TITLE

MICROWAVE HEATING MODELLING OF A GREEN SMOOTHIE. EFFECTS ON ITS BIOACTIVE COMPOUNDS CHANGES DURING STORAGE

RUNNING TITLE

Quality changes modelling of a purple smoothie during its shelf life

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ABSTRACT

BACKGROUND: The heating of a green smoothie during an innovative semi-continuous microwave treatment (MW; 9 kW for 15 s) was modelled. Thermal and dielectric properties of the samples were previously determined. Furthermore, the heating effect on the main chemopreventive compounds of the smoothie and during its subsequent storage up to 30 days at 5 or 15 ºC were studied. Such results were compared to conventional pasteurization (CP; 90 ºC for 45 s) while unheated fresh blended samples were used as control (CTRL).

RESULTS: A procedure was developed to predict the temperature distribution in samples inside the MW oven with the help of numerical tools. MW-treated samples showed the highest sulforaphane formation after 20 days, regardless of the storage temperature, while its content was 2-fold reduced in CP samples. Storage of the smoothie at 5 ºC is crucial for maximizing the levels of the bioactive compound *S*-methyl cysteine sulphoxide.

CONCLUSION: The proposed MW treatment can be used by the food industry to obtain an excellent homogeneous heating of a green smoothie product, and probably similar products as well, retaining high levels of bioactive compounds during subsequent retail/domestic storage up to one month at 5 ºC.

Keywords: thermal processing; dielectric properties; sulforaphane; glucosinolates; isothiocyanates; *S*-methyl cysteine sulphoxide.

INTRODUCTION

At present, worldwide consumption of fruit and vegetables is below the recommended daily intake.[1] Accordingly, beverages, and more recently smoothies, represent an excellent and convenient alternative that can be used to promote the daily consumption of fruit and vegetables.[2] One of the most-commonly used vegetables is broccoli, although its bitterness should be managed in order to make the smoothie more palatable. The broccoli's bitterness is mainly linked to glucosinolates/isothiocyanates,[3] and such bitterness may be reduced with the sweet taste[4] of fruit, i.e. grapes, in the smoothie preparation. Besides glucosinolates/isothiocyanates, broccoli is a rich source of other health-promoting compounds such as polyphenols, vitamin C, lutein, folates, etc.[5], but thermal processes may highly reduce their contents.[6, 7] Isothiocyanates are bioactive compounds that may be synthetized, among other compounds, after myrosinase hydrolysis of glucosinolates. Sulforaphane is an isothiocyanate which is formed in broccoli after the myrosinase conversion of the glucosinolate glucoraphanin. The potential anticarcinogenic and antiproliferative properties of sulforaphane have been reported together with other biological activities, such as anti-inflammatory and antibacterial properties.[8] Another compound found in broccoli, *S*-methyl cysteine sulfoxide (SMCSO), is an amino acid derivate with potential anti-carcinogenic, anti-diabetic and cardiovascular effects.[9, 10] SMCSO is found in higher concentrations in *Brassica* vegetables (1–2% dry weight) than all glucosinolates combined (0.1–0.6 % dry weight; dw).[10] However, there are no studies on the effects of high power/short time semi-industrial microwave treatments on the glucosinolates/isothiocyanates and SMCSO contents of *Brassicas* products.

Heat is transferred in conventional heating methods to the product's surface by conduction, convection or radiation, and to the inner part by thermal conduction. However, latter heating techniques are sometimes inefficient for food industries as related to processing time and energy consumption. On the other hand, when microwave (MW) heating is used, the energy is absorbed volumetrically, with the heat generated inside the product leading to faster heating. Accordingly, innovative high power/low time MW treatments may be applied to food products using continuous industrial MW ovens. In that sense, such mild, but effective and efficient MW treatments are excellent alternatives to conventional heating systems, and can be used by the food industry to obtain high-quality products with reduced nutritional/bioactive losses during processing while ensuring the food safety of the final product. Nevertheless, homogeneous food heating with these high power/short time MW treatments must be ensured. Accordingly, the numerical methods used to predict and understand the high temperature increases during MW treatments of food products need to be studied. In addition, it is important to understand the dielectric behaviour of the product in order to determine both the energy propagation within the product and the transformation of the MW energy into heat inside the dielectric element.

