# Effect of heat and thermosonication treatments on watercress (*Nasturtium officinale*) vitamin C degradation kinetics

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## Abstract

The use of ultrasound in food processing creates novel and interesting methodologies, which are often complementary to classical techniques. In this work, the effect of heat and the combined treatment heat/ultrasound (thermosonication) on the thermal degradation kinetics of vitamin C in watercress (*Nasturtium officinale*) was studied in the temperature range of 82.5 to 92.5 °C. First order reaction kinetics adequately described the vitamin C losses during both blanching processes.

The activation energies and the reaction rates at 87.5 °C for heat (H) and thermosonication (Ts) treatments were, respectively, $E_{a_{vitC_H}} = 150.47 \pm 42.81$ kJ mol$^{-1}$ and $E_{a_{vitC_{Ts}}} = 136.20 \pm 60.97$ kJ mol$^{-1}$, and $k_{87.5 \ °C vitC_H} = 0.75 \pm 0.10$ min$^{-1}$ and $k_{87.5 \ °C vitC_{Ts}} = 0.58 \pm 0.11$ min$^{-1}$. No significant differences ($P > 0.05$) were detected between both treatments. The thermosonication treatment was found to be a better blanching process, since it inactivates watercress peroxidase at less severe blanching conditions and consequently retains vitamin C content at higher levels. The present findings will help to optimise the blanching conditions for the production of a new and healthy frozen product, watercress, with heat and a new blanching process methodology.

*Industrial relevance:* Thermosonication blanching can be useful since it reduces processing times, and consequently minimizes the adverse effects of heating on watercress quality. This new application will provide good material, in terms of vitamin C, for further processes, and can be an excellent alternative to the traditional heat treatment.

## Introduction

Watercress (*Nasturtium officinale*) is a hardy perennial herb of the family *Cruciferae* (mustard family) that grows in and around water. It has an essential range of minerals and vitamins and beneficial phytochemicals. Normally, it is commercialised in fresh and consumed in salads, soups and other recipes. Its short shelf life of nearly 7 days could be extended through freezing, allowing a longer period for distribution and storage.

Vegetables are a major source of ascorbic acid (AA), a nutrient that besides its vitamin action is valuable for its anti-oxidant effect, stimulating the immune system and other health benefits. During processing, distribution and storage of frozen vegetables, ascorbic acid oxidizes to dehydroascorbic acid (DHAA), which retains vitamin C activity. Afterwards, it can be irreversibly hydrolyzed to 2,3-diketogulonic acid (DKGA), which possesses no biological activity. This last oxidation step is found to be much more temperature sensitive than the oxidation of ascorbic acid to dehydroascorbic acid (Belitz & Grosch, 1999; Cooke & Moxon, 1981; Esteve, Farré, Frigola, & Garcia-Cantabella, 1997; Giannakourou & Taoukis, 2003; Karhan, Aksu, Tetik, & Turhan, 2004; Tannenbaum, 1976; Vieira, Teixeira, & Silva, 2000).

Vitamin C is the least stable of all vitamins and is easily destroyed during processing and storage. The rate of destruction is increased by the action of metals, especially copper and iron, and enzymes. Availability of oxygen, prolonged heating in the presence of oxygen and exposure to light are all harmful factors

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to vitamin C content of foods (Deman, 1990). When oxygen is present, the contribution of the anaerobic degradation to the total vitamin C loss is small or not noticeable, compared to the aerobic degradation which has a much higher degradation rate (Villota & Hawkes, 1992).

Enzymes containing copper or iron in their prosthetic groups are efficient catalysts of vitamin C decomposition. The most important enzymes of this group are ascorbic acid oxidase, cytochrome oxidase, phenolase and peroxidase. The severity of processing conditions can often be judged by the percentage of vitamin C that has been lost (Deman, 1990).

