Impact of Thermal Blanching and Thermosonication Treatments on Watercress (*Nasturtium officinale*) Quality: Thermosonication Process Optimisation and Microstructure Evaluation

Rui M. S. Cruz · Margarida C. Vieira · Susana C. Fonseca · Cristina L. M. Silva

Abstract The objectives of the present work were to optimise watercress heat and thermosonication blanching conditions, in order to obtain a product with better quality for further freezing, and to evaluate the effects of thermosonication on the microstructure of watercress leaves. In a chart of optimal time–temperature conditions for a 90% peroxidase inactivation (imposed constraint), vitamin C (objective function) and *a*-value (improvement toward green) were mathematically predicted for both heat and thermosonication blanching treatments. Two optimal thermosonication combinations were selected: 92 °C and 2 s, retaining 95% of vitamin C content and 5% *a*-value improvement, and a better condition in terms of practical feasibility, 86 °C and 30 s, allowing a 75% vitamin C retention and 8% *a*-value improvement. The experimental values, for each thermosonication optimal time–temperature zone, were in good agreement with the models' predicted responses. In terms of microstructure, thermosonicated watercress at 86 and 92 °C showed similar loss of turgor and release of chloroplasts. The proposed optimal thermosonication blanching conditions allow the improvement of the blanched watercress quality and consequently contribute for the development of a high-quality new frozen product. However, a suitable scale-up is mandatory for industrial implementation.

Keywords Watercress · Thermosonication · Optimisation · Peroxidase · Colour · Vitamin C · Microstructure

R. M. S. Cruz · S. C. Fonseca · C. L. M. Silva (✉)
CBQF/Escola Superior de Biotecnologia,
Universidade Católica Portuguesa,
Rua Dr. António Bernardino de Almeida,
Porto 4200-072, Portugal
e-mail: clsilva@esb.ucp.pt

R. M. S. Cruz
e-mail: rcruz@ualg.pt

S. C. Fonseca
e-mail: scfonseca@mail.esb.ucp.pt

M. C. Vieira
Instituto Superior de Engenharia,
Universidade do Algarve, Campus da Penha,
Faro 8005-139, Portugal
e-mail: mvieira@ualg.pt

Nomenclature

a	Colour co-ordinate, represents red to green in the Hunter Lab colour space
C	Value or concentration of the dependent variable (peroxidase activity, colour or vitamin C)
E_a	Activation energy (kJ mol^{-1})
k	Reaction rate constant (min^{-1})
fw	Fresh weight

Subscripts

e	Value at equilibrium
0	Initial value at time equal to zero
1	Relative to heat-labile enzyme fraction
2	Relative to heat-resistant enzyme fraction
84.6 °C	At the reference temperature of 84.6 °C
87.5 °C	At the reference temperature of 87.5 °C

Introduction

Watercress (*Nasturtium officinale*) is a green vegetable of the family Cruciferae that grows in and around water. Normally, it is consumed fresh, in soups or other recipes, and it has a short shelf-life (approximately 7 days) that can

be extended throughout freezing, allowing a longer period for distribution and storage.

Pre-treatments are common in most processing operations to improve product quality or process efficiency (Jha and Prasad 1996). Blanching is one of the pre-treatments used before freezing to inactivate enzymes and prevent biochemical reactions that might contribute to the development of off-flavours and discoloration in the frozen product (Allali et al. 2008; Mountney and Gould 1988). However, blanching also causes undesirable changes in the food properties, such as loss of colour, flavour, texture and nutrients (Pala 1983; Pizzocaro et al. 1995).

The safety and impact of industrial operations on food quality involves, in most cases, conflicting and opposing influences and, consequently, it is necessary to balance these out to optimise the process. The nutrient value retention of processed food products is an example of a situation where a compromised solution is required. Basically, the optimisation methods seek to determine the most suitable processing conditions for achieving a desired objective (Holdsworth 1985).

In the blanching process, precise conditions (time and temperature) must be evaluated for the raw material, and usually represent a balance between retaining the quality characteristics of the raw material and avoiding over-processing (Grandison 2006).

During food processing, the cell walls undergo several modifications in terms of physical state, macrostructure, microstructure, and composition. The external stress caused by several processing treatments also favours the softening and solubilisation of pectin and reduces adhesion between cells. Modifications in the composition and structure of the cell wall, as well as in the tissue structure, cause changes in plant texture (Kunzek et al. 1999).

Many studies report the impact of thermal and non-thermal treatments, such as thermosonication, on several quality parameters. Ultrasound alone or combined with other preservation methods have been applied for enzyme inactivation in foodstuffs (Knorr et al. 2004). This inactivation is achieved, probably, as a result of the phenomenon called cavitation (Mason et al. 1996; Vercet et al. 2001). The synergistic effect of the combined heat and ultrasound treatment (thermosonication) allows the inactivation of several enzymes at lower temperatures and/or in shorter amounts of time (Vercet et al. 2001). Thermosonication has a synergistic effect in watercress peroxidase inactivation (Cruz et al. 2006), since the enzyme activity decreases at a higher rate compared to the heat treatment. Thermosonication treatment (72 °C and 20 kHz) also increased the inactivation rate of orange juice pectinmethylesterase 25 times (Raso and Barbosa-Cánovas 2003). The reduction of specific activity is related to the conformation changes in the tertiary structure, as in the active site three-dimensional structure, and affects the enzyme–substrate interaction (Lemos et al. 2000).

