A green fresh vegetables smoothie rich in health-promoting compounds was developed. Two thermal treatments to reduce microbial load and preserve quality were assayed: T1 (3 min at 80°C) and T2 (45 s at 90°C). Fresh blended unheated samples were used as control (CRL). The smoothie presented a viscoelastic behaviour. Thermal treatments reduced initial microbial loads by 1.3-2.4 and 1.4-3.1 log units, respectively. Samples were stored in darkness at 5 and 15°C. Colour and physicochemical changes were measured.
reduced in thermal-treated samples throughout storage, which were better preserved at 5°C rather than at 15°C. Vitamin C changes during storage were fitted with a Weibullian distribution. Total vitamin C losses of T1 and T2 samples during storage at 15°C were greatly reduced when they were stored at 5°C. Initial total phenolics content (151.1 mg kg\(^{-1}\) fw) was 44 and 36% increased after T1 and T2 treatments, respectively. The 3-p-coumaroyl quinic and chlorogenic acids accounted the 84.7% and 7.1% relative abundance, respectively. Total antioxidant capacity (234.2 mg Trolox equivalent kg\(^{-1}\) fw) remained constant after the thermal treatments and was better maintained during storage in thermal-treated samples. Glucobrassicin accounted the 81% of the initial total glucosinolates content (117.8 mg kg\(^{-1}\) fw) of the smoothie. No glucosinolates losses were observed after T2 treatment being better preserved in thermal-treated samples. Conclusively, a short time–high temperature mild thermal treatment (T2) showed better quality and bioactive compounds retention in a green fresh vegetable smoothie during low temperature storage.

Keywords: phenolics; glucosinolates; vitamin C; antioxidants; quality; beverages.

1. Introduction

Clinical and epidemiological research indicates that at least 80% of current chronic diseases and premature deaths are preventable with changes in diet and consumer lifestyle (Anand et al., 2008). Fruit and vegetables contain a high content of phytochemicals responsible of preventative effects on cardiovascular disease, cancers, hypertension and other chronic conditions such as diabetes and obesity derived from (Boeing et al., 2012). However, fruits and vegetables consumption consistently is below the 400 g of fruits and vegetables daily intake which has been worldwide promoted by
several programs such as ‘5 A Day’ (WHO/FAO, 2003). Latter fact may be explained by the current lifestyle which does not allow the time needed for the preparation of these products particularly vegetables needing more preparation time. Accordingly, smoothies represent an excellent and convenient alternative to promote the daily consumption of fruit and vegetables. Smoothies are non-alcoholic beverages prepared from fresh or frozen fruit and/or vegetables, which are blended and usually mixed with crushed ice to be immediately consumed. Often, some smoothies may include other components like yogurt, milk, ice-cream, lemonade or tea. They have a milk shake-like consistency that is thicker than slush drinks (Rodríguez-Verástegui, Martínez-Hernández, Castillejo, Gómez, Artés & Artés-Hernández, 2015). Recent research has shown that daily consumption of green smoothies may enhance health quality of consumers (Maeda, 2013). The main issue of the smoothie processing is the limited shelf-life of these products since they are susceptible to spoilage (Buzrul, Alpas, Largeteau & Demazeau, 2008) and quality degradation. For that reason, mild thermal treatments must be used during processing in order to increase the shelf-life while keeping quality (Di Cagno, Minervini, Rizzello, De Angelis & Gobbetti, 2011). Furthermore, storage at low temperature up to 5°C is recommended. However, the treatment should not be much aggressive to preserve its nutritional and sensory quality. Thermal treatment (generally in the range of 80 °C to 95 °C) is applied for the inactivation of spoilage enzymes in smoothies, fruit purées and juices (Barba, Esteve & Frigola, 2012). However, thermal treatments may reduce the phytochemical content of smoothies, such as antioxidants among others, in detriment of related health-promoting properties. Studies about the effects of thermal processing and subsequent storage on bioactive compounds and quality changes of fresh vegetable smoothies are very scarce and no previous data for green smoothies are so far reported. For that reason, the aim of
this work was to study the effect of two different mild conventional heat treatments on quality changes, as well as on selected bioactive compounds of a green fresh vegetable smoothie throughout storage at 5 and 15°C.

2. Materials and methods

2.1 Plant material and smoothie preparation

Fresh vegetables were purchased at a local supermarket in January. The raw material was sanitized with 75 mg L⁻¹ NaClO during 2 min and then rinsed with cold tap water for 1 min. Cucumbers were peeled and all vegetables were then cut and blended (MX2050 blender, Braun, Germany). The green smoothie composition was 77.2% cucumber, 12% broccoli and 6% spinach. The smoothie composition was selected among several pre-formulations according to sensory evaluations conducted by an informal sensory panel. The nutritional composition of the smoothie was determined with the software DIAL 1.0 (Ortega-Anta, López-Sobaler, Andrés-Carvajales, Requejo-Marcos, Aparicio-Vizuete & Molinero-Casares, 2008) and it is presented as Supplementary material 1. Citric acid (4.8%) was added in order to decrease the pH below 4.5 and reduce microbial growth of the smoothie during subsequent storage.