The main objectives of this study were to model the heating of a green smoothie during an innovative semi-continuous microwave treatment and to compare its effects, along with a conventional heat treatment and unheated samples, on the main bioactive compounds as well as during subsequent storage at 5 or 15ºC for up to 30 days.

MATERIALS AND METHODS

Plant material and smoothie preparation

The vegetables and fruit proportions for the smoothie preparation were: 50.9% grapes, 35.0% kalian-hybrid broccoli (Bimi®), 13.8% cucumber, 0.2% freshly ground (with a coffee grinder and sieved to 30 mesh) yellow mustard and 0.15% ginger. The smoothie composition was selected among several formulations according to sensory pre-evaluations conducted by a sensory panel, focusing on the maximum quantity of broccoli. Fresh vegetables at their optimal maturity stage were purchased at a local supermarket in June. The raw material was sanitized with 75 mg L^{-1} NaClO for 2 min and then rinsed with cold tap water for 1 min. Cucumbers and ginger were previously peeled. Then, the smoothie was prepared with all prepared vegetables in a food processor (Model Robot Cook®, Robot Coupe, Vincennes Cedex, France) and immediately kept cold (2 ºC) in an ice-water bath until subsequent thermal treatments. The nutritional composition of the smoothie was determined with the software DIAL 1.0[11] and is presented in the Supplementary material 1.

Thermal and dielectric properties of the smoothie

The thermal conductivity (κ) of the smoothie was measured at several temperatures (20, 40 and 75 ºC) with the modified transient plane source method with a thermal conductivity analyser (Model C-Therm TCi, Mathis Instruments Ltd., Danville, Canada). Heat capacity (c_p) was determined using a differential scanning calorimeter (Model DSC 822e, Mettler-Toledo, Schwerzenbach, Switzerland), using an oscillating method with a sapphire standard (optimized method based on the steady state and sapphire methods previously described,[12] consisting of successive isothermal 2-min-steps followed by 2.5 ºC min^{-1} heating steps for 2 min up to 75 ºC.

The dielectric constant (ε') and dielectric loss factor (ε'') were determined using a dielectric coaxial probe (Model DAK-12/3.5, SPEAG, Zurich, Switzerland) at different temperatures in the frequency range (0.01-3 GHz). A dielectrometer (Model Dielkitv, DIMAS, ITACA, Valencia, Spain) was used to verify the latter measurements.

Conventional heat treatment

Conventional pasteurization (CP) was applied using the same Mastia thermoresistometer device as previously described.[13] The sterilized vessel of the thermoresistometer was filled with 400 mL of smoothie immediately after its preparation. The thermoresistometer was programmed to increase the initial smoothie temperature with a heating rate of 30 ºC min^{-1} up to 90 ºC, then maintained for 45 s and cooled down to a final temperature of 40 ºC (cooling rate of 30 ºC min^{-1}). The smoothie temperature was reduced below 10 °C within 5 s after the treatment by submerging the vessel in an ice-water bath, with continuous agitation programmed in the thermoresistometer. Subsequently, sterile polyvinyl chloride squeeze-pouches (9 cm×13 cm; 118 mL; Infantino, San Diego, USA) were filled with approximately 80 g of heat-treated smoothie in aseptic conditions through the thermoresistometer sampling port. The remaining air in the pouches was removed before closing them by pressing the pouches by hand. Samples were stored in darkness at 5 and 15 ºC simulating optimal and inappropriate temperature during domestic/retail storage of the smoothie product. Fresh-blended unheated samples were used as control (CTRL). Sampling was conducted on processing day (0) and up to 30 days with different sampling times depending on the treatment and storage temperature. Five replicates per treatment, storage temperature and sampling day were prepared.

