mol^{-1}); DF is the dilution factor, ε is the molar absorptivity (L mol^{-1} cm^{-1}) and L is the path length (cm) (Stintzing and Carle 2008b). In order to support the discussion, the results are presented as mg/100 ml instead of mg/l.

The degradation of betalain during the period of storage was evaluated through the degradation rate (DR), which is related to the variation of betalain concentration of extract over storage time. The DR (mg of betanin/100 ml of extract per day) is calculated using Eq. (3):

$$\mathrm{DR} = \frac{dC}{dt} \quad (3)$$

where C is the betalain concentration of the extract (mg of betanin/100 ml of extract) and t is the storage time (day).

Peroxidase activity

The assay of POD activity used guaiacol as a substrate, because the rate of formation of oxidized guaiacol is a measure of POD activity in a spectrophotometric analysis (Neelwarne and Rudrappa 2013).

The activity of the peroxidase enzyme in each extract was determined with a UV/VIS spectrophotometer (Agilent 8453) according to the method described by Vetal and Rathod (2015), with some modifications as described by Santos et al. (2016). The assay was performed in duplicate and the results were expressed in % RA—residual activity of POD, which is calculated using Eq. (4):

$$RA = \frac{POD\ activity\ of\ the\ sample\ after\ the\ treatment}{POD\ activity\ of\ the\ control\ sample} \quad (4)$$

Statistical analysis

The statistical analysis in this study was conducted using analysis of variance (ANOVA) and Tukey test, with 95% confidence level. The software used was Statistica® 7.0 for Windows (Statistica® 7.0, Stat Soft).

Results and discussion

The betalain concentration in the red beet stalks ranged from 0.21 to 0.25 mg of betanin/g of stalks. Due to the low concentration of pigments in stalks, the results were shown in mg of betanin/100 ml of extract.

High pressure processing

For all HPP treatment of samples of red beet extracts, the pressurization was less than 3 min and the depressurization was faster than the pressurization. The temperature increased with the pressurization, but it was kept near 25 °C (room temperature) or 40 °C (moderate temperature) during most of the treatment in all tested conditions.

Preliminary tests

Preliminary tests were carried out to determine the most suitable operating conditions of the HPP treatment for red beet stalk extracts related to time and pressure applied. In this sense, Fig. 1a, b shows the influence of HPP treatment on the betalain concentration. It was observed that the HPP treated samples have got lower betalain concentrations than the untreated control sample. This could be due to the HPP treatment itself and the subsequent sample preparation that would have caused over exposure to oxygen and/or light. Further it was shown that the samples treated for a shorter