2.2. Thermal treatments and storage conditions

Thermal treatments were applied by using a Mastia thermoresistometer described by Conesa, Andreu, Fernández, Esnoz and Palop (2009). Immediately after blending, the sterilized vessel of the thermoresistometer was filled with 400 mL of the smoothie. For treatment T1, the thermoresistometer was programmed to increase the initial smoothie temperature (8±2°C) with a heating rate of 30°C/min to 80°C, then maintained for 3 min and cooled down to a final temperature of 40°C (heating rate of 30°C/min). Then, the
smoothie temperature was further cooled down to 4ºC submerging the vessel in an ice-
water bath while continuously agitation was programmed in the thermoresistometer. For
treatment T2, the thermoresistometer was programmed to increase the initial smoothie
temperature with a heating rate of 30ºC/min to 90ºC, then maintained for 45 s and
cooled down to a final temperature of 40ºC (heating rate of 30ºC/min). The smoothie
temperature was cooled down to 4ºC similarly to T1. Subsequently, 15-mL aliquots of
thermal treated samples were taken in aseptic conditions in sterile Falcon tubes through
the thermoresistometer sampling port. Samples were stored in darkness at 5 and 15ºC.
Fresh blended unheated samples were used as control (CTRL). Visual appearance,
flavour, texture, off-colours, off-odours, lumpiness, turbidity, precipitation/phase
separation and overall quality of CTRL smoothie conducted by an informal sensory
panel test of 8 persons were reported to be over the limit of acceptability up to 21 days
at 5ºC (data not shown). Thermally-treated smoothies maintained their sensory
acceptation up to 49 days at 5ºC (data not shown). Unappropriated storage conditions of
smoothies were also studied for a 7 days period at 15ºC of shelf life. Accordingly, the
shelf-lives of the smoothies were established based on those sensory analyses. Then,
sampling was conducted on processing day (0) and after 7, 11, 21, 35 and 49 days
depending of the treatment and storage temperature. Five replicates per treatment and
sampling day, for each storage temperature, were prepared. Samples of each treatment
were taken on each sampling day to be analysed storing also samples for bioactive
compounds at -80ºC until further analysis.

2.3. Rheological properties of smoothies
Rheological measurements were executed using the same instrument and methodology
as previously described by Castillejo, Martínez-Hernández, Gómez, Aguayo, Artés and
Three repetitions of the dynamic-mechanical experiments were performed.

2.4. Total dietary fibre and mineral content

The contents of pectin, hemicellulose, cellulose, lignin and ash in the smoothie were studied using the same instrument and methodology as previously described by Castillejo et al. (2016). The weight percentage of each component was obtained as the mass loss produced during volatilization. The mineral contents of the samples were analysed by X-ray fluorescence (XRF) using the same methodology and device as Martínez-Hernández, Gómez, Artés and Artés-Hernández (2015). Mineral contents were expressed as g kg\(^{-1}\) fresh weight (fw) and mg kg\(^{-1}\) fw for major and trace minerals, respectively. Each of the five replicates was analysed by duplicate.

2.5. Microbial analysis

Mesophilic, psychrophilic, Enterobacteria, and yeast and mould growth was determined using standard enumeration methods according to Castillejo et al. (2016). All microbial counts were reported as log colony forming units per gram of product (log CFU g\(^{-1}\)). Each of the five replicates was analysed by duplicate. The presence of Salmonella spp., Listeria monocytogenes and generic Escherichia coli was monitored according to the European legislation (Regulation EC 1441/2007, 2007).

2.6. Physiochemical analyses

The pH, titratable acidity (TA), total soluble solids content (SSC) and colour of smoothies were determined according to Castillejo et al. (2016). TA and SSC were expressed as g citric acid 100 mL\(^{-1}\) and °Brix, respectively. Total colour differences
(ΔE) throughout storage compared to their respective initial values were calculated according to equations previously described (Walkling-Ribeiro, Noci, Cronin, Lyng & Morgan, 2010).

2.7. Chlorophylls content
Sample preparation for total chlorophyll determination was conducted according to Martinez-Hernández, Gómez, Pradas, Artés and Artés-Hernández (2011). An UV-visible spectrophotometer (8453, Hewlet Packard, Columbia, USA) was used to registered absorbances at 662 and 644 nm. The equations developed by Wellburn (1994) were used to determine chlorophyll a (Cha = 10.05×A662 – 0.766×A644) and chlorophyll b (Chb = 16.37×A644 – 3.14×A662). Then, total chlorophyll content was expressed as the sum of Cha and Chb (Ca + Cb). Chlorophyll content was expressed as mg kg⁻¹ fw. Each of the five replicates was analysed by duplicate.

2.8. Bioactive compounds
2.8.1. Vitamin C
The ascorbic (AA) and dehydroascorbic (DHA) acids were measured according to the method of Zapata and Dufour (1992) with modifications from Martínez-Hernández, Artés-Hernández, Colares-Souza, Gómez, García-Gómez and Artés (2013). Derivatized samples (20 µL) were injected onto a Gemini NX (250 mm×4.6 mm, 5 µm) C18 column (Phenomenex, Torrance CA, USA), using an HPLC (Series 1100 Agilent Technologies, Waldbronn, Germany) equipped with a G1322A degasser, G1311A quaternary pump, G1313A autosampler, G1316A column heater and G1315B photodiode array detector. The HPLC system was controlled by the software ChemStation Agilent, v. 08.03. AA and DHA were quantified using commercial standards (Sigma, St Louis, MO, USA).
Calibration curves were made with at least six data points for each standard. Total vitamin C was calculated as the sum of AA and DHA and expressed as mg kg\(^{-1}\) fw. Each of the five replicates was analysed by duplicate.