The thermosonication treatment is also more effective in watercress green colour enhancement (Cruz et al. 2007). These colour changes may be related to the replacement of the gases inside the intercellular spaces by the blanching medium, altering light refraction from the cell surface (Bowers 1992). Moreover, the thermosonication treatment and the heat treatment have similar effects in what concerns the vitamin C content degradation (Cruz et al. 2008). Similar results were also reported by Tiwari et al. (2009) in a study with orange juice. The sonicated and the thermal treated samples showed ascorbic acid values in the same range. Thermosonication treatment also retained high levels of ascorbic acid in a study with orange juice reported by Walkling-Ribeiro et al. (2007). Murcia et al. (2000), in a study in which broccoli florets were industrially processed, showed that blanching for 60 s at 92–96 °C maintained about 50% of vitamin C content and reduced 99% of peroxidase activity. A blanching process in butternut squash at 80 °C for 30 s, for 90% peroxidase inactivation and 50% vitamin C retention, was reported by Agüero et al. (2008).

A process optimisation and a suitable scale-up are usually required for industrial processing. The optimisation of a process (set of process conditions that produces the best results) requires various steps: (1) definition of the objective function, which is normally the maximisation of the quality parameter that will work as the limiting factor; (2) identification of the independent decision variables, affecting the objective function, which can be adjusted to improve the process (e.g. treatment temperature); (3) setting of constraints that normally impose safety or quality levels (e.g. reduction of a microorganism or an enzyme; the constraints can also limit the values of the decision variables in order to deal with equipment conditions); (4) development of a mathematical model that simulates conditions (taking into account the decision variables limits) and predicts process results and (5) the implementation of the optimisation technique, by which the combinations of the decision variables are studied within the constraint limits to achieve the best solution imposed by the objective function (Teixeira and Shoemaker 1989).

There are many studies on food process optimisation with thermal treatments (Banga et al. 1991; Quintero-Ramos et al. 1998; Saguy and Karel 1979), but none so far for watercress thermosonication blanching.

The objectives of this study were to optimise watercress heat and thermosonication blanching conditions in order to obtain a product with better quality, in terms of colour and vitamin C, for further freezing process, and to evaluate the effects of thermosonication on the microstructure of watercress leaves.

Materials and Methods

Modelling and Blanching Process Optimisation

Vitamin C degradation and peroxidase inactivation by thermosonication followed first-order kinetics. Peroxidase inactivation by heat treatment followed a biphasic first-order reaction model, due to the presence of two isoenzymes with different thermal stabilities.

Watercress *a*-value changes followed a reversible first-order model, since, after an initial increase, the values reached a "plateau". The goodness of fit of the used models, within a temperature range of 82.5–92.5 °C, was assessed in previous work (Cruz et al. 2006, 2007, 2008) (Table 1). The used models allow quantifying watercress vitamin C content, peroxidase activity, and colour *a*-value at any processing temperature, within the used temperature range.

For blanching process optimisation, vitamin C retention maximisation was set as the objective function. A 90% inactivation in watercress peroxidase was established as the constraint for each treatment; once in order to retain the quality of vegetables during frozen storage, the activity of the target enzyme should be reduced by at least 90% with the blanching treatment (Bahçeci et al. 2005).

Optimal combinations of blanching times and temperatures were obtained and selected through the kinetic prediction models and observed by the lines intersection of each evaluated parameter within the temperature range. Afterwards, the selected optimal thermosonication blanching combinations were experimentally checked.

Vegetable Material

For the experimental analysis, watercress leaves were kindly supplied by Vitacress, a company that grows

Table 1 Studied watercress kinetic parameters for heat and thermosonication treatments