Microwave treatment

The microwave (MW) treatment was conducted using an improved semi-industrial prototype continuous-flow microwave oven (Model SI MAQ0101, Sairem Iberica S.L., Barcelona, Spain). The unit consisted of 4 adjustable magnetrons (0.5-3.0 kW; 2450 MHz), a polytetrafluoroethylene (PTFE) feed belt able to work on continuous or back-and-forth movement mode (semi-continuous), an optimized heating chamber, new energy economizing filters, a computer interface and a fibre optic slip ring for online temperature measurements inside the microwave oven. As with the CP treatment, approximately 80 g of smoothie were filled (Infantino Squeeze station, Infantino, San Diego, USA) under aseptic conditions into a sterile squeeze-pouch immediately after smoothie preparation. The MW treatment had been previously optimized in order to achieve a fast and homogenous heating of the filled smoothie pouches. Accordingly, the temperature of the filled smoothie pouches was continuously recorded under different conditions (power of every magnetron, treatment time and feed belt speed/movement mode) with a portable fibre optic thermometer (Model Neoptix NOMAD-Fiber NMD, Neoptix, Quebec, Canada) and with a thermographic camera (Fluke TI25, Fluke Corporation, Washington, USA). The selected MW treatment consisted of a semi-continuous mode with back-and-forth movement of 2 m min^{-1} belt speed at 9 kW (3+2+2+2 kW) for 15 s. The reflected power from each magnetron was 360 W resulting in a final MW power of 7.56 kW (2.64+1.64+1.64+1.64 kW). Four smoothie pouches were always treated at the same time in every treatment batch. Treated smoothie pouches were immediately cooled down to 15 or 5 ºC in an ice-water bath. Storage and sampling conditions were conducted as described for CP treatment.

Modelling of the electromagnetic field distribution inside the microwave oven

A model of the industrial continuous flow MW oven was developed to simulate the electromagnetic field distribution inside the oven (Figure 1). The previously-determined thermal and dielectric properties were used for modelling. It included a PTFE transport belt, 4 smoothie samples and 4 MW waveguide ports with the WR-340 section to model the power feeding of the oven. The simulation was conducted with the CST Microwave Studio software (v. 2016; CST-Computer Simulation Technology, Darmstadt, Germany) that uses the Finite Integration Technique to solve the Maxwell equations.[14] Other numerical methods commonly used for this purposes are the Finite-Differences Time-Domain method[15] and the Finite Element Method.[16] Open boundaries were selected to simulate the openings and the absorbing ferrites at both sides of the belt.

Results were obtained for four regular samples measuring 75×15×105 mm^3. Nine equally-spaced positions were selected to discretize the back-and-forth movement of the samples and to ensure the accuracy of the solution. The section of the electric field strength distribution was obtained with a model using approximately 20,000,000 cells solved within 24 h in an Intel Xeon CPU E5-2603 v3 1.36GHz with 48 Gb RAM and 12 threads.

Glucoraphanin content

Glucoraphanin extraction and analysis were conducted based on Francisco *et al.*[17] but with slight modifications. A 500 mg freeze-dried sample was homogenized (Ultra Turrax® model 18T, IKA-Werke GmbH & Co. KG, Germany) for 10 s in 10 mL 70% methanol under an ice-water bath to avoid enzymatic activation. Immediately, samples were heated at 70 ºC for 30 min in a water bath under continuous agitation to inactivate myrosinase. Then, the samples were centrifuged (13,000×g, 15 min, 4 ºC). The supernatants were collected and filtered through 0.22 µm PTFE syringe filters.