### 2.8.2. Simultaneous analysis of phenolic compounds and intact glucosinolates

Simultaneous extraction, analysis and identification of phenolic and intact glucosinolates were based on Fernández-León, Fernández-León, Lozano, Ayuso, Amodio, Colelli and González-Gómez (2013) with some modifications. A smoothie sample of 9 g was homogenized (Ultra-turrax T-25, Ika-Labortechnik, Staufen, Germany) in 7 mL 70% MeOH under an ice-water bath to avoid enzymatic activations. Immediately, samples were heated at 70ºC for 15 min in a water bath under continuous agitation to inactivate myrosinase. Then, the samples were centrifuged (13,000×g, 10 min, 4ºC). The supernatants were collected and filtered through 0.20 µm syringe PTFE filters.

Samples of 20 µL were analysed using an Ultra High-Performance liquid chromatography (UPLC) 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 UPLC system was controlled by the software LabSolutions (Shimadzu, v. 5.42 SP5). Chromatographic analyses were carried out onto 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 40ºC. 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 a linear gradient starting increasing from 5% B to 15% B at 6.6 min, 35% B at 7.92 min, 35% B in 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 at 330 nm for phenolics and 227 nm for
glucosinolates. Phenolic acids were quantified as equivalents of chlorogenic acid (5-
caffeoylquinic acid; Sigma, St Louis, MO, USA) and sinapic acid derivates (Sigma, St
Louis, MO, USA). Glucosinolates were quantified as sinigrin equivalents. The
calibration curves were made with at least six data points for each standard. The results
were expressed as mg kg$^{-1}$ fw. Each of the five replicates was analysed by duplicate.

LC/UV-PAD/ESI-MSn analyses were carried out in an Agilent HPLC 1100 series
equipped with a photodiode array detector and mass detector in series (Agilent
Technologies, Waldbronn, Germany). The HPLC consisted of a binary pump (model
G1312A), an autosampler (model G1313A), a degasser (model G1322A), and a
photodiode array detector (model G1315B). The HPLC system was controlled by a
ChemStation software (Agilent, v. 08.03). The mass detector was an ion trap
spectrometer (model G2445A) equipped with an electrospray ionisation interface and
was controlled by LCMSD software (Agilent, v. 4.1). The ionisation conditions were
adjusted at 350ºC and 4 kV for capillary temperature and voltage, respectively. The
nebulizer pressure and flow rate of nitrogen were 65.0 psi and 9 L min$^{-1}$, respectively.
The full scan mass covered the range from m/z 150 up to m/z 900. Collision-induced
fragmentation experiments were performed in the ion trap using helium as the collision
gas, with voltage ramping cycles from 0.3 up to 2 V. Mass spectrometry data were
acquired in the negative ionisation mode. MSn is carried out in the automatic mode on
the more abundant fragment ion in MS (n$^{-1}$).

2.8.3. Total antioxidant capacity
Total antioxidant capacity (TAC) extraction and analysis were conducted according to Rodríguez-Verástegui et al. (2015) using three different methods: free radical scavenging capacity with 2,2-diphenyl-1-picrylhydrazil (DPPH) (Brand-Williams, Cuvelier & Berset, 1995), ferric reducing antioxidant power (FRAP) (Benzie & Strain, 1999) and 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (Cano, Hernández-Ruíz, García-Cánovas, Acosta & Arnau, 1998). All TAC data were expressed as mg of Trolox equivalents kg\(^{-1}\) fw. Each of the five replicates was analysed by duplicate.

2.9. Statistical Analysis

The experiment was 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 the level \(P=0.05\), and Tukey’s multiple range test was used to separate means.

3. Results and discussion

3.1. Rheological properties

The texture of a smoothie has to provide a balance between desired mechanical stability (for storage and handling) and desired instability (to elicit a specific texture attribute during mastication). Rheological properties are useful in determining the most ingredients proportions in the product development, quality control, and correlation of food texture to sensory attributes. Smoothies are viscoelastic food materials that exhibit both solid-like and fluid-like behaviour. The rheological properties of the smoothie are presented as Supplementary material 2. The storage modulus (\(G'\)) of smoothies was greater than the loss modulus (\(G''\)) at any given point in the frequency sweep tests (see
Supplementary material 2). This fact indicates a dominant contribution of the elastic component to the viscoelasticity of the smoothie, behaviour typical for a viscoelastic solid. This means that the attractive forces become dominant due to the strong hydrogen bond and hydrophobic association (Basu, Shivhare, Singh & Beniwal, 2011). The $\tan\delta$ value (ratio between loss and storage modulus, also known as loss tangent) is a direct measure of the relative importance of viscous and elastic effects in the sample. The $\tan\delta$ of samples was lower than 1 thus indicating a gel-like behaviour. Apparent viscosity of the green smoothie was not greatly changed after thermal treatment as observed in Supplementary material 2. The effective shear rate range in the mouth is 40-50 s$^{-1}$, which would have implied actual sensory consistency (Wood & Goff, 1973). The viscosity of T1 samples was slightly higher than T2 samples within the shear rate range 40-50 s$^{-1}$.