Quality parameter	Treatment	Model	Kinetic parameter		Reference
Peroxidase	Heat	*Biphasic first-order*	C_{01} (μmol min^{-1} mg protein^{-1})	0.49±0.08	Cruz et al. (2006)
			$k_{1\ 84.6°C}$ (min^{-1})	18.01±13.98	
			E_{a1} (kJ mol^{-1})	420.72±114.94	
			C_{02} (μmol min^{-1} mg protein^{-1})	0.48±0.06	
			$k_{2\ 84.6°C}$ (min^{-1})	0.24±0.14	
			E_{a2} (kJ mol^{-1})	351.65±80.91	
			R^2	0.94	
	Thermosonication[a]	*First-order*	C_0 (μmol min^{-1} mg protein^{-1})	1.01±0.05	
			$k_{87.5°C}$ (min^{-1})	9.64±2.21	
			E_a (kJ mol^{-1})	496.45±65.52	
			R^2	0.97	
Colour (*a* parameter)	Heat	*Reversible first-order*	C_0	0.96±0.01	Cruz et al. (2007)
			C_e	1.01±0.02	
			E_a (kJ mol^{-1})	422.37±126.63	
			$k_{87.5°C}$ (s^{-1})	0.028±0.024	
			R^2	0.99	
	Thermosonication[a]	*Reversible first-order*	C_0	1.01±0.01	
			C_e	1.09±0.01	
			E_a (kJ mol^{-1})	187.70±160.07	
			$k_{87.5°C}$ (s^{-1})	0.28±0.18	
			R^2	0.99	
Vitamin C	Heat	*First-order*	C_0	1	Cruz et al. (2008)
			E_a (kJ mol^{-1})	150.47±42.81	
			$k_{87.5°C}$ (min^{-1})	0.75±0.10	
			R^2	0.98	
	Thermosonication[a]	*First-order*	C_0	1	
			E_a (kJ mol^{-1})	136.20±60.97	
			$k_{87.5°C}$ (min^{-1})	0.58±0.11	
			R^2	0.97	

[a] Ultrasound at 20 kHz and 125 W

watercress in Almancil, Algarve-Portugal. The leaves were selected (intact and dia=1.4 cm), washed thoroughly, processed and analysed within 24 h.

Thermosonication Blanching Process

Two optimal thermosonication time and temperature combinations were used: 30 s at 86ºC and 2 s at 92ºC. Each sample of watercress (3 g) was blanched in individual conical flasks with 100 ml of distilled water, in a water bath (Grant W14, Cambridgeshire, UK) (±0.5ºC). The combination of heat and ultrasound was carried out using an ultrasound horn (Coleparmer V1A; 13 mm dia) at 20 kHz and an ultrasound generator (Coleparmer 4710 Series, Chicago, IL, USA) radiating 50% of power (125 W). After each treatment, the samples were cooled in an iced water bath. The temperature was monitored with a digital thermometer (Ellab ctd 87, Roedovre, Denmark) and a thermocouple (1.2 mm needle dia; T-type constantan).

Ten replications were run to experimentally determine peroxidase activity, colour (*a*-value) and vitamin C content.

Peroxidase Assay

After each blanching treatment, peroxidase activity was determined following the methodology reported by Cruz et al. (2006).

Colour Measurement

Colour was evaluated with a tristimulus spectro-colorimeter (Dr Lange, Düsseldorf, Germany) in the Hunter system (Hunter Lab 2000). The colorimeter (d/8º geometry, illuminant D65, 10º observer) was calibrated against a standard ceramic white tile (X=84.60, Y=89.46, Z=93.85) and a standard ceramic black tile (X=4.12, Y=4.38, Z=4.71). Each sample (ten replicas were used) was measured 20 times against a white surface.

Vitamin C Analysis by Reverse-Phase Ion Interaction High-performance Liquid Chromatography

Ascorbic acid content was determined, based on a method previously reported by Zapata and Dufour (1992) by high-performance liquid chromatography ultraviolet detection using isoascorbic acid as internal standard. Dehydroascorbic acid was as well detected as fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-b]quinoxaline-1-one, after pre-column derivatization with 1,2-phenylenediamine dihydrochloride (Sigma, St. Louis, MO, USA). The vitamin C final value was assumed to be the sum of the two biological active forms (ascorbic and dehydroascorbic acids).

Microstructure Quality Evaluation

After thermosonication blanching at 86°C (30 s), 92°C (2 s) and 50°C (for 60 s; control temperature) and heat blanching (control samples—without ultrasound) at 86°C (30 s) and 92°C (2 s), the leaves were stored in a vertical freezer (Snijders Scientific, Tilburg, Netherlands) at −80°C overnight. Afterwards, based on the method reported by Fonseca et al. (2005), the samples were transversally cut with a surgery thin blade and observed in a scanning electron microscope (JEOL JSM-5600 LV, Tokyo, Japan) at low vacuum with an acceleration voltage of 15 kV and a cryo-chamber set at −25°C. The experiments were run with six replicates.

Statistical Analysis

Calculations were performed using the Microsoft Excel 2007 (Microsoft Corporation 2007) data analysis tool package. Evaluations were based on a significance level of 5%.

Results and Discussion

Identification of Optimal Thermal and Thermosonication Blanching Conditions

Figure 1 presents the relationship between time and temperature for (1) a 90% peroxidase inactivation (constraint) with heat and thermosonication treatments, (2) different levels of vitamin C retention and (3) 5% and 8% of *a*-value improvement. The vitamin C time–temperature plots for different retention levels were the same for heat and thermosonication since, for this parameter, there are no significant differences (P>0.05) between these two treatments (Cruz et al. 2008).