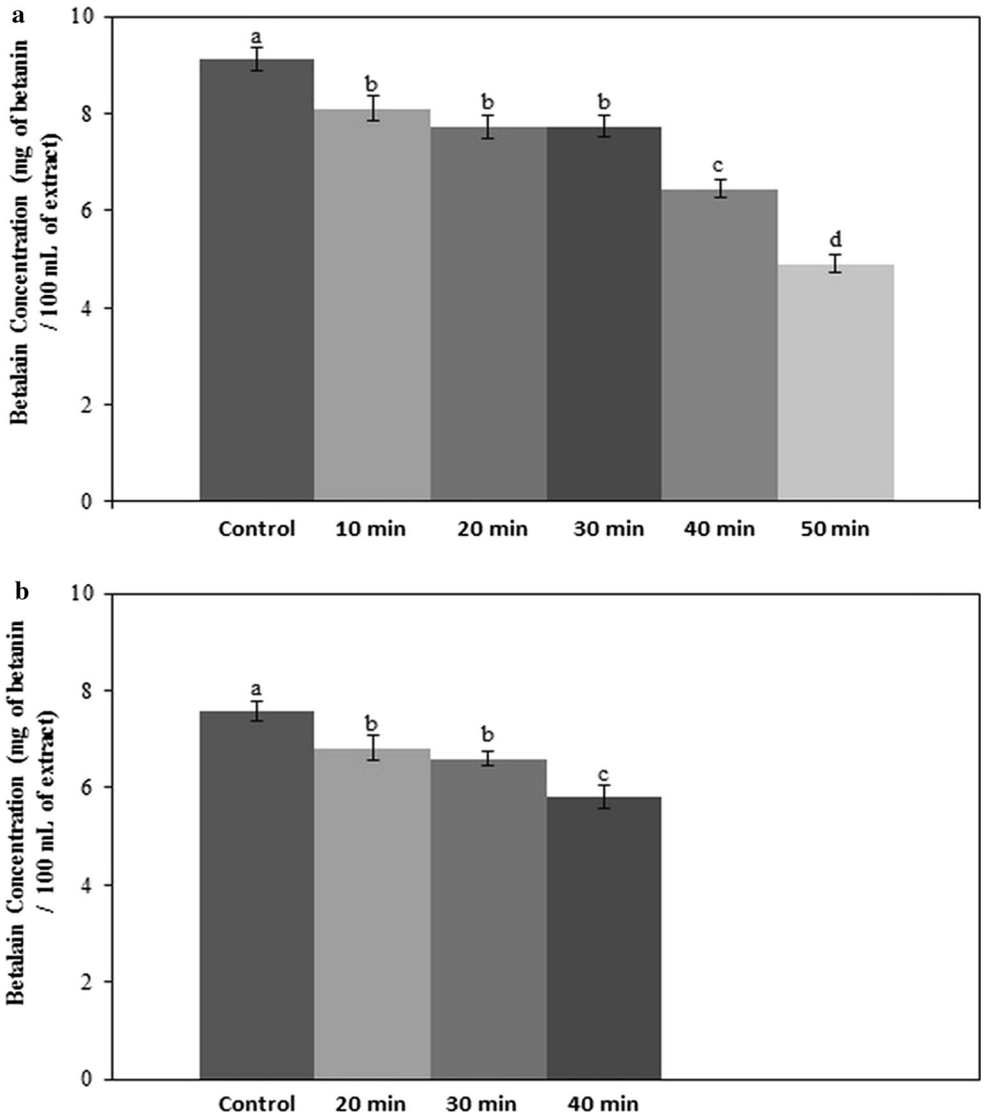

Fig. 1 Betalain concentration of red beet stalk extract when subjected to HPP treatment during short and long processing time at moderate temperature (40 °C) and under 4000 bar (**a**) and 6000 bar (**b**) of pressure, for different treatment times

time ($t \leq 30$ min), presented higher betalain concentrations than the samples treated for 40 and 50 min.

The processing times of 40 and 50 min were deemed too long due to the reduction in betalain concentration of 30.2 and 46.9%, respectively, to treatment at 4000 bar (Fig. 1a); and 23.2% at 40 min for treatment at 6000 bar (Fig. 1b). Further, the increase in pressure did not cause a drop in betalain concentration of red beet stalks extract, as the betalain concentration reduction of the sample treated at 4000 bar during 30 min was 16.2% and the sample treated at 6000 bar was 12.8%.

These preliminary studies show that the concentration of HPP-treated betalain extract is a balance between the initial concentration, degradation caused by the processing and the preparation of samples and the HPP treatment conditions used. Therefore, a pressure of 6000 bar at treatment times ≤ 30 min were selected to treat extracts of two different concentrations for the stability tests with HPP treatment.

Stability of betalains with HPP

Figure 2a, b shows the stability of betalain concentration of the samples when treated by HPP (6000 bar) for 10, 20 and 30 min, with low and high initial betalain concentration, and their respective control samples during the 1st, 2nd and 4th day of storage. It confirms the degradation of betalain during HPP treatment when compared to the control. As mentioned previously, the decrease of the betalain concentration on the first day may be due to oxidation and to the exposure to light during the preparation of the samples. The absorption of light causes an excitation of electrons of the betalain chromophore, raising these electrons to a higher energy level and leaving more reactive molecule (Herbach et al. 2006; Jackman and Smith 1996), favoring the oxidation of the pigments, causing their degradation. Moreover, Fig. 2 also shows that betalains get degraded with storage time irrespective of initial betalain