3.2. Total dietary fibre and mineral content

The total dietary fibre (DF) content of the smoothie was 4.4% wet basis (wb) (data not shown). Pectin and hemicellulose contents of the smoothie were 0.8 and 1.9% wb, respectively. The smoothie accounted 1.8% wb of cellulose. According to The Code of Federal Regulations (FDA, 2015), food products which contain 20% or more of the recommended daily nutrient intakes (RNIs) for fibre (25 g day$^{-1}$) are considered as an ‘excellent source of fibre’. Accordingly, this green smoothie can be considered as an ‘excellent source of fibre’ since a portion of 250 g provides approximately 50% of the RNIs for fibre.

The P, S, Na, K, Ca, Mg, Cl, Al and Si contents of the smoothie were 0.75, 0.48, 0.47, 4.24, 0.41, 0.30, 0.48, 0.01 and 0.04 g kg$^{-1}$ fw, respectively (data not shown). The Fe, Mn and Zn contents of the smoothie were 8.72, 3.11 and 5.93 mg kg$^{-1}$ fw, respectively.
(data not shown). A 250 g portion of this smoothie provides 29-34, 9-15, 7-12 and 15-21% of the RNIs for Mg, Ca, Fe and Zn, respectively, covering population groups with special nutritional requirements such as elders, pregnant women or adolescents (FAO/WHO, 2004).

3.4. Microbial analysis

Microbial load is a major quality criterion to determine the shelf life of food products. The unit operations of the smoothie processing, which includes several injury stresses such as peeling, cutting, shredding or blending greatly increase the risk of microbial development. The mesophilic load of CTRL smoothie on processing day was 4.4 log CFU g⁻¹ (Figure 1A). Thermal treatments reduced mesophilic load by 1.7-1.8 log units without differences among them. Zhao et al. (2014) reported 2 log units mesophilic reductions in cucumber juice heat treated at 85°C for 15 s. The similar microbial reduction in the juice with shorter treatment time may be owed to the fibres and other particles contained in the smoothie which may difficult the heat transmission contrary to the juice. However, Walkling-Ribero et al. (2010) reported higher mesophilic reductions (3.5 log units) in a fruit smoothie treated with a milder heat treatment (70°C for 15 s). The dynamic system used by Walkling-Ribero et al. (2010) during heat treatment compared to our static system may explain the better microbial reductions achieved by those authors. Great mesophilic increases of 4.7 and 5.2 log units were observed in CTRL and thermal-treated smoothies after 7 days at 15°C, respectively. However, storage at 5°C greatly reduced mesophilic growth of CTRL and heat-treated samples compared to those stored at 15°C since increments of 3.6-3.8 and 0.7 log units were registered after 21 days at 5°C. Heat-treated samples stored at 5°C showed a great mesophilic growth (2.9-3.1 log units) in the first 11 days remaining those loads almost
unchanged (<0.4 log units changes) until day 35. However, those microbial loads of T1 and T2 samples were increased by 1.4 and 1.9 log units, respectively, from day 39 to day 49. The higher mesophilic growth of heat-treated samples could be explained by different hypotheses: 1) the vegetative or spore cells which resisted to the thermal treatment, due to their higher thermal resistance and/or the protecting effects of the smoothie matrix, could grow better due to the lower microbial competence for the nutrients. 2) The used heat treatment completely inactivated the initial myrosinase activity (163.0 nmoles sinigrin transformed per g fw of sample; data not shown), which is responsible for the glucosinolates conversion to isothiocyanates. Isothiocyanates from broccoli have shown high antimicrobial activities contrary to glucosinolates (Vig, Rampal, Thind & Arora, 2009). Accordingly, the glucosinolate-isothiocyanate conversion was possible in untreated unheated samples, contrary to heat-treated samples, with the observed preserving benefits from the isothiocyanates throughout storage of smoothies. Therefore, our previous preliminary non-published data showed that mesophilic increase of 2 log units in untreated smoothie after 28 days at 5ºC was doubled when that untreated smoothie was prepared without broccoli (data not shown).

Initial psychrophilic count of CTRL smoothie was 5.1 log CFU g⁻¹ (Figure 1B). Psychrophilic counts were reduced by 2.4 and 3.1 log units after T1 and T2 treatments, respectively. During storage at 15ºC, psychrophilic counts of CTRL, T1 and T2 samples augmented by 3.1, 2.8 and 3.5 log units after 7 days, respectively. However, and similarly to mesophilic data, although heat-treated samples showed great increases of 2.6-3.0 log units after 11 days at 5ºC loads changes were lower than 2.4 log units in the last 38 days of storage. In contrast, CTRL samples did not significantly change after 21 days at 5ºC. CTRL and heat-treated samples registered psychrophilic counts of 5.5 and 6.7-7.0 log CFU g⁻¹ after 21 and 49 days at 5ºC, respectively.
Initial *Enterobacteriaceae* counts of 3.8 log CFU g$^{-1}$ were only significantly reduced after T2 treatment by 1.6 log units (Figure 1C). The *Enterobacteriaceae* levels of CTRL and T1 samples stored at 15ºC increased by 2.2-2.3 after 7 days while T2 samples increased in a greater extend with 3.5 log units increments after 7 days. CTRL and thermal-treated samples registered *Enterobacteriaceae* counts of 5.0 and 4.6-4.8 log CFU g$^{-1}$ after 21 and 49 days of at 5ºC, respectively.