The synergistic effect generated by thermosonication due to heat and cavitation decreased peroxidase activity at a higher rate. In order to produce the same degree of peroxidase inactivation (reduction of 90%), for example, at 90°C, the heat blanching requires 70 s, that is about 14-fold the processing time of the thermosonication treatment (5 s) at the same temperature. This huge difference leads to a higher retention of vitamin C in the thermosonication treatment (about 94%) as compared to heat blanching, the content of which is reduced to 29% (Fig. 1, rectangular markers).

As it was demonstrated that the thermosonication blanching is more efficient on peroxidase inactivation, without higher fallout on the product quality, the optimal time–temperature combinations for this treatment were determined (Fig. 1). Watercress thermosonication at 92°C

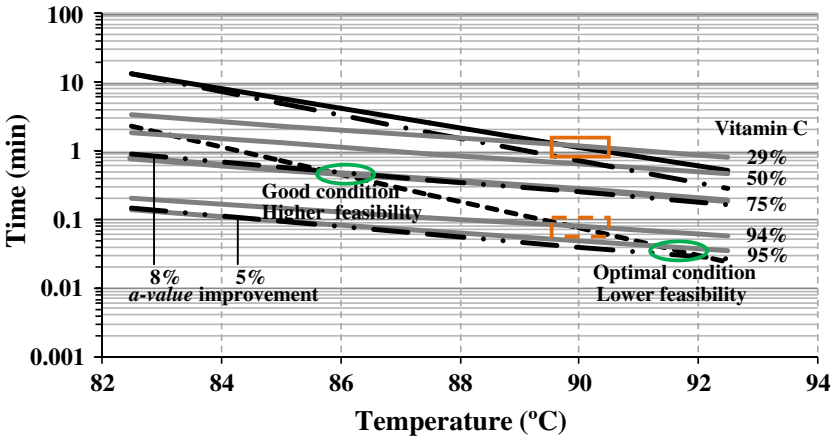

Fig. 1 Graphical optimisation for heat and thermosonication watercress blanching in the temperature range of 82.5 to 92.5 °C: (*solid line*) 90% of peroxidase inactivation with heat treatment; (*broken line*) 90% of peroxidase inactivation with thermosonication treatment. The *grey lines* correspond to 29%, 50%, 75%, 94% and 95% of vitamin C retention for both treatments. Five percent of greenness improvement with heat treatment is represented by the *dashed dotted line*; 5% and 8% of greenness improvement with thermosonication treatment are represented by the *dashed double-dotted lines*. The *continuous* and *dashed orange rectangular markers* compare two time–temperature combinations at 90 °C for heat and thermosonication, respectively. The *elliptic green markers* indicate two time–temperature thermosonication combinations: 92 °C, 2 s, and 86 °C, 30 s

was chosen as an optimal condition, since vitamin C retention can be maximised (about 95%) and green colour improvement can be achieved (5%). Nevertheless, the high temperature and very short time period (about 2 s) is poor in terms of industrial process feasibility. In order to gather all the necessary conditions, a second time–temperature combination was selected. In this approach, the combination of 86 °C and 30 s proved to be a better choice in terms of product quality [about 75% vitamin C content; about 8% *a*-value (improvement toward green)] and practical feasibility (higher), since the 30 s allows the application of these conditions at an industrial level. According to the initial watercress characteristics for peroxidase activity (0.02 ± 0.002 μmol min^{-1} mg $protein^{-1}$), *a*-value (−9.66 ± 1.08) and vitamin C [50.40 ± 5.77 mg 100 g fw^{-1}], the thermosonicated watercress quality at 86 °C and 30 s should present the following range of predicted values (confidence intervals of 95%): peroxidase remaining activity 0.0015 to 0.0025 μmol min^{-1} mg $protein^{-1}$, colour (*a*-value improvement) −10.36 to −10.46 and vitamin C remaining content 37.93 to 41.36 mg 100 g fw^{-1}.

Experimental Responses at Optimal Thermosonication Conditions and their Effect on Watercress Microstructure

Figures 2, 3 and 4 present the experimental data for peroxidase remaining activity, colour *a*-value and vitamin C content, respectively, at the two optimal thermosonication

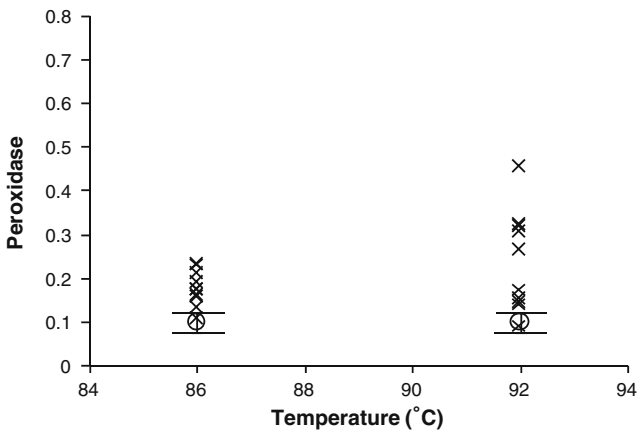

Fig. 2 Peroxidase activity in thermosonicated watercress after 2 s at 92 °C and 30 s at 86 °C. Normalised experimental values (*crosses*); model prediction (*circles*) with 95% confidence interval