Twenty microliter samples were analysed using an Ultra High-Performance liquid chromatography (UHPLC) instrument (Shimadzu, Kyoto, Japan) equipped with a DGU-20A degasser, LC-30AD quaternary pump, SIL-30AC autosampler, CTO-10AS column heater and SPDM-20A photodiode array detector. The UHPLC system was controlled with LabSolutions software (Shimadzu, v. 5.42 SP5). Chromatographic analyses were carried out with a Kinetex C18 column (100 mm×4.6 mm, 2.6 µm particle size; Phenomenex, Macclesfield, UK) with a KrudKatcher Ultra HPLC guard column (Phenomenex, Macclesfield, UK). The column temperature was maintained at 37 ºC. The mobile phase was a mixture of (A) formic acid 0.1 % and (B) methanol. The flow rate was 1.5 mL min^{-1} in an increasing linear gradient starting from 5 % B to 15 % B at

6.6 min, 35 % B at 7.92 min, 35 % B from 7.92-12.32 min, 46 % B at 14.08 min, 50 % B at 16.28 min and 5 % B at 20.68 min. Then, column equilibration was conducted at 5 % B for 2.2 min. Chromatograms were recorded using a wavelength of 227 nm and glucoraphanin was identified and quantified with a commercial standard using a calibration curve prepared with at least six data points. The results were expressed as mg kg^{-1} dw. Each of the five replicates was analysed in duplicate.

Endogenous sulforaphane content

The endogenous sulforaphane content was analysed according to previous methods.[18,19] Briefly, 0.25 g of freeze-dried sample were suspended in 5 mL of acidic water (HCl; pH 6.0) at 45 ºC for 2.5 h in a shaking water bath. After glucoraphanin conversion to sulforaphane, 20 mL of dichloromethane was added to the mixture followed by sonication for 1 min. Anhydrous sodium sulphate (6.5 g) was added, filtered through filter paper (Whatman No. 41) and the eluent was collected. The filtered solid residue was washed twice with 3 mL of dichloromethane and the three eluted portions were collected together. A solid phase extraction with activated (3 mL of dichloromethane) Strata SI-1 silica gel 3-mL disposable columns was performed. Briefly, the previous extract was passed through the cartridge, washing the cartridge with 3 mL of ethylacetate (which was then discarded) and eluting the sulforaphane with 3 mL of methanol. The methanol extract was evaporated to dryness in a vacuum oven at 45 ºC for 2 h. Subsequently, the residue was re-dissolved with 2 mL of acetonitrile. Then, the purified sulforaphane extract was evaporated to dryness with a vacuum oven set at 45 ºC for 2 h. Finally, the residue was dissolved in 2 mL of acetonitrile, sonicated for 30 s and filtered through a 0.45 μm PTFE membrane filter.

Sulforaphane was analysed using a C18 (250 mm×4.6 mm, 5 μm) Gemini NX column (Phenomenex, Torrance CA, USA) as the stationary phase. UPLC analyses were carried out with 30/70 acetonitrile/water isocratic elution and a flow rate of 1 mL min^{-1}. Chromatograms were recorded using a wavelength of 202 nm and sulforaphane was identified and quantified with a commercial standard using a calibration curve prepared with at least six data points. Results were expressed as μmoles g^{-1} dw. Each of the five replicates was analysed in duplicate.