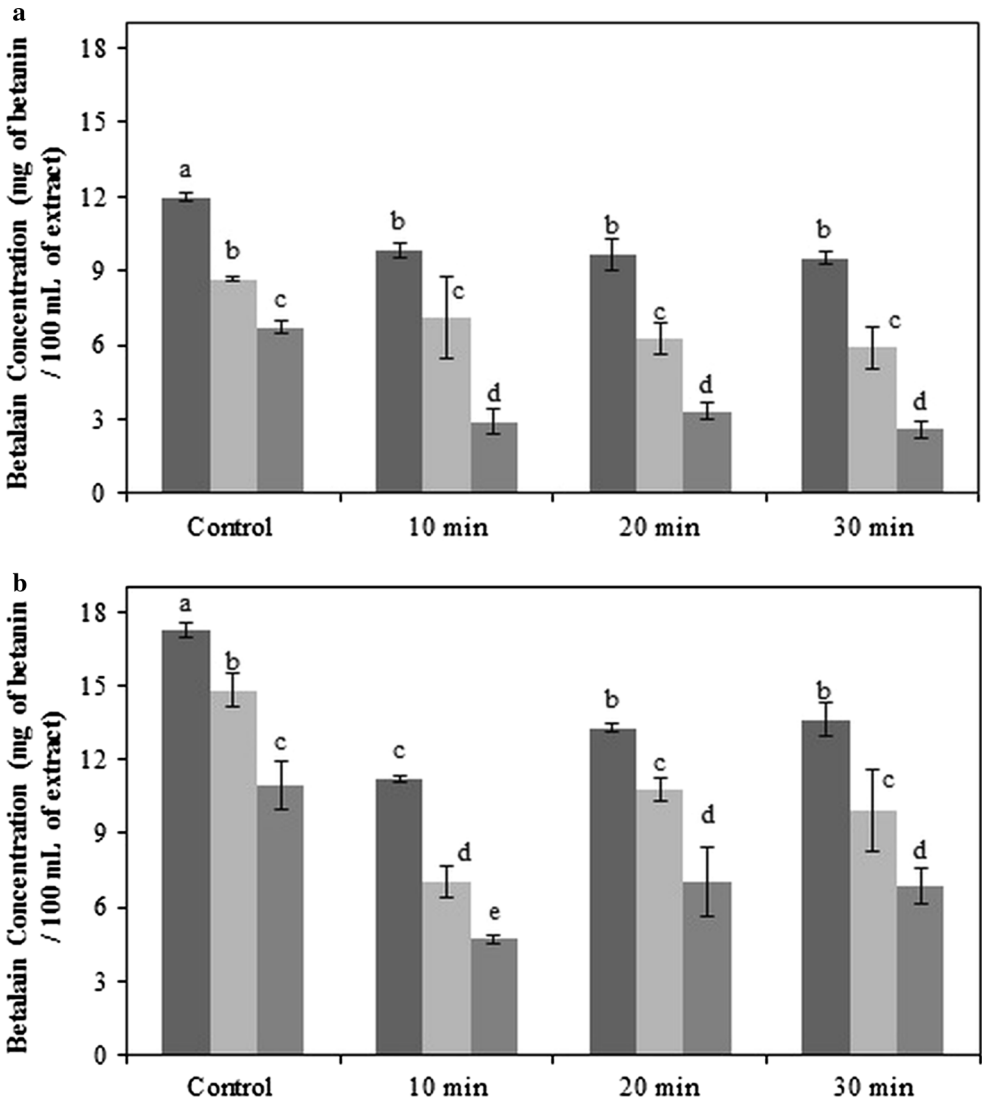

Fig. 2 Betalain concentration of red beet stalk extract, with low (**a**—12.0 ± 0.2 mg of betanin/100 ml of extract) and high (**b**—17.3 ± 0.3 mg of betanin/100 ml of extract) initial betalain concentration, over 1st, 2nd and 4th day of storage, for control and after subjected to HPP treatment at 6000 bar, for different treatment times. In this figure: ■ is 1st day, ■ is 2nd day and ■ is 4th day

concentration. The calculated betalain DR as given in Eq. (3) of the control and the treated samples at 10, 20 and 30 min were: 1.8, 2.3, 2.1 and 2.3 mg of betanin/100 ml of extract per day, respectively, to samples with low initial betalain concentration and 2.1, 2.2, 2.1 and 2.3 mg of betanin/100 ml of extract per day, respectively, to samples with high initial betalain concentration. This shows that HPP treatment neither enhance extraction nor assist in maintaining the stability of betalain during the storage period considered.