Some studies have been reported on the degradation of vitamin C in thermally processed fruits and vegetables like grapefruit juice (Saguy, Kopelman, & Mizrahi, 1978), orange juice (Johnson, Braddock, & Chen, 1995), lime, lemon, grapefruit and tangerine (Alvarado & Viteri, 1989), cupuaçu nectar (Vieira et al., 2000), tomatoes (Dewanto, Wu, Adom, & Liu, 2002), green asparagus (Esteve, Frígola, Martorell, & Rodrigo, 1999), amla (Nisha, Singhal, & Pandit, 2004), drumstick (Bineesh, Singhal, & Pandit, 2005) and strawberry products (Castro, Teixeira, Salengke, Sastry, & Vicente, 2004). However, few works have been reported on kinetic data for vitamin C degradation in leafy vegetables during thermal processing.

The application of heat treatment is the most common method for stabilizing foods, because of its capacity to destroy microorganisms and inactivate enzymes. However, since heat can impair as well many organoleptic properties and reduce the contents or bioavailability of some nutrients, there is a growing interest in searching for new technologies able to reduce the intensity of the heat treatments needed for food preservation (López, Sala, Fuente, Condón, Raso, & Burgos, 1994).

Over the years, researchers have optimised time/temperature profiles to minimise the exposure of food to heat. Further, the newer process technologies may have the potential to reduce or even eliminate heat exposure. Some of these processes are not new, but have recently made significant advances towards commercialisation (Vikram, Ramesh, & Prapulla, 2005).

The use of ultrasound in processing creates novel and interesting methodologies, which are often complementary to classical techniques. It has been proven to be particularly useful in sterilisation, extraction, freezing and filtration processes, providing reduced processing times and increased efficiency (Mason, Paniwnyk, & Lorimer, 1996).

The thermosonication is a good alternative to the heat treatment, since it is in favour of less severe heat blanching conditions. Previous work demonstrated that thermosonication is more effective in watercress peroxidase inactivation (Cruz, Vieira, & Silva, 2006) and green colour enhancement (Cruz, Vieira, & Silva, 2007).

Since there are few research studies on the thermal degradation of vitamin C in leafy vegetables (Bineesh et al., 2005; Negi & Roy, 2000), and none at all for watercress thermosonication blanching, the objective of this work was to determine the degradation kinetics of vitamin C in watercress with a combined method of heat and ultrasound. Thus, the findings will help to optimise the design of watercress blanching conditions.

## Materials and methods

### Raw material

Raw watercress (*N. officinale*) was kindly supplied from a local producer. The leaves were selected, washed thoroughly and analyzed within 24 h.

### Thermal and thermosonication blanching processes

Heat treatments were carried out with temperatures ranging from 82.5 to 92.5 °C, with different times of exposure. Each watercress sample (3 g) was processed in individual conical flasks with 100 ml of distilled water, in a water bath Grant W14.

The blanching vessel temperature was monitored with a thermocouple in order to control the blanching water at the desired temperature. Sample's weight and blanching water volume were controlled for good experimental reproducibility.

The combination of heat/ultrasound was applied to the watercress for the same range of temperatures. The samples were blanched with an ultrasound horn (Coleparmer V1A; 13 mm dia) at 20 kHz, and an ultrasound generator (Coleparmer 4710 Series) radiating 50% of power (125 W). The blanching water volume for both treatments was adjusted for the ultrasound horn capacity in order to maintain the same processing conditions.

After each treatment, the samples were cooled in an iced water bath. The experiments were run in triplicate (Cruz et al., 2006).

### Vitamin C analysis by reversed phase ion interaction high performance liquid chromatography

Ascorbic acid (AA) (Riedel-de Haën) content was determined based on a method previously reported (Zapata & Dufour, 1992), by HPLC UV detection using isoascorbic acid (IAA) (Fluka) as internal standard. Dehydroascorbic acid (DHAA) (Sigma) was as well detected as fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-b] quinoxaline-1-one (DFQ), after pre-column derivatization with 1,2-phenylenediamine dihydrochloride (OPDA) (Sigma).