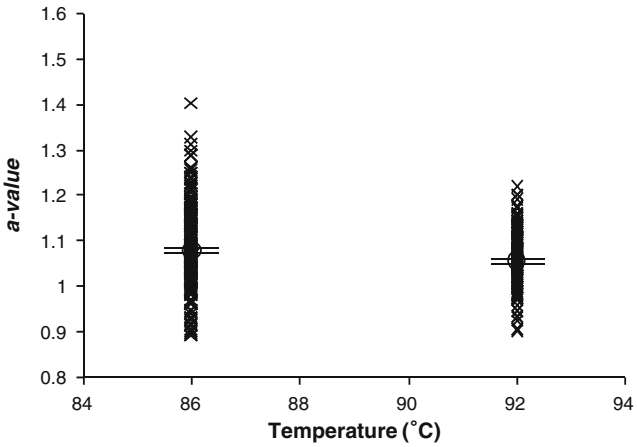

Fig. 3 Colour *a*-value improvement in thermosonicated watercress after 2 s at 92 °C and 30 s at 86 °C. Normalised experimental values (*crosses*); model prediction (*circles*) with 95% confidence interval

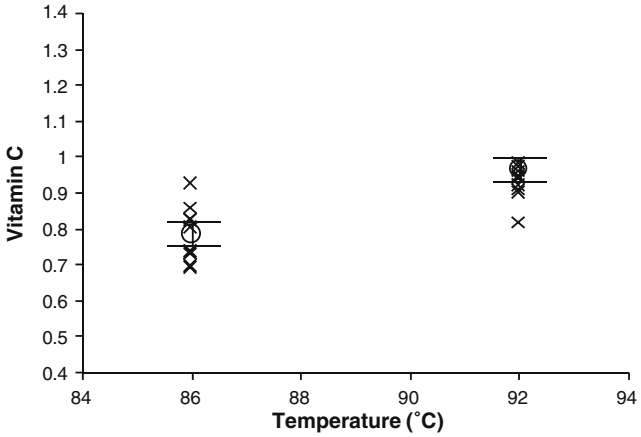

Fig. 4 Vitamin C content in thermosonicated watercress after 2 s at 92 °C and 30 s at 86 °C. Normalised experimental values (*crosses*); model prediction (*circles*) with 95% confidence interval

conditions (92 °C, 2 s, and 86 °C, 30 s). Although the results showed that thermosonication at 92 °C and 2 s induced higher experimental error on peroxidase activity due to its short processing time, the experimental values of the studied parameters, at the two defined optimum scenarios, showed a good agreement with the models predicted values. These results revealed that the developed models were capable of describing the selected responses behaviour for each optimal time and temperature thermosonication zone, confirming the advantages of the thermosonication treatment.

In order to evaluate the effect of thermosonication on watercress microstructure quality, a study at optimal conditions, thermosonication at 50 °C (control temperature) and heat blanching (control samples—without ultrasound) was also performed. According to Kerr (2004), heat treatment disrupts the normal cell structure, and the cell membrane may be damaged, allowing water to enter the cell. The internal organelles may be distorted and begin to