Recently, Paciulli et al. (2016) studied the impact of thermal processing and HPP on the quality of beetroot slices. The authors observed an improvement in the extraction of betanin when the HPP was applied until 7 min and they considered that the increase of the betanin content was due to cell breaking. The samples treated for 15 and 30 min had higher betalains concentration than the raw sample but lower than the samples treated for 3 and 7 min. The authors speculated that the baro-induced intensification of the partial pressure of oxygen in the samples treated with HPP during 15 and 30 min was the cause of the decrease in betanin content. The extraction by HPP may have been higher in the beetroot slices than in the red beet stalks extracts because HPP treatment would have been effective in opening the cells of the plant tissue. Whereas in this study, the higher percentage of betalain was already extracted during sample preparation using crushing followed by mixing and blending. Also, the addition of water during the extraction could have an effect on the concentration and a_w, whereas Paciulli et al. (2016) study was done on solid beet slices.

As can be seen in Table 1, it was not possible to inactivate POD using HPP treatment for all conditions tested. In contrast, HPP treatment increased its activity significantly. These results agree with the observation of Anese et al. (1995) on activation of POD from carrots by HPP. Their results showed an increase in activity when subjected to treatment with high pressure, particularly when the

Table 1 Residual Activity (RA %) of POD in the samples treated by HPP treatment with low (12.0 ± 0.2 mg of betanin/100 ml of extract) and high (17.3 ± 0.3 mg of betanin/100 ml of extract) initial betalain concentrations and by HTST thermal treatment with low (12.2 ± 0.1 mg of betanin/100 ml of extract) and high (17.3 ± 0.3 mg of betanin/100 ml of extract) initial betalain concentration

Initial betalain concentration	POD RA (%)*					
	HPP 10 min	HPP 20 min	HPP 30 min	HTST 80 s/75.7 °C	HTST 100 s/81.1 °C	HTST 120 s/85.7 °C
Low	130.0 ± 28.9^{ab}	134.9 ± 28.4^{ab}	117.0 ± 20.2^{b}	20.1 ± 5.8^{c}	1.2 ± 0.7^{c}	0.7 ± 0.3^{c}
High	175.1 ± 9.8^{a}	184.3 ± 1.0^{a}	170.0 ± 13.8^{ab}	5.9 ± 1.9^{c}	1.4 ± 0.5^{c}	0.9 ± 0.1^{c}

* The results followed by different letters indicate a significant difference ($p < 0.05$) according to the Tukey test

treatment was applied at pH 6, with different pressures (4000, 5000 and 6000 bar). They further reported that POD inactivation starts only at very high pressure of about 9000 bar.

Similarly, Huang et al. (2013), observed the activation of the POD by HPP in apricot nectar. Their results showed that, in the highest pressure tested, 400 MPa (4000 bar) and 500 MPa (5000 bar), the HPP caused activation, i.e., an increase in POD activity in all treatment times tested, 5, 10, 15 and 20 min. The highest value of RA observed by the aforementioned authors was 146.8% at 500 MPa/5 min. They speculated that the increase in POD activity can be due to changes in the constituents of the extract, the release of enzymes that were in the membranes or activation of enzymes that were inactive or latent.

In food processing, sometimes a technology can be effective when both, product quality and its shelf life are improved, even if enzymes are not inactivated. This was also observed by Woolf et al. (2013). They analyzed the impact of the HPP in quality of avocado slices through analysis of color, respiration rate, ethylene production, PPO and POD activities. The results of their study showed that the HPP had an effect on inactivation of cellular respiration and ethylene production although the process was ineffective in inactivation of enzymes. Woolf et al. (2013) concluded that despite the HPP had moderate effects with relation to enzymatic activity, the treatment contributed to the increase in shelf life of avocado slices. In contrast, the same result was not observed in the present study since there was no reduction in the DR of betalain for any sample after HPP treatment. This may be due to the high residual % POD of HPP treated samples that has a strong contribution towards the degradation of betalain.