For the mobile phase, 13.61 g of potassium dihydrogen phosphate (Merck) and 3.64 g of cetrimide (Fluka) were added to 2 l of methanol (Merck, LiChrosolv)-ultrapure water (5:95, v/v). The eluent was filtered in a 0.45 μm membrane (Macherey-Nagel, Porafil) and degassed in an ultrasonic bath for 15 min. The HPLC system consisted of a controller (LKB-2152, Bromma), a solvent pump (LKB-2150, Bromma), an injection valve with a 20 μl sample loop, a guard pre-column (Macherey-Nagel, Chromcart Nucleosil 100-10 C18) followed by a reversed phase column (Macherey-Nagel, Chromcart 100-10 Nucleosil, 250×4.6 mm), and an UV detector (LKB-2153, Bromma).

After heat and thermosonication treatments, each sample (3 g) was homogenised with an Ultra-turrax (IKA T25 Janke & Kunkel) in 20 ml of methanol-ultra pure water (5:95, v/v) through 5 min at 8000 rpm. Each sample (5 ml) was transferred to a 20 ml volumetric flask and 1 ml of IAA standard solution (0.03 g/50 ml) was added. The pH was adjusted with HCl (Merck) to obtain final values between 2.20 and 2.45. The volume was completed to 20 ml with methanol-ultra pure water (5:95, v/v). The content was
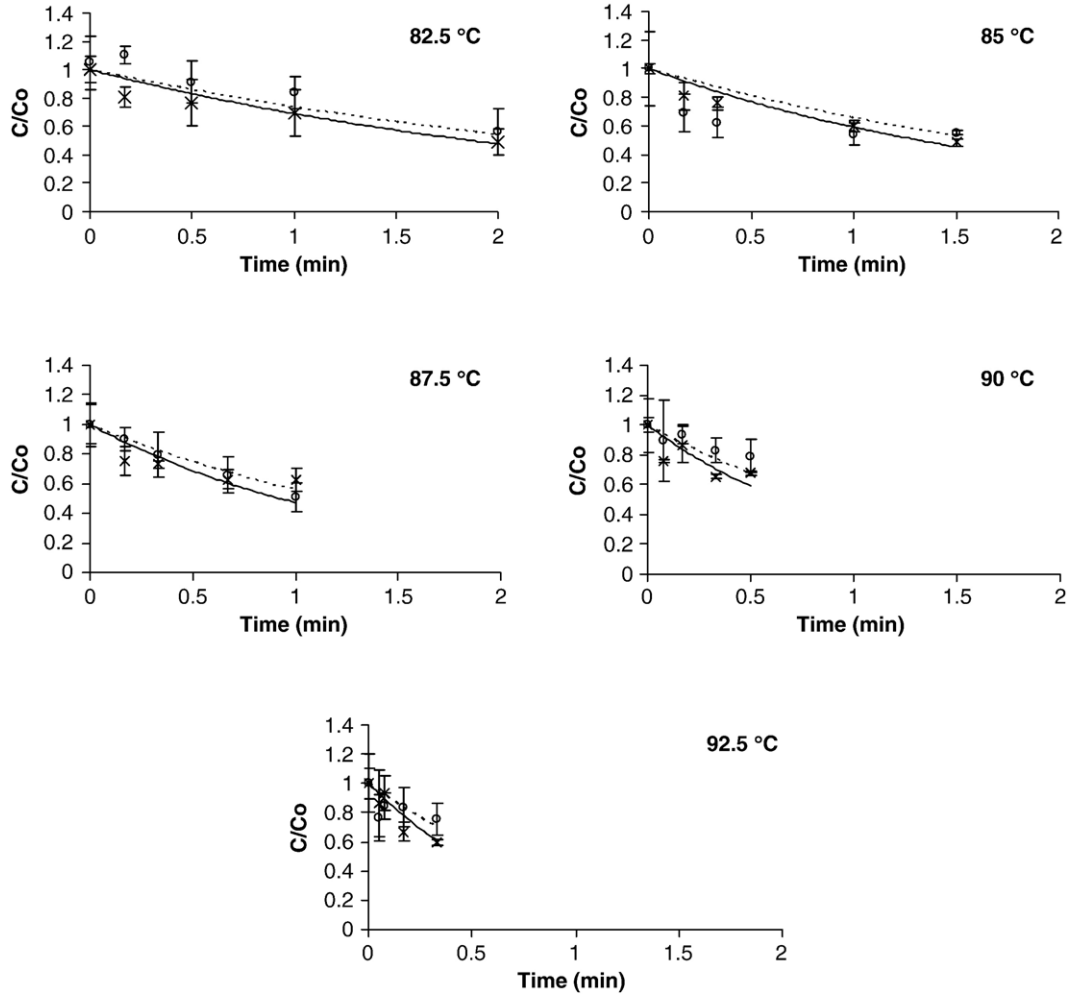
Fig. 1. Effect of temperature, ultrasound and time on watercress vitamin C, in the temperature range of 82.5 to 92.5 °C: (x) experimental values with heat blanching processes; (−) model predicted values for heat blanching processes; (o) experimental values with thermosonication blanching processes; (---) model predicted values for thermosonication blanching processes. Bars represent mean±standard deviation.