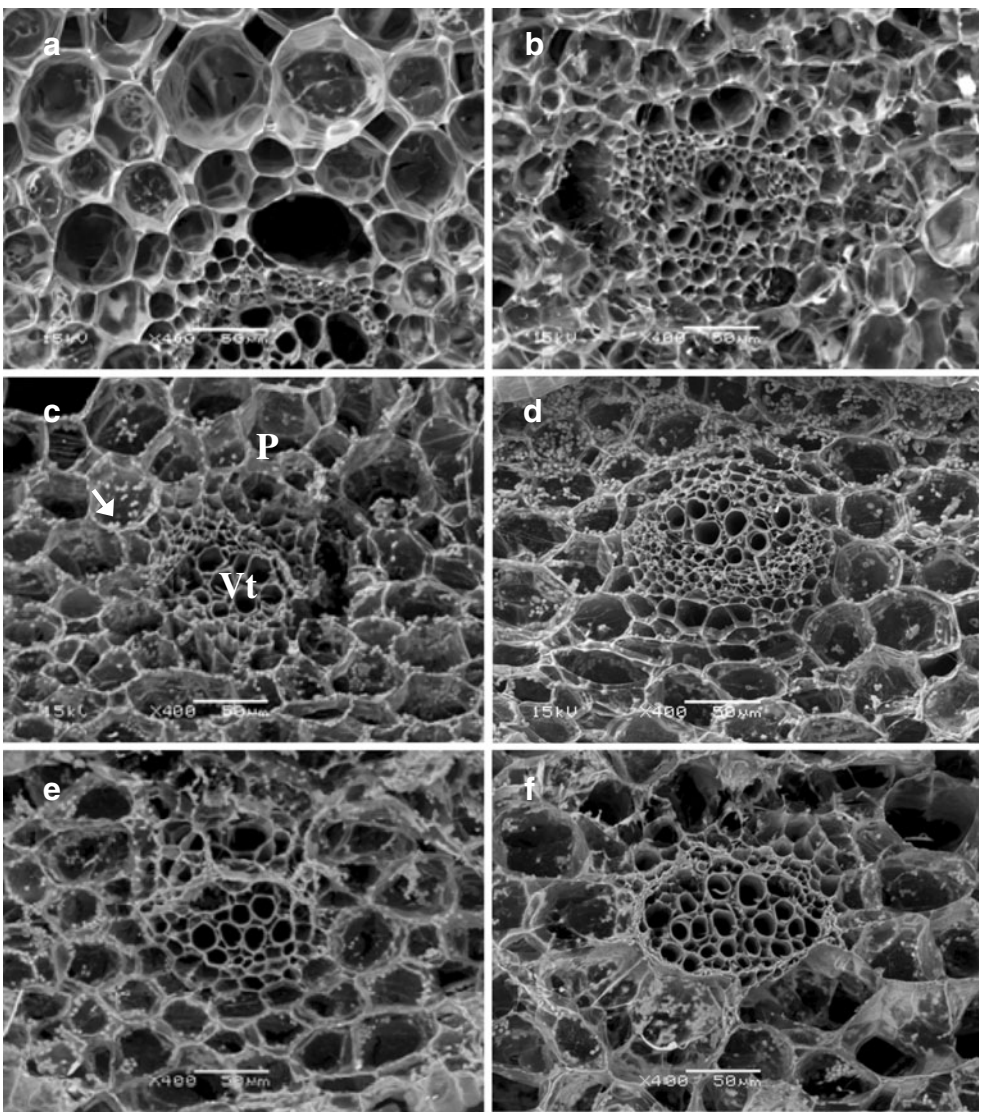

Fig. 5 Scanning electron microscope micrographs of watercress cross section tissue at 400× magnification level: **a** Fresh watercress; **b** thermosonicated watercress at 50 °C, 60 s; **c** thermosonicated watercress at 92 °C, 2 s; **d** heat-blanched watercress at 92 °C, 2 s (control); **e** thermosonicated watercress at 86 °C, 30 s; **f** heat-blanched watercress at 86 °C, 30 s (control). *P* parenchyma cells, *Vt* vascular tissue, *white arrow* indicates the chloroplasts

leak their contents. Moreover, another impact is the reduction of cell turgidity caused by the loss of cell membrane function. During blanching, pectic substances are released from the cell wall. The middle lamella is affected causing less cohesion between adjacent cells and thus leading to a breakdown of the structure.

In the microstructure qualitative evaluation, the micrographs of fresh samples (Fig. 5a) showed the vascular tissue and the parenchyma cells to be turgid, compact and well defined. On the other hand, the microstructure of thermosonicated samples, for both optimal temperatures, presented the above mentioned release phenomenon, as the micrographs (Fig. 5c and e) showed the parenchyma cells filled with spherical structures [chloroplasts (Fig. 6)]. Thermosonicated samples at 92 or 86 °C seemed to have the same degree of damage, independent from the thermosonication treatment extent. The cells' shape definition and chloroplast release were very similar in both conditions. Moreover, the control samples at 92 and 86 °C without ultrasound (Fig. 5d and f) also showed the microstructure with chloroplasts. This indicates that the treatment high temperature was probably responsible for this outcome.

Corroborating these results was the fact that the thermosonicated watercress at 50 °C for 60 s (Fig. 5b) did not show the referred chloroplasts, confirming that high temperature was the main factor for this result.

The verified loss of turgor in the processed watercress leaves could also be observed by the softness of the watercress after the blanching treatment.

Also, in a study reported by Préstamo and Arroyo (1998), spinach leaves treated with high pressure showed similar cellular disruption; the cells lost their turgor and firmness.

Conclusion

The thermosonication treatment (water immersion plus ultrasound at 20 kHz and 125 W) was found to be better than the heat blanching process, since it inactivates watercress peroxidase at less severe thermal conditions and, consequently, retains vitamin C content at higher levels and enhances green colour. Watercress thermosonication at 92 °C and 2 s proved to be an optimal condition, since vitamin C retention can be maximised (about 95%) and greener samples can be achieved (5% improvement). Nevertheless, the high temperature and very short time period (about 2 s) is poor in terms of industrial process feasibility. Thus, a combination of 86 °C and 30 s proved to be a better choice in terms of product quality [about 75% vitamin C content; about 8% *a*-value (improvement toward green) and higher feasibility].