_S_-methyl cysteine sulphoxide (SMCSO) content

SMCSO content was determined as previously described[18] with slight modifications. Briefly, 2 g frozen smoothie were steeped overnight (4 ºC in darkness) in 30 mL acidified (10 mM HCl) cold 90 % methanol and subsequently homogenized (Ultra Turrax®) for 10 s. Then, the sample was incubated at 70 °C for 10 min using a vortex mixer every 2-3 min. After centrifugation (3,400×g, 4 ºC for 15 min) the methanolic fraction was aliquoted into a separate tube. The remaining homogenate was further extracted using 2×30 mL of boiling (70 ºC) acidified (10 mM HCl) 90% methanol with 10 min incubation at 70 ºC with a vortex. The combined methanolic extracts were concentrated to 2-3 mL under reduced pressure (40 °C) and adjusted to 5 mL by addition of 20 mM borate buffer (pH 9.2). The extract was stored at -20 ºC until derivatisation. Dansyl derivatives were prepared by mixing 100 μL of the sample extract with 250 μL 10mM dansyl chloride (prepared in acetonitrile) and 0.65 mL of 20 mM borate buffer (pH 9.2). The mixture was briefly shaken, allowed to stand at room temperature for 30 min, centrifuged at 16,200×g for 10 min and analysed by UHPLC. Dansyl derivatives were analysed using a C18 (250 mm × 4.6 mm, 5 μm) Gemini NX column (Phenomenex, Torrance CA, USA). The mobile phase was a mixture of (A) 50 mM pH 5 ammonium acetate buffer and (B) methanol. The flow rate was 0.9 mL min^{-1}, using a linear gradient. It increased from 30 % B to 40% over 35 min, to 75 % B over 60 min, and then maintained for 5 min at 75 % B before finally re-equilibrating to 30 % B for 5 min. The chromatograms were recorded using a wavelength of 250 nm and SMCSO was quantified with a commercial standard using a calibration curve prepared with at least 6 data points. The results were expressed as μmol kg^{-1} fresh weight (fw). Each of the five replicates was analysed in duplicate.

Statistical Analysis

The experiment had a two-factor (treatment×storage time) design subjected to analysis of variance (ANOVA) using Statgraphics Plus software (vs. 5.1, Statpoint Technologies Inc., Warrenton, USA). Statistical significance was assessed at p=0.05, and Tukey's multiple range test was used to separate the means.

RESULTS AND DISCUSSION

Modelling of the electromagnetic field distribution inside the microwave oven

The heating characteristics of a product is dependent on several thermal properties such κ, c_p and ρ. The smoothie showed κ, c_p and ρ values of 0.5354 W m^{-1} ºC, 2580 J kg^{-1} ºC and 1,040.3 kg m^{-3} at 20 ºC, respectively (Table 1). The dielectric properties of a product to be treated by microwaves need to be measured, as these properties determine both the energy propagation within the product and the transformation of the MW energy into heat inside the dielectric element. MW heating is based on the higher or lower capacity of the dielectric element to polarize its charges through its volume against an external electric field. The polar molecules cannot follow the fast changes of the electric field, sot that the energy is dissipated as heat. The complex relative permittivity can be obtained as described in Eq. (1):

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \quad (1)$$

The dielectric constant (ε') and the loss factor (ε'') were determined in the smoothie at different temperatures in the frequency range 0.01-3 GHz (Figures 2A and 2B, respectively). The frequency range 0.01-3GHz includes the radiofrequency and MW frequencies employed for heating purposes. At the working frequency (2450 MHz) ε' decreased with an increase in temperature of up to 60 ºC where it stabilised and ε'' decreased with an increase of temperature with a behaviour similar to the one found in vegetable purees.[21,22] This phenomenon can be attributed to the predominance of the dispersion resulting from the dipole rotation of water molecules at 2,450 MHz, and at high temperatures, fewer hydrogen bonds are formed, causing a decrease in ε''.[19,20] Those dielectric property measurements were verified with a dielectrometer, obtaining similar results at specific frequencies near 2 GHz. As observed in Figures 2A and 2B, both results were in agreement and in the same order of magnitude.

A mean value for the permittivity at 2.45 GHz ($\varepsilon^* = 62.4 - j12.99$) was used to obtain the electric field data for the smoothie in the simulation model. Due to the high simulation times, it was not possible to vary neither the permittivity nor the thermal parameters with the increasing temperatures. Since the parameters did not vary in excess, as shown in Figures 2A and 2B, these estimates should not affect the validity of the results.

The electric field of a sample that is in motion or under the influence of a moving stirrer is usually approached obtaining the desired results of the discretized positions of the moving sample/stirrer.[19] The total averaged electric field when discretizing the movement can be obtained[20-22] with Eq. (2):

$$\left|\vec{E}_{avg}(x,y,z)\right| = \sqrt{\frac{\sum_{i=1}^{N}\left|\vec{E}_i(x,y,z)\right|^2}{N}} \quad (2)$$

where, $\vec{E}_i$ is the electric field on the sample under study for the *i*-th position of the movement, *N* represents the number of positions and is directly related to the discretization step. In our case *N*=9.