centrifuged (Sigma 3 K20) for 5 min. at 8.720×g and 4 °C. Afterwards, 3 ml were transferred to another tube with 1 ml of OPDA (Sigma) (0.03 g/50 ml) daily prepared and maintained in dark. The mixture was then vortexed and placed in dark at room temperature for 40 min. Then the mixture was filtered in a 0.45 µm membrane (Millipore), the first millilitre was discarded and 20 µl were injected in the HPLC. The wavelength detector was set to 348 nm and after elution of DHAA, the wavelength was shifted to 262 nm for AA and IAA detection.

### Kinetics modelling

Vitamin C in watercress was considered to follow first order degradation kinetics:

$$\frac{C}{C_0} = e^{-kt} \tag{1}$$

where $C_0$ is the initial vitamin C concentration at time equal to zero, $C$ is the content at time $t$, blanching time is represented by

$t$, and $k$ is the reaction rate. The vitamin C final value was assumed to be the sum of the two biological active forms (ascorbic and dehydroascorbic acids). In order to minimise differences in watercress initial content, the experimental data were standardised (dividing each batch samples result by the corresponding initial concentration).

The reaction rate temperature dependence followed the Arrhenius behaviour:

$$k = k_{ref} e^{\left[ -\frac{E_a}{R} \left( \frac{1}{T} - \frac{1}{T_{ref}} \right) \right]} \tag{2}$$

where $k_{ref}$ is the reaction rate at the reference temperature, $E_a$ the activation energy, $R$ the universal gas constant, $T$ the absolute temperature and $T_{ref}$ the reference temperature.

By substitution, Eq. (1) can be expressed as

$$\frac{C}{C_0} = e^{\left\{ -k_{ref} e^{\left[ -\frac{E_a}{R} \left( \frac{1}{T} - \frac{1}{T_{ref}} \right) \right]} t \right\}} \tag{3}$$

Experimental data points were normalised, dividing the vitamin C content by the corresponding initial value at time zero, before any processing. A one step non-linear regression was performed, fitting Eq. (3) to the normalised vitamin C experimental data (Arabshahi & Lund, 1985). The reaction rate, at reference temperature, and activation energy were estimated using the statistical software STATA version 6.0 (Stata Corporation, 1999). For the parameters estimation, all the measurements were used, thus taking into account the samples variability. The reference temperature used was the mean value of the range considered (i.e. $T_{ref} = 87.5$ °C), aiming at improving parameters' estimation. Parameters' precision was evaluated by confidence intervals at 95%, and the quality of the regression was assessed by the coefficient of determination ($R^2$), and randomness and normality of residuals, thus allowing best model selection.