Yeast and moulds counts of CTRL smoothie of 3.9 log CFU g$^{-1}$ were reduced by 1.3-1.4 log units after heat treatments without significant differences among them (Figure 1D). Similarly to mesophiles, dynamic heating system during a milder heat treatment (70ºC for 15 s) induced greater yeasts and moulds reductions (3.7 log units) in a fruit smoothie (Walkling-Ribero et al., 2010). Furthermore, data from Zhao et al. (2014) showed better heat transmission in cucumber juice compared to our smoothie since initial yeasts and moulds (4 log CFU g$^{-1}$) were greatly reduced below the detection limit (1 log CFU g$^{-1}$). Yeast and moulds counts of CTRL and T2 samples were incremented by 3.6 and 3.4 log units, respectively, after 7 days at 15ºC while T1 samples only increased in 0.5 log units. Similarly to mesophilic and psychrophilic, yeasts and moulds counts greatly augmented by 2.5 and 2.8 log units after 11 days followed by a 1.3-1.4 log units increment in the last 38 days of storage at 5ºC. CTRL and thermal-treated samples showed final yeasts and moulds counts of 5.4 and 6.5-6.6 log CFU g$^{-1}$ after 21 and 49 days at 5ºC, respectively.

Conclusively, thermal treatments, with better initial microbial reductions achieved by T2, combined with low temperature storage, kept microbial loads below 6 log units after 39 days at 5ºC. Although CTRL samples showed a similar microbial behaviour to heat-treated samples during low temperature storage, thermal treatment is needed to inactivate quality-degradation enzymes, as recently reported in vegetable smoothies.
(Rodríguez-Verástegui et al., 2015), in order to reduce colour changes of smoothies during storage as shown later.

3.5. Soluble solids content, pH and titratable acidity

Physicochemical quality of smoothies can be evaluated based on SSC, pH and TA. Physicochemical data of the smoothie during storage is shown in Table 1. CTRL smoothie showed an initial SSC of 4.3 °Brix (Table 1). Di Cagno et al. (2011) reported a higher SSC of 10.8 °Brix in a green smoothie due to the high fruit content (40% kiwifruits, 7% fennels, 8% spinach and 15% papaya). Thermal treatment did not induce SSC changes. SSC of all smoothies remained quite constant after 7 days of storage at 15°C. Contrary, CTRL smoothie showed a SSC increase of 0.7 °Brix after 7 days at 5°C followed by a decrease. Accordingly, CTRL smoothie registered 4.6 °Brix after 21 days at 5°C with no differences regarding its respective initial level. SSC levels of T1 and T2 smoothies remained stable up to 35 days of storage at 5°C followed by a decrease of 0.9 and 0.7 °Brix, respectively, from day 35 to day 49. The observed SSC decreases during storage may be owed to the sugars and other soluble solids used by microorganisms and enzymatic systems as substrates in several metabolic reactions.

The initial pH and TA of CTRL smoothie were 4.49 and 0.22 mg citric acid 100⁻¹ g fw, respectively (Table 1). Data from Di Cagno et al. (2011) showed a higher TA of 0.6 mg citric acid 100⁻¹ g fw in a green smoothie owed to the high kiwifruit content (40%) showing this fruit a high TA. Similar to SSC, pH and TA did not register significant differences after thermal treatments. The pH of CTRL and T1 samples was slightly reduced by 0.2 and 0.4 pH units, respectively, after 7 days at 15°C although pH of T2 samples remained stable. Correspondingly, TA of CTRL and T1 samples increased 0.1 and 0.4 mg citric acid 100⁻¹ g fw, respectively, after 7 days at 15°C while T2 did not
register differences during this period. No great pH and TA changes in CTRL samples were observed after 21 days at 5°C. The pH of thermal-treated samples was constant throughout storage at both storage temperatures. Similarly, no pH changes were observed in untreated and heat treated (100°C for 60 s) spinach puree after 43 days at 4°C (Wang et al., 2013). Correspondingly, TA values of T1 and T2 did not change for 35 days at 5°C followed by an increase from day 35 to day 49 registering values 0.38 and 0.20 mg citric acid 100⁻¹ g fw higher, respectively, compared to their respective initial levels. As previously observed, microbial growth may be greatly reduced by thermal treatment and subsequent low temperature storage. Microorganisms consume sugars and other soluble solids during growth producing metabolic acidic products. Accordingly, SSC and pH decreased, and TA increased during storage of the smoothie being these changes greatly reduced in thermal-treated samples and during cold storage.