HTST thermal treatment

Determination of effective temperature

Figure 3 shows the temperature profile during the heating of red beet extracts using an oil bath, maintained at 120 °C. As can be seen in this figure, the heating of the samples did not occur instantaneously and their temperatures were lower than the temperature of the oil bath during the short treatment time. Then, the use of oil bath average temperature or final bath temperature does not properly report the temperature in which the treatment happened. In order to show the actual temperature that the samples were subjected, it was essential to use an effective treatment temperature as outlined earlier.

Using the data presented in Fig. 3 and Eq. (1), it was determined that the T_{eff} for HTST treatment for 80, 100 and 120 s were 75.7, 81.1 and 85.7 °C, respectively.

Stability of betalains with HTST

Thermal treatment is a widely used technology and it can be indispensable for conventional food processing. Previous studies presented in the literature have verified the impact of thermal processing on betalain from red beetroot and the use of these pigments as food dye (Güneşer 2016; Paciulli et al. 2016; Saguy 1979). Saguy (1979) investigated the influence of temperature on betanin half-life (min) in beet juice at pH 5.8. His study showed that the half-life of betanin at 61.5 °C was 154.3 min. However, when the temperature was increased, the half-life of betanin decreased. At 75.5, 85.5 and 100 °C, the betanin half-lives were 47.4, 21.7 and 7.3 min, respectively. This study shows that there is a considerable reduction in half-life of betanin with the increase in temperature, for instance, when the temperature was increased from 61.5 to 75.5 °C, the half-life decreased by about 69%. These results agree with Güneşer (2016), who investigated the stability of betalain color in cow milk and observed an increase in degradation of betalain with the increase of temperature. Güneşer (2016) also pointed out the need for further studies to investigate the color change during processing and/or shelf life of pigments like betalain.

However, in the study of Paciulli et al. (2016), beetroot slices were subjected to conventional thermal blanching by water immersion at 90 ± 2 °C for 7 min and then chilled

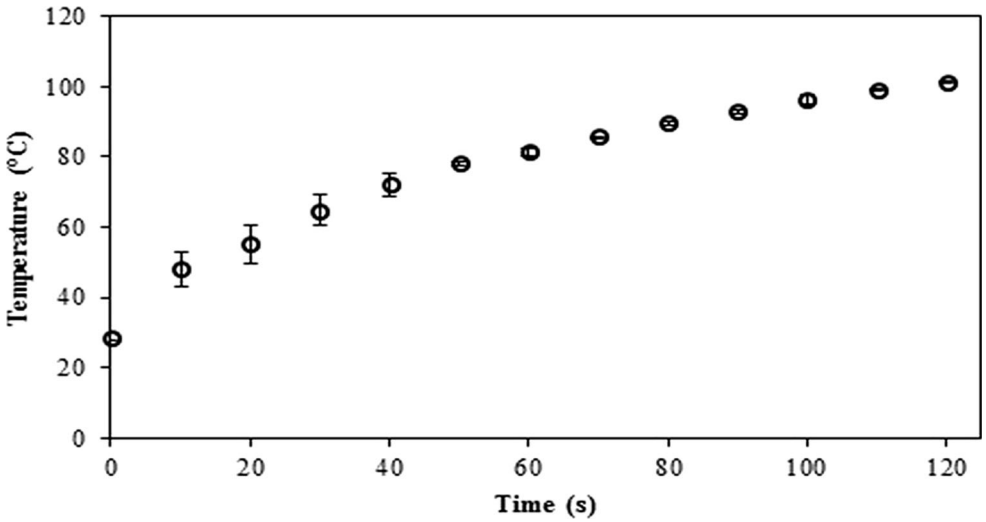

Fig. 3 Temperature profile of betalain extract subjected to heating in a preheated bath at 120 °C for 120 s

in ice water bath for 2 min. The authors observed during this short heat a significant increase in the betalain concentration, from 6.4 ± 0.0 to 13.1 ± 0.2 mg of betanin/g d_w. In this context, it is worthwhile to study the stability of betalain treated by HTST treatment.