A section of the electric field strength distribution obtained using Eq. (2) at the height of the surface of the sample is shown in Figures 3 A-C when the powers 2.64 kW, 1.64 kW, 1.64 kW and 1.64 kW were applied to the four ports (magnetrons). Latter figures represent three different positions (initial, 3A; middle, 3B; and final, 3C) of the 9 positions employed to discretize the back-and-forth movement. As observed, the multimode distribution shows several maxima and minima inside the cavity. The field levels inside the sample were much lower than outside due to the energy reflected at the air-smoothie interface. The latter finding explains the high levels of power needed to reach the temperature of 80 ºC in a short period of time. However, the uniformity of the field inside the sample was much higher than the uniformity in the rest of the cavity due to the high values of ε'' and to the attenuation of the electric field inside the sample.

The solution obtained from the electromagnetic problem was used to obtain the dissipated power $P_v(x,y,z)$ (Eq. 3) and this term was included in the heat equation as the source used to generate the temperature increase.[14]

$$P_v(x,y,z) = \pi f \varepsilon_0 \varepsilon'' \left|\vec{E}_{avg}(x,y,z)\right|^2 \quad (3)$$

where $\varepsilon_o$is the vacuum permittivity (8.8512×10-12 F/m), $\left|\vec{E}_{avg}(x,y,z)\right|$ (V m^{-1}) is the averaged electric field strength obtained from a linear average of the absorbed power

(Eq. 3) and directly related to the temperature increase, f (Hz) is the frequency and $\tan\delta$ is the loss tangent of the smoothie samples. $\tan\delta$ can be obtained with Eq. (4):

$$\tan\delta = \frac{\varepsilon''}{\varepsilon'} \quad (4)$$

Once the averaged field strength was determined, the temperature distribution within the time was obtained from the heat Eq. (5):

$$\rho c_p \frac{\delta T}{\delta t} = \nabla \cdot (k\nabla T) + P_v(x, y, z) \quad (5)$$

where T (ºC) is the temperature. Equation (5) was solved using finite differences with Matlab (Mathworks, Natick MA, USA) using an initial sample and ambient temperatures of 10 and 23 ºC, respectively. Figure 4 shows the results for the temperature after 15 s on the samples surfaces using the previously reported powers. As can be observed in the thermography of Figure 5, the experimental temperature distribution shows a similar profile tending to overheat the edges of the sample in the equivalent experiment using the microwave oven. However, the edge overheating observed in the thermography was not so important as that obtained from the model due to the cooling of samples, which occurred within the few seconds after taking the thermography next to the MW device. Conclusively, the proposed procedure has been successfully applied to predict the power levels and the conditions of the oven to provide the expected temperature increase in the smoothies. This could have been experimentally done, but many trials, samples and time would have been needed.

Glucoraphanin and sulforaphane contents

Initial glucoraphanin content of untreated smoothies was 4.5 µmol g^{-1} dw (Table 2). Since the green smoothie was 35 % kalian-hybrid broccoli, glucoraphanin levels were within previously-reported ranges for kalian-hybrid broccoli.[23] As previously described, glucosinolates themselves are not bioactive until they are transformed by plant myrosinase into isothiocyanates. Low glucoraphanin conversion into sulforaphane has been reported in broccoli florets homogenized in water, with non-bioactive compound sulforaphane nitrile being the predominant product due to ephithiospecifier protein (ESP) activity.[24] However, higher sulforaphane formation (after complete endogenous glucoraphanin hydrolysis) was observed in CTRL smoothies with a sulforaphane:glucoraphanin ratio of 1:5 on processing day (Table 2) as compared to conversion rates observed in other broccoli cultivars.[25] Isothiocyanate formation has been reported to be inhibited by Fe ions at pH 4.5-5.5 (the pH of the smoothie and fresh broccoli is approximately found within this pH range).[29,30] Kalian-hybrid broccoli has 70 % lower Fe content as compared to common broccoli cv. Parthenon.[26] Accordingly, the greater sulforaphane formation in the smoothie compared to other broccoli cultivars may be due to the lower Fe content. Great differences of sulforaphane:glucoraphanin rates among seven broccoli cultivars have been reported.[25] Consequently, the higher sulforaphane formation in the smoothie could also be explained by a higher myrosinase and/or lower ESP activities in the kalian-hybrid broccoli.