### Results and discussion

Watercress samples presented an initial value of $50.40 \pm 5.77$ mg vitamin C 100 g $fw^{-1}$, which is in the same range of other reported values (62 mg 100 g $fw^{-1}$, (Holland, Unwin, & Buss, 1991)) and confirms watercress as a good source of this nutrient.

Fig. 1 shows vitamin C degradation for both treatments, with no evidence of a broken line, which would imply the existence of two different degradation rates. Therefore, it seems that the anaerobic pathway was not followed, since the experiments were run in an opened blanching vessel, ensuring the presence of oxygen throughout the duration of all the blanching treatments.

Table 1
Published vitamin C activation energies ($E_a$'s) for different heating processes

| Heating process | $E_a$ (kJ $mol^{-1}$) | Reference |
|---|---|---|
| Conventional | 115.3 | Saguy et al., 1978 |
| | 38.9–46.5 | Johnson et al., 1995 |
| | 52.8 | Lima, Heskitt, Burianek, Nokes, and Sastry, 1999 |
| Ohmic | 52.3 | Lima et al., 1999 |
| | 29.3 | Assiry, 1996 |

The treatments caused a vitamin C reduction of 40 to 60% to the fresh product value and its degradation was faster at higher temperatures, as expected, since this vitamin is thermolabile. Previous studies on savoy beet, amaranth and fenugreek leaves reported the same effect with hot water blanching (Negi & Roy, 2000). Many studies indicate that the vitamin C content loss during blanching can be attributed to the fact that this vitamin is very soluble in water (occurrence of the leaching phenomenon) and is not stable at high temperatures (Lee & Kader, 2000; Lyimo, Nyagwegwe, & Mnkeni, 1990; Nagy & Smooth, 1977; Oboh, 2005). The watercress vitamin C could have been washed away by the blanching water, however, since processing times used in this study were very short (maximum 2 min), the reduction of this vitamin was probably mainly due to the heat effect.

The results showed no significant differences ($P > 0.05$) between heat and thermosonication treatments. Vitamin C degradation followed first order kinetics for both conventional and thermosonication treatments. According to many authors (Castro et al., 2004; Esteve et al., 1999; Nisha et al., 2004;
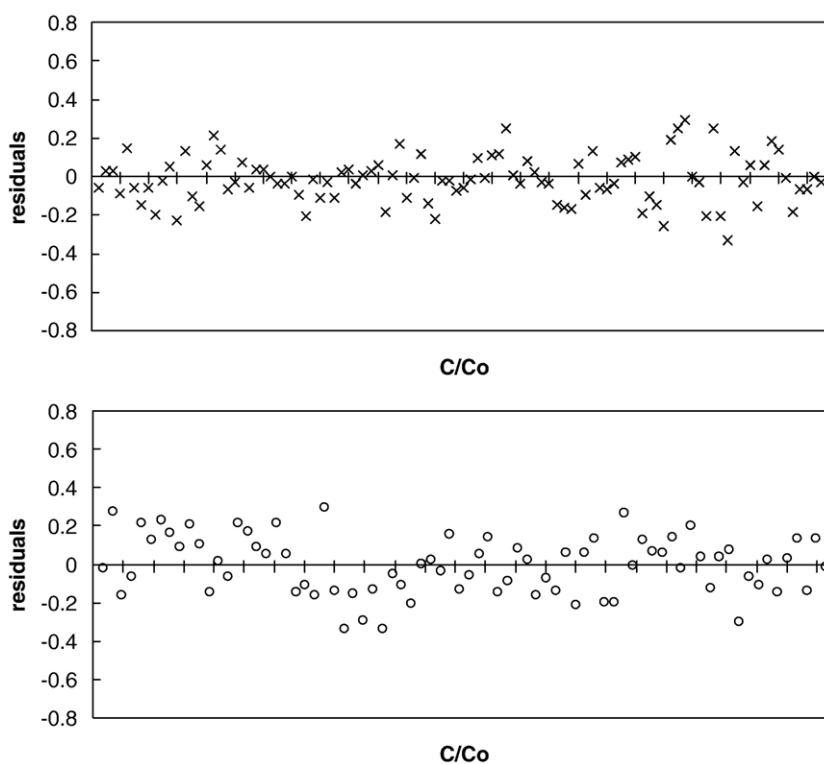