However, one drawback of the thermosonication treatments, at these conditions, as well as on the heat blanching treatments (control), is the loss of turgor showed by the watercress microstructure. The proposed optimal thermosonication blanching conditions will improve the blanched watercress quality and, consequently, contribute to the

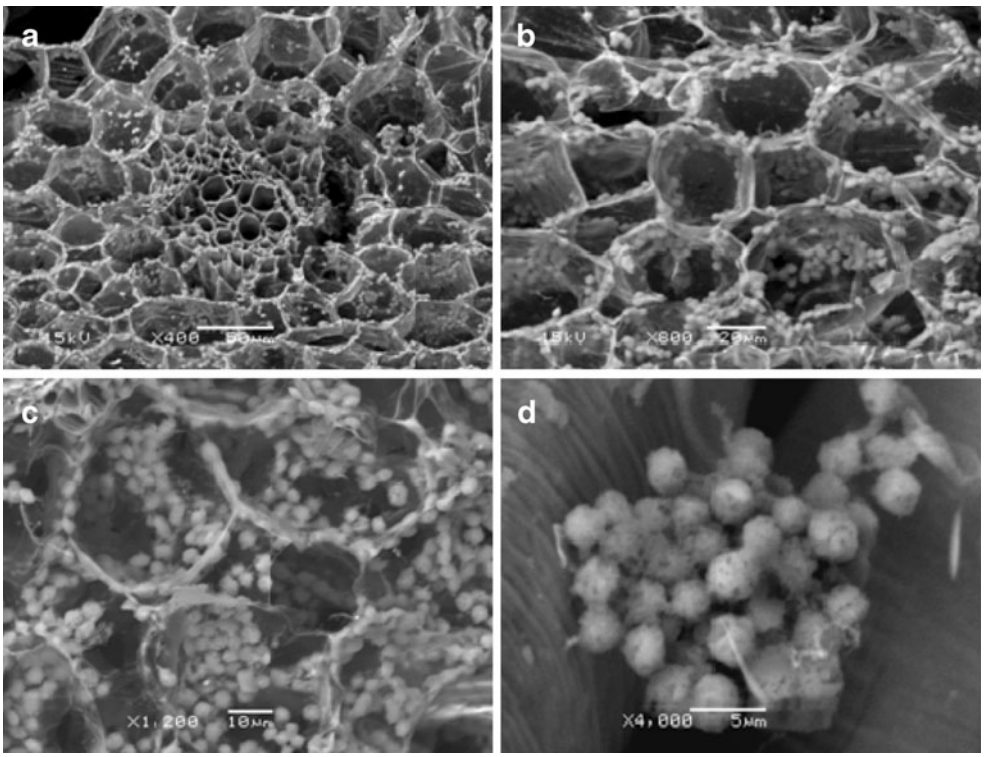

Fig. 6 Scanning electron microscope micrographs of watercress chloroplasts at different magnification levels (thermosonication at 92 °C, 2 s): **a** at 400×; **b** at 800×; **c** at 1,200×; **d** at 4,000×

development of a new high-quality frozen product. The optimisation can be accomplished regarding the studied quality parameters; however, a suitable scale-up study is mandatory for industrial production.

Acknowledgments The author Rui M. S. Cruz gratefully acknowledges his Ph.D. grant SFRH/BD/9172/2002 to *Fundação para a Ciência e a Tecnologia* (FCT) from *Ministério da Ciência e do Ensino Superior*. The authors thank the Vitacress Company for supplying the raw watercress.

References

Agüero, M. V., Ansorena, M. R., Roura, S. I., & del Valle, C. E. (2008). Thermal inactivation of peroxidase during blanching of butternut squash. *LWT-Food Science and Technology, 41*, 401–407.

Allali, H., Marchal, L., & Vorobiev, E. (2008) Blanching of strawberries by ohmic heating: Effects on the kinetics of mass transfer during osmotic dehydration. *Food and Bioprocess Technology*. doi:10.1007/s11947-008-0115-5.

Bahçeci, K. S., Serpen, A., Gökmen, V., & Acar, J. (2005). Study of lipoxygenase and peroxidase as indicator enzymes in green beans: change of enzyme activity, ascorbic acid and chlorophylls during frozen storage. *Journal of Food Engineering, 66*(2), 187–192.

Banga, J. R., Perez-Martin, R. I., Gallardo, J. M., & Casares, J. J. (1991). Optimization of the thermal processing of conduction-heated canned foods: study of several objective functions. *Journal of Food Engineering, 14*, 25–51.

Bowers, J. (1992). *Food theory and applications* (pp. 738–739). New York: Macmillan.

Cruz, R. M. S., Vieira, M. C., & Silva, C. L. M. (2006). Effect of heat and thermosonication treatments on peroxidase inactivation kinetics in watercress (*Nasturtium officinale*). *Journal of Food Engineering, 72*(1), 8–15.

Cruz, R. M. S., Vieira, M. C., & Silva, C. L. M. (2007). Modelling kinetics of watercress (*Nasturtium officinale*) colour changes due to heat and thermosonication treatments. *Innovative Food Science & Emerging Technologies, 8*, 244–252.