Glucoraphanin content was not significantly ($p<0.05$) changed after the CP treatment, while MW reduced glucoraphanin content by 25 %. However, microwave treatments of 5 min/900 W and 2.5 min/1000 W (domestic microwave) induced higher glucoraphanin degradation of 46 and 63 % in fresh-cut broccoli cv. Youxiu and kalian-hybrid, respectivel.[18,32] Accordingly, the innovative short time/high power MW treatment achieved lower glucoraphanin degradation compared to domestic microwave treatments. Nevertheless, a slightly higher sulforaphane formation was observed after MW with a sulforaphane:glucoraphanin ratio of 1:6. The latter results of high sulforaphane formation may be due to the lower MW treatment time as compared to CP.

Glucoraphanin content was reduced during storage at 5 ºC with CTRL samples showing the highest decrease of 44 % after 7 days. Similar reductions of glucoraphanin content were previously reported in fresh-cut broccoli cv. Marathon after 7 days at 1 ºC.[27] However, an increasing trend in glucoraphanin content was observed in all samples after 20 days at 5 ºC. Glucoraphanin levels have been reported to increase under an ethylene-enriched atmosphere.[28] Fresh-cut kalian-hybrid broccoli has been shown to emit ethylene at a rate of 3.0-8.3 nmol kg^{-1} s^{-1}.[29] Accordingly, the ethylene accumulation within the closed recipients containing the smoothie could trigger the observed glucoraphanin biosynthesis. MW samples showed glucoraphanin increments of 18 and 38 % after 7 and 20 days at 15 and 5 ºC, respectively. The latter finding was in accordance with the significant sulforaphane increments of 134 and 230 % observed in MW samples after 7 and 20 days at 15 and 5 ºC, respectively. The delayed sulforaphane peak formation from 7 to 20 days at the lower temperature was in accordance with the reduced sulforaphane formation at 4 ºC as compared to 14 ºC like previously reported.[30] Sulforaphane content of heated samples decreased by 80 % in the last 10 days of storage at 5 ºC while CTRL remained unchanged. Heat treatments led to plant cell disruption as observed in kalian-hybrid broccoli after different cooking methods.[7] Accordingly, greater availability of substrates for isothiocyanate-degrading reactions may have occurred in heat-treated samples, leading to the observed sulforaphane degradation of CP and MW samples after 10 days. However, latter degrading reactions may be increased at higher storage temperatures since sulforaphane contents of CTRL samples stored at 15 ºC decreased by 70 % after 7 days. The latter finding may be explained since sulforaphane, contrary to their relatively inert precursor glucoraphanin, is a highly reactive compound that is very unstable in aqueous solutions, with its degradation rates increased as the storage temperature increases.[30, 31]

Conclusively, MW treated samples showed the highest sulforaphane content after 20 days, without significant differences among storage temperatures, while the content of this bioactive compound was reduced 2-fold in CP samples after the same storage time. Although recommended smoothie consumption could be up to 20 days, heat-treated samples still allowed sulforaphane formation of 0.2-0.3 μmol g^{-1} dw after 30 days of storage.