Figure 4a, b shows the betalain concentration of HTST treated samples, with low and high initial betalain concentration over 1 week in storage. As can be seen in Fig. 4a, the betalain was degraded by the HTST treatment applied for all the periods tested. The control (untreated sample by HTST treatment) presented higher betalain concentration than the treated samples for in the 1st and 2nd day of storage. After a week of storage (8th day), the betalain DR of the control was 1.0 mg of betanin/100 ml of extract per day and of the treated samples at 80, 100 and 120 s were 1.0, 1.2 and 1.1 mg of betanin/100 ml of extract per day, respectively. This shows that the HTST did not improve the betalain stability with low initial betalain concentration.

In extracts with high initial betalain concentration (Fig. 4b), the preparation and manipulation of the samples caused degradation of betalain at all times of treatment, similar to low initial betalain concentration. However, despite the pigment degradation caused by the HTST treatment, the sample treated for 120 s at 85.7 °C showed improved stability. In this case, the control sample presented a higher betalain concentration than the treated samples only up to the 2nd day.

The high betalain concentration in the second day of the treated sample at 120 s may be related to an event known as betalain regeneration and this phenomenon happens when betalains are thermally treated. Huang and von Elbe (1985) explain that upon heating, betanin in solution get hydrolyzed to betalamic acid and Cyclodopa-5-*O*-glucoside (Damodaran et al. 2008). However, this reaction may be reversible, causing the regeneration of betalains. The regeneration occurs with a Schiff-base condensation between the Cyclodopa-5-*O*-glucoside nucleophilic amine and betalamic acid aldehyde group (Damodaran et al. 2008, Czapski 1990). Huang and von Elbe (1985) observed that the samples treated with high temperature had greater percentage of betanin regeneration, mainly when they were immediately and rapidly cooled after heat treatment. They also mentioned that the activation energy (E_a) of the degeneration reaction of betanin is relatively high (17.3 kCal), while the regeneration has a low E_a value of 0.64 kCal. This further explains the low temperature dependency of the regeneration reaction that assisted in the retention of betanin when the reaction mixture was cooled rapidly.

As a result, the combination of time and temperature treatment (120 s/85.7 °C) probably allowed the regeneration of a larger amount of betanin in comparison with the other treatment conditions. This increase in stability was observed from the second day onwards which is further confirmed by their DR values. After 1 week of storage (8 days), the DR of the control sample was 1.2 mg of betanin/100 ml of extract per day, while the DR of treated sample (120 s) was 0.4 mg of betanin/100 ml of extract per day. Moreover, the betalain concentration of the control in the 4th day was similar to the treated sample (120 s) at 8th day. On contrary, the treated samples at 80 and 100 s were more unstable than the control, since the DR were 1.5 and 1.4 mg of betanin/100 ml of extract per day, respectively. This shows that there is a specific time—temperature combination at or above which regeneration is most noticeable.

Further, the HTST thermal treatment was effective in reducing POD activity of all samples, as shown in Table 1. POD is one of the elements responsible for the