3.6. Colour differences and total chlorophylls and carotenoids contents

Colour is one of the most important quality parameters of smoothies to evaluate its storage quality. It also influences, to a great extent, whether or not consumers prefer the stored product. Green colour of vegetables is mainly due to chlorophylls which may be degraded due to certain degrading-enzymes (chlorophyllase, Mg-dechelatase and POD). The initial L*, a* and b* values of CTRL smoothie were 41.9, -14.0 and 22.7, respectively (data not shown). The initial total chlorophylls content of CTRL smoothie was 58.9 mg kg⁻¹ fw (Table 1) accounting chlorophyll a 82% of total content (data not shown). Meng, Zhang, Zhan and Adhikari (2014) found approximately 75 mg kg⁻¹ fw total chlorophylls content in fresh-cut cucumber. The higher total chlorophylls content of our smoothie may be owed to the spinach contribution which has high chlorophylls content. Total chlorophyll content decreased by 68-64% after heat treatments without
differences among them (Table 1). Accordingly, an initial $\Delta E$ of 4.97-5.02 was observed after thermal treatments without significant differences among them (Table 1). However, milder thermal treatment (72°C for 15 s) reported lower $\Delta E$ value (1.2) in fruit smoothie (Walkling-Ribeiro et al., 2010). Accordingly, $a^*$ value, the most important index evaluating instrumental colour in green vegetables, was increased from -11.2 to -6.5 in spinach puree after heat treatment at 100°C for 60 s (Wang et al., 2013). Storage of CTRL samples either at 15°C or 5°C induced total chlorophyll losses of approximately 26 and 53% after 7 and 21 days at 5°C, respectively (Table 1). Although T1 and T2 samples stored at 5°C showed higher total chlorophylls degradation trends (67-71%), these differences were not significant after 7 days at 5°C regarding CTRL samples. In general, thermally treated samples did not show total chlorophylls changes during storage at 5°C registering similar levels after 49 days compared to their respective initial levels. Accordingly, thermal treatments reduced colour changes during storage since CTRL smoothie registered $\Delta E$ of 9.44 and 9.83 after 7 days at 15°C and 21 days at 5°C, respectively, while T1/T2 registered $\Delta E$ values of 6.47-6.20/7.66-7.46 after 49 days at 5°C. Similarly, spinach puree heat treated at 100°C for 60 s only showed $a^*$ changes of 2.3 while untreated spinach puree reported $a^*$ changes of 6.5 after 43 days at 4°C (Wang et al., 2013). As observed, these heat treatments can reduce colour changes related to chlorophylls levels in the green smoothie due to inactivation of colour-degrading enzymes. Accordingly, great to nearly complete inactivations have been reported in broccoli and spinach puree after similar thermal treatments (Wang et al., 2013; Wang, Wang, Zheng, Hu, Zhang & Liao, 2012).

3.7. Total vitamin C
The initial total vitamin C (ascorbic acid+DHAA) of CTRL smoothie was 731.5 mg kg⁻¹ fw (Figure 2). Ascorbic acid is easily oxidized by the enzymes ascorbate oxidase and ascorbic acid peroxidase to DHAA which exhibits antioxidant properties in addition to antiscorbutic activity equivalent to that of ascorbic acid (Munyaka, Makule, Oey, van Loey & Hendrickx, 2010). During smoothie preparation blending disrupts plant cells allowing enzymes to access their substrates located in different plant cell locations. Accordingly, no ascorbic acid was detected in CTRL or thermally-treated smoothies on processing day due to the rapid ascorbic acid to DHAA enzymatic conversion (data not shown). The ascorbic acid to DHAA conversion was possible since the applied thermal treatments may not completely inactivate the vitamin C oxidative enzymes as previously reported (Munyaka et al., 2010). It is well known that vitamin C is a very thermostable vitamin (Lee & Kader, 2000). Accordingly, heat treatments reduced by approximately 50% the initial total vitamin C level of the smoothie without significant differences among treatments. Lower vitamin C content (430 mg L⁻¹) has been reported in a green smoothie (40% kiwifruits, 7% fennels, 8% spinach and 15% papaya) which was treated at 80ºC for 10 min (Di Cagno et al., 2011).

Total vitamin C losses of 76-87% were observed after 7 days at 15ºC without significant differences among samples (data not shown). However, latter great total vitamin C loss was reduced by 50% when CTRL samples were stored at 5ºC. Similarly, storage of T1 and T2 samples at 5ºC reduced by 1.5 and 2-fold, respectively, the total vitamin C losses (85-91%) observed after 11 days at 15ºC. CTRL and heat-treated samples showed total vitamin C contents of 42.7 and 14.9/15.7 mg kg⁻¹ fw after 21 and 49 days at 5ºC, respectively, without differences among thermal treatments.