_S_-methyl cysteine sulphoxide content

The untreated smoothie showed an initial SMCSO content of 110.2 μmoles kg^{-1} fw which increased by approximately 100 % after CP treatment (Table 2). The higher SMCSO in CP-treated samples could be due to a better extraction with this longer treatment. Generally, the SMCSO contents of samples increased throughout storage. CTRL samples achieved higher SMCSO increments throughout storage compared to heat-treated samples. Accordingly, a SMCSO increase of 160 % was observed in CTRL samples after 30 days at 5 ºC while CP and MW samples achieved SMCSO increments of 50 and 70 %, respectively, after the same time period. When samples were stored at 15 ºC, SMCSO increments were observed early, with increases of 160 % for CTRL after 7 days and 54 and 120 % for CP and MW, respectively, after 20 days. SMCSO biosynthesis has not been elucidated well yet, although cysteine and serine could be the two possible amino acid substrates as recently reviewed.[10] Serine is one of the major amino acids present in broccoli and its concentration has been reported to increase during storage probably due to an increase of proteinase activity even at low storage temperature.[32] Accordingly, the SMCSO increments could be due to the increased serine contents through the action of proteinases. The activity of the latter enzyme may be greater at higher storage temperatures leading to the observed earlier SMCSO increments in samples stored at 15 ºC. The lower SMCSO increments in heat-treated samples could be due to the partial heat-inactivation of the proteinase enzyme. Among heat treatments, the longer treatment time of CP could induce higher proteinase inactivation rates as compared to MW, leading to the lower SMCSO increments in CP samples compared to MW throughout storage.

CONCLUSIONS

This results show that specific modelling of a heating profile according to the product to be treated is highly important, as shown by the use of an innovative semi-continuous microwave pasteurization treatment with a high power/low treatment time applied to a broccoli-based green smoothie. The thermal conductivity, heat capacity, density and relative complex permittivity of the green smoothie were shown at different treatment temperatures. The electromagnetic-thermal coupled problem was successfully solved using numerical methods to predict and to understand the high temperature increase in the samples. Temperature measurements during the heating process and thermographic images verified the procedure. The determination of the needed power levels, number and distribution of samples, time duration and the entire set-up of the MW oven were crucial for the research work, as it avoided previous time- and sample-consuming trials. Attending to the evolution of the studied health-promoting properties throughout the storage of the smoothies, the MW treatment led to better sulforaphane biosynthesis as compared to the conventional pasteurization treatment (CP) with increments of sulforaphane content of 230 % after 30 days at 5 ºC, and 130 % after 7 days at 15 ºC. *S*-methyl cysteine sulphoxide (SMCSO) increased with storage time at both temperatures. The MW-treated samples showed higher SMCSO increments as compared to samples treated with CP. Accordingly, this MW treatment could be used by the food industry to obtain a green smoothie with a good quality profile and high levels of health-promoting compounds that are more stable during subsequent retail/domestic storage up to one month at 5 ºC.

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TABLE AND FIGURE CAPTIONS

Table 1. Heat capacity (c_p) and thermal conductivity (κ) of a green smoothie at different temperatures (n=5±SD). Different letter denotes significant differences ($p<0.05$) among different temperatures.

Table 2. Glucoraphanin, sulforaphane and *S*-methyl cysteine sulphoxide (SMCSO) of an untreated green smoothie (CTRL) or thermally treated (conventional pasteurization, CP; and semi-continuous microwave treatment, MW) and stored at 5 or 15 ºC up to 30 days (n=5±SD). Different capital letter denotes significant differences ($p<0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p<0.05$) among different sampling times for the same treatment.

Figure 1. Semi-industrial continuous-flow microwave oven.

Figure 2. Dielectric constant (ε'; A) and dielectric loss factor (ε''; B) of a green smoothie at 0.01-3GHz.

Figure 3. Electric field strength distribution on the surface of the samples at the initial (A), intermediate (B) and final position (C).

Figure 4. Temperature distribution on the surface of the samples obtained through simulations.

Figure 5. Thermography with temperature distribution on the surface of the samples obtained experimentally.

SUPPLEMENTARY MATERIAL

Supplementary material 1. Nutritional composition of the green smoothie.