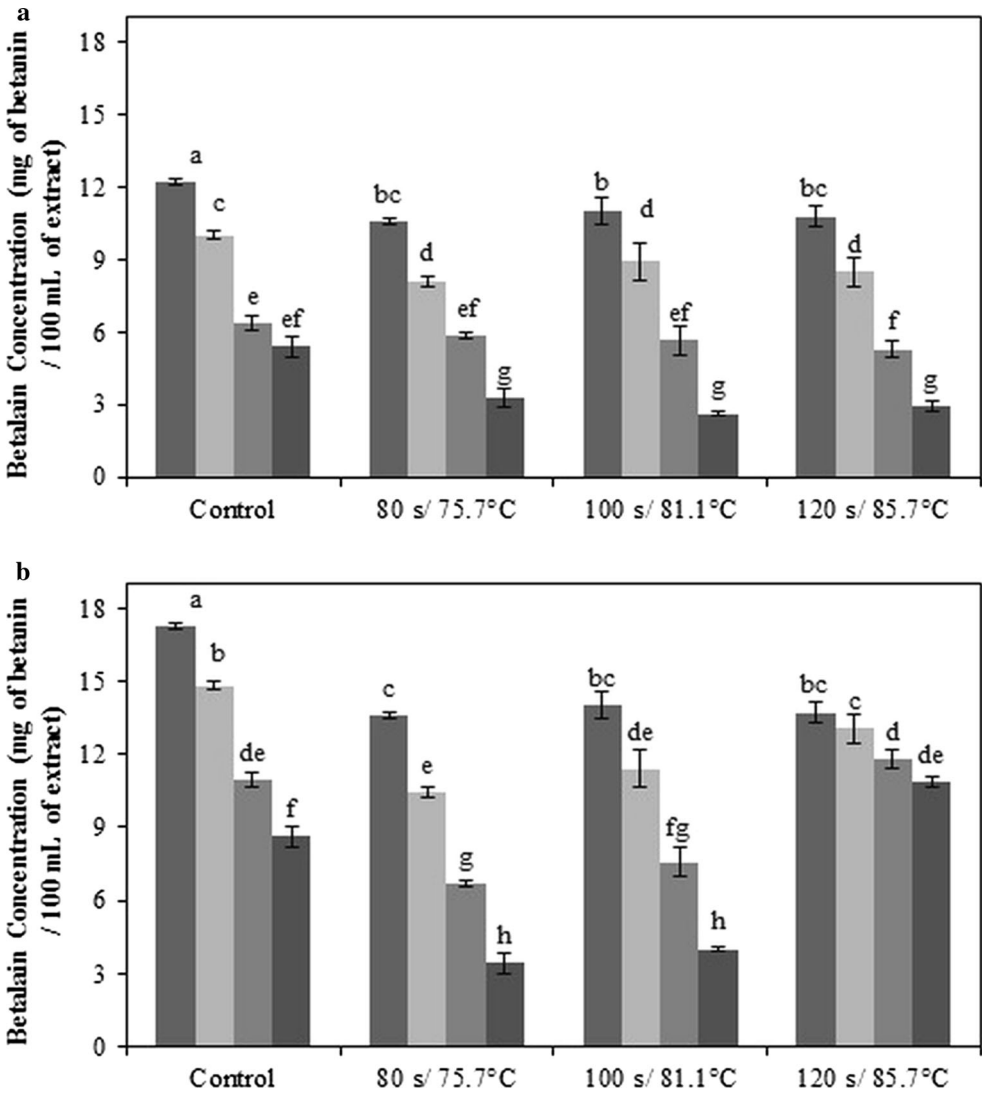

Fig. 4 Betalain concentration of red beet stalk extract, with low (**a**—12.2 ± 0.1 mg of betanin/100 ml of extract) and high (**b**—17.3 ± 0.3 mg of betanin/100 ml of extract) initial betalain concentration, during a storage period of 1 week, for control and after submitted to HTST treatment. In this figure: ■ is 1st day, ■ is 2nd day, ■ is 4th day and ■ is 8th day

betalain degradation, but the stability of this pigment depends upon many other factors such as pH, temperature, other enzymes and water content (Manchali et al. 2013; Damodaran et al. 2008; Herbach et al. 2006). The results for low betalain concentration show that the POD activity was reduced by over 99% (120 s); but this was insufficient to keep the extract stable. However, at high betalain concentration, the significant reduction of POD activity would have prevented the oxidation catalyzed by the enzyme and contributed towards extract stability. Due to the low number of studies about red beet stalks, the literature does not display any discussion related to the behavior of compounds present in the extract when submitted to different treatments. Nevertheless, the difference in stability could be because at high concentrations, solutions have less mobility of food components than at low concentration solutions; disfavoring the oxygen solubility and, consequently, increasing the betalain stability (Stintzing and Carle 2008a).

Conclusion

HTST thermal treatment caused a small degradation of betalains, but it contributed to the stability of pigments over the days when it was applied in samples with high initial betalain concentration. No results were found to indicate that the HPP can be used for improving the stability of the extract in conditions that they were tested.

Despite positives results of HTST treatment to improve betalain stability in red beet stalks extracts with high initial betalain concentration, further studies would be required on the stability of low betalain concentration extracts, since the variation of pigment content is a natural characteristic of the plant.

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