Fig. 2. Plot of residuals for C/Co experimental data against model predicted values: (x) heat blanching process; (○) thermosonication blanching process.

Vikram et al., 2005; Villota & Hawkes, 1992), for a wide number of foodstuffs vitamin C degradation appears to follow first order kinetics, independently of the pathway followed.

The rate constants dependence on temperature followed an Arrhenius behaviour (Eq. (2)). The activation energies and the reaction rates at the reference temperature of 87.5 °C were, respectively, $E_{avitC_H} = 150.47 \pm 42.81$ kJ mol$^{-1}$ and $E_{avitC_{Ts}} = 136.20 \pm 60.97$ kJ mol$^{-1}$, and $k_{87.5\ °CvitC_H} = 0.75 \pm 0.10$ min$^{-1}$ and $k_{87.5\ °CvitC_{Ts}} = 0.58 \pm 0.11$ min$^{-1}$.

Fig. 2 presents the residuals plot, with no defined pattern, meaning that the model is adequate to the experimental data. This is also corroborated by the high coefficients of determination obtained for both blanching treatments ($R^2_{vitC_H} = 0.98$; $R^2_{vitC_{Ts}} = 0.97$).

Previous studies reported activation energy values for vitamin C degradation in foodstuffs of 65 to 160 kJ mol$^{-1}$ (Hallström, Skjöldebrand, & Trägårdh, 1988) and 7.54 to 125.6 kJ mol$^{-1}$ (Lee & Labuza, 1975).

Furthermore, research studies using different heating methods reported also values within the range of those obtained for watercress (Table 1). Moreover, in a study with manothermosonication (Vercet, Burgos, & López-Buesa, 2001) the results demonstrated that manothermosonication did not affect significantly the nutrient content when compared with heat treatment. Nevertheless, the results are always dependent on the experimental set up (e.g. combination of time and temperature) as well as on the raw material used.

## Conclusion

The thermosonication is a good alternative to the thermal treatment, since it is in favour of less severe heat blanching conditions. Previous work demonstrated that thermosonication is more effective in watercress peroxidase inactivation (Cruz et al., 2006). In order to produce the same degree of peroxidase inactivation (90% reduction), for example at 90 °C, the heat blanching requires 70 seconds, that is about 14-fold the processing time of the thermosonication treatment (5 s). This huge difference can lead to a higher retention of vitamin C in the thermosonication treatment (about 94%) as compared to heat blanching, which reduces the content to 29%.

The vitamin C losses due to processing are of great concern and this new pre-treatment will allow good vitamin C preservation, and consequently better raw material for further freezing processes. These studies are important to predict quality losses during thermal processing, even more when there is a lack of studies on the vitamin C thermal degradation kinetics in green vegetables.

The present findings will help to optimise the design of watercress blanching conditions with heat and thermosonication.

## Symbols

$C$       Vitamin C content (mg 100 g fw$^{-1}$)
$E_a$      Activation energy (kJ mol$^{-1}$)
$k$       Reaction rate (min$^{-1}$)
R      Universal gas constant (8.314 J mol$^{-1}$ K$^{-1}$)
t      Time (min)
T      Absolute temperature (K)

## Subscripts

H      Relative to heat blanching
ref     At the reference temperature
Ts     Relative to thermosonication blanching
vitC    Relative to vitamin C
87.5 °C   At the reference temperature of 87.5 °C
0       Initial value at time equal to zero

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