According to FAO/WHO, vitamin C intake is required to promote optimal health (FAO/WHO, 2004). A 250 g portion of this smoothie provides approximately 400% of...
the RNIs for vitamin C for adults and 260% for lactating women which is the
population group with the highest RNIs for vitamin C (FAO/WHO, 2004). However,
vitamin C of fruit and vegetables beverages may greatly decrease during storage due to
oxidative and enzymatic degradative processes, among others (Lee & Kader, 2000).
 Accordingly, it is important to predict the vitamin C degradation during the smoothie
storage to know the maximum storage time that ensures the minimum vitamin C RNIs.
Experimental data related to total vitamin C changes during storage at 5°C were well
fitted (R²_{\text{ADJ}} > 95%; Table 2) with the cumulative form of the Weibull distribution (Eq.
1). Calculations were estimated with the GInaFiT application (version 1.6) for
Microsoft Excel (Geeraerd, Valdramidis, & Van Impe, 2005). However

\[ \log_{10} X = \log_{10} X_0 - \left( \frac{t}{\delta} \right)^p \]  

Eq. 1

Where X is the vitamin C content, X_0 is the initial vitamin C content, t is the storage
time (days), \( \delta \) represents the time needed for the first decimal reduction (days) and p is
the shape parameter. Table 2 shows the calculated parameters \( \delta \) and p for the vitamin C
curves determined with the Weibull model. While vitamin C curves of CTRL smoothie
stored at 5°C showed downward concavity (p>1), T1 and T2 samples showed upward
concavity (p<1). Since total vitamin C content did not significantly change during
storage at 15°C and no intermediate data were analyzed between processing and 7th day
of storage these data were not modelled. The maximum storage time at 5°C of a
smoothie portion of 250 g that ensured the minimum vitamin C RNI (45 mg day⁻¹) for
CTRL T1 and T2 samples was 15.2, 10.7 and 10.8 days, respectively. At the end of
CTRL and T1-T2 smoothies shelf lives the vitamin C contents still represented 30 and
10% of the RNIs, respectively. As observed, total vitamin C degradation of thermal-
treated smoothies was higher than CTRL samples. DHAA can be rapidly and
irreversibly hydrolyzed to 2,3-diketogulonoic acid (2,3-DKG) hence losing its antiscorbutic activity (Deutsch 2000). The applied thermal treatments may increase the extraction of those compounds involved in the vitamin C degradation to 2,3-DKG increasing its reaction rates according to the observed reduced vitamin C levels of thermal-treated samples during storage.

3.8. Phenolic compounds

The phenolic compounds of the smoothie were identified by their chromatographic behaviour, UV spectra and HPLC/MS (Supplementary material 4). A characteristic chromatogram of phenolic acids of the smoothie is presented in Figure 3. Initial total phenolic content (calculated as the sum of identified phenolics) of CTRL smoothie was 151.1 mg kg\(^{-1}\) fw (Table 3). Latter total phenolic content is lower than previous reported data for fresh cucumber, the main vegetable of our smoothie (Kaur & Kapoor, 2002). Cucumber peel has approximately 7-fold higher total phenolic content than pulp (Ji, Wu, Gao, Wei, Yang & Guo, 2011). Accordingly, cucumber peel removal during our smoothie preparation led to the observed lower total phenolic content compared to whole cucumber. The main phenolic acids found in the smoothie were 3-p-coumaroyl quinic acid (84.7% relative abundance; sum of both found isomers 3-p-coumaroyl quinic acid (1) and 3-p-coumaroyl quinic acid (2)), chlorogenic acid (7.1%), sinapic acid (3.1%), 1,2,2’-trisinapoylgentiobioside (2.9%), 1-sinapoyl-2-feruloylgentiobioside (1.0%), 1,2’-disinapoyl-2-feruloylgentiobioside (0.9%) and 1,2-disinapoylgentiobioside (0.3%; Table 3). These phenolic compounds have been also previously reported in cucumber, broccoli and spinach (Abu-Reidah, Arráez-Román, Quirantes-Piné, Fernández-Arroyo, Segura-Carretero & Fernández-Gutiérrez, 2012; Bunea et al., 2008; Martínez-Hernández et al., 2011).
The initial total phenolic content of CTRL was increased by 44 and 36% after T1 and T2 treatments, respectively. The apparent increases of these phenolic compounds could be primarily due to the cell membrane and wall ruptures of plant material, releasing phytochemicals from the insoluble portion of the smoothie. That breakdown of plant cell structures increases the pool of phenolics, making them more accessible in the extraction procedure (Martínez-Hernández et al., 2013). The lower phenolic increment of T2 samples may be owed to the lower treatment time which did not produce great cell disruption as observed in T1 samples. Accordingly, the content of the main phenolic acids (3-p-coumaroyl quinic, chlorogenic and sinapic acids) remained unchanged after T2 treatment. The greatest phenolic acids increments after T1/T2 treatments were those corresponding to 1-sinapoyl-2-feruloylgentiobioside, 1,2,2’-trisinapoylgentiobioside, 3-p-coumaroyl quinic acid (1) and 1,2’-disinapoyl-2-feruloylgentiobioside with 153/98, 95/110, 82/94 and 82/83% compared to the respective initial contents of CTRL samples.