Cruz, R. M. S., Vieira, M. C., & Silva, C. L. M. (2008). Effect of heat and thermosonication treatments on watercress (*Nasturtium officinale*) vitamin C degradation kinetics. *Innovative Food Science & Emerging Technologies, 9*, 483–488.

Fonseca, S. C., Silva, C. L., & Malcata, F. X. (2005). Microstructural analysis of fresh-cut red bell pepper (*Capsicum annuum* L.) for postharvest quality optimization. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 4*(5), 1081–1085.

Grandison, A. S. (2006). Postharvest handling and preparation of foods for processing. In J. G. Brennan (Ed.), *Food processing handbook*. Weinheim: Wiley-VCH Verlag.

Holdsworth, S. D. (1985). Optimisation of thermal processing—A review. *Journal of Food Engineering, 4*, 89–116.

Jha, S. N., & Prasad, S. (1996). Determination of processing conditions on gorgon nut (*Euryale ferox*). *Journal of Agricultural Engineering Research, 63*(2), 103–112.

Kerr, W. L. (2004). Texture in frozen foods. In C. Hui, L. Legaretta & N. Murrell (Eds.), *Handbook of frozen foods* (pp. 149–150). New York: Marcel Dekker.

Knorr, D., Zenker, M., Heinz, V., & Lee, D. (2004). Applications and potential of ultrasonics in food processing. *Trends in Food Science & Technology, 15*, 261–266.

Kunzek, H., Kabbert, R., & Gloyna, D. (1999). Aspects of material science in food processing: Changes in plant cell walls of fruits and vegetables. *Zeitschrift für Lebensmittel untersuchung und-Forschung A, 208*, 233–250.

Lemos, M. A., Oliveira, J. C., & Saraiva, J. A. (2000). Influence of pH on the thermal inactivation kinetics of horseradish peroxidase in aqueous solution. *LWT-Food Science and Technology, 33*, 362–368.

Mason, T. G., Paniwnyk, L., & Lorimer, J. P. (1996). The uses of ultrasound in food technology. *Ultrasonics Sonochemistry, 3*, 253–260.

Mountney, G. J., & Gould, W. A. (1988). Low-temperature food preservation. In G. Mountney (Ed.), *Practical food microbiology and technology* (pp. 112–115). New York: Van Nostrand Reinhold.

Murcia, M. A., López-Ayerra, B., Martinez-Tomé, M., Vera, A. M., & García-Carmona, F. (2000). Evolution of ascorbic acid and peroxidase during industrial processing of broccoli. *Journal of the Science of Food and Agriculture, 80*, 1882–1886.

Pala, M. (1983). Effect of different pretreatments on the quality of deep frozen green beans and carrots. *International Journal of Refrigeration, 6*, 237–246.

Pizzocaro, F., Senesi, E., Querro, O., & Gasparoli, A. (1995). Blanching effect on carrots. Study of the lipids stability during the frozen conservation. *Industrie Alimentari, 34*, 1265–1272.

Préstamo, G., & Arroyo, G. (1998). High hydrostatic pressure effects on vegetable structure. *Journal of Food Science, 63*(5), 1–4.

Quintero-Ramos, A., Bourne, M. C., Barnard, J., & Anzaldúa-Morales, A. (1998). Optimization of low temperature blanching of frozen jalapeño pepper (*Capsicum annuum*) using response surface methodology. *Journal of Food Science, 63*(3), 519–522.

Raso, J., & Barbosa-Cánovas, G. V. (2003). Nonthermal preservation of foods using combined processing techniques. *Critical Reviews in Food Science and Nutrition, 43*(3), 265–285.

Saguy, I., & Karel, M. (1979). Optimal retort temperature profile in optimizing thiamin retention in conduction-type heating of canned foods. *Journal of Food Science, 44*(5), 1485–1490.

Teixeira, A. A., & Shoemaker, C. F. (1989). Process optimization. In S. Teixeira (Ed.), *Computerized food processing operations* (pp. 169–170). New York: Van Nostrand Reinhold.

Tiwari, B. K., O' Donnell, C. P., Muthukumarappan, K., & Cullen, P. J. (2009). Ascorbic acid degradation kinetics of sonicated orange juice during storage and comparison with thermally pasteurised juice. *LWT-Food Science and Technology, 42*, 700–704.

Vercet, A., Burgos, J., Crelier, S., & López-Buesa, P. (2001). Inactivation of proteases and lipases by ultrasound. *Innovative Food Science & Emerging Technologies, 2*, 139–150.

Walkling-Ribeiro, M., Noci, F., Riener, J., Cronin, D. A., Lyng, J. G., & Morgan, D. J. (2007). The Impact of thermosonication and pulsed electric fields on *Staphylococcus aureus* inactivation and selected quality parameters in orange juice. *Food and Bioprocess Technology*, . doi:10.1007/s11947-007-0045-7.

Zapata, S., & Dufour, J. (1992). Ascorbic, dehydroascorbic and isoascorbic acid simultaneous determinations by reverse phase ion interaction HPLC. *Journal of Food Science, 57*(2), 506–511.