Attending to phenolic acids changes during storage, the levels of 3-p-coumaroyl quinic acid (1), 1-sinapoyl-2-feruloylgentiobioside, 1,2,2’-trisinapoylgentiobioside and 1,2’-disinapoyl-2-feruloylgentiobioside smoothies decreased throughout storage for both treatments and storage temperatures registering the greatest losses in the first 7 days of storage. Storage at 5°C of CTRL smoothies greatly reduced the 3-p-coumaroyl quinic acid (2) and 1-sinapoyl-2-feruloylgentiobioside losses of 57 and 65% at 15°C to 16 and 27%, respectively. However, 3-p-coumaroyl quinic acid (1) and 1,2’-disinapoyl-2-feruloylgentiobioside showed the opposite behaviour with losses of 18-57 and 72-70% in those CTRL smoothies stored for 7 days at 15 and 5°C, respectively. On the other side, 1,2,2’-trisinapoylgentiobioside and 1,2-disinapoylgentiobioside levels of CTRL samples decreased by approximately 85 and 33%, respectively, after 7 days.
independently of the storage temperature. Among thermally-treated samples sinapic acid contents did not change after 49 or 7 days at 5 or 15°C, respectively. In general, T1 samples registered 7-fold lower 3-p-coumaroyl quinic acid (1) losses and 1.1-1.6 fold lower losses for 1-sinapoyl-2-feruloylgentiobioside, 1,2,2’-trisinapoylgentiobioside and 1,2’-disinapoyl-2-feruloylgentiobioside regarding T2 samples after 7 days either at 5 or 15°C. Accordingly, among thermal treatment conditions, the lower temperature treatment better retained latter four phenolic acids during storage compared to higher temperature treatment time. 3-p-coumaroyl quinic (2) and sinapic acids did not registered great changes throughout storage in all samples at 5°C or thermally-treated samples stored at 15°C. However, 3-p-coumaroyl quinic (2) and sinapic acids decreased by 57 and 27%, respectively, in CTRL samples stored for 7 days at 15°C. Attending to chlorogenic acid, no significant changes in CTRL samples were observed for 7-11 days at 15 or 5°C. Chlorogenic acid of CTRL samples stored at 5°C increased from day 11 to day 21 by 38%. However, T1 and T2 samples stored at 15°C registered 16 and 26% chlorogenic acid losses after 7 days, respectively. PAL is the key enzyme in the phenols biosynthesis pathway which is activated under abiotic stresses (Cisneros-Zevallos, 2003) such as the wounding produced during smoothie blending. Accordingly, PAL was activated in untreated red smoothies after 10 days of storage at 5°C being this enzyme activation, retarded to 20-30 days either at 5 or 20°C in thermally-treated samples (similar conditions as T1) (Rodriguez-Verástegui et al., 2015). Furthermore, PAL activation of CTRL samples was double of that from heat-treated samples (Rodriguez-Verástegui et al., 2015). Accordingly, the observed chlorogenic acid increment in CTRL samples may be owed to PAL activation. However, a lower PAL activation of heat-treated samples may lead to the observed unchanged levels in T1 and T2 samples stored at 5°C and reduced levels in those samples stored at 15°C as a negative counterbalance.
between chlorogenic acid biosynthesis and its degradation at this high storage temperature. As it has been previously reviewed the changes of the phenolic profile of fruit blends during storage greatly depend on the phenolic compound and storage conditions as also observed in our smoothie data (Chen, Yu & Rupasinghe, 2013).

Conclusively, phenolic contents increased after thermal treatment, in a greater extend in T1 samples, being this phenolic increment associated with subsequent enhanced bioaccessibility in the gastrointestinal tract (Bugianesi et al., 2004). In general, phenolic levels decreased during storage, except chlorogenic and sinapic acids, registering the greatest losses in the first 7-11 days showing T1 samples lower degradation rates.

3.9. Total antioxidant capacity (TAC)

The initial TAC of CTRL smoothie obtained by FRAP, ABTS and DPPH were 234.2, 395.7 and 54.4 mg Trolox equivalents kg\(^{-1}\) fw, respectively (Table 4). Phenolic compounds are the major contributors to the antioxidant properties of fresh produce (Cisneros-Zevallos, 2003). Antioxidant capacity of a food product may greatly differ depending on the analytical method used (Prior, Wu & Schaich, 2005). Accordingly, a Pearson correlation using total phenolic content and TAC data during storage was used to determine which TAC method was better correlated to total phenolic content. FRAP method achieved the best correlations \(r^2=0.67\) closely followed by ABTS \(r^2=0.53\). Consequently, only FRAP data is discussed.

Contrary to the apparent increase of total phenolic content after thermal treatment, TAC did not register significant differences after heating. Similarly, Keenan, Brunton, Gormley, Butler, Tiwari, and Patras (2010) did not find significant TAC changes after heat treatment (70°C for 10 min) of a fruit smoothie while total phenolic content increased. Vitamin C also plays an important contribution to the TAC of the smoothie.
Accordingly, the unchanged TAC may be a result of the above described vitamin C reduction after thermal treatment. The TAC levels of all samples remained quite constant throughout storage at both temperatures, except CTRL samples stored at 5°C which showed a TAC decrease of 45% after 11 days followed by an increase registering final levels of 174.4 mg Trolox equivalents kg⁻¹ fw. Similarly, Keenan et al. (2010) reported TAC decreases in heat-treated (70°C for 10 min) fruit smoothies after 10 days at 4°C. Accordingly, thermal treatments avoided TAC losses of the smoothie during storage at both temperatures probably due to the heat inactivation of enzymes involved in the degradation of antioxidant compounds.

3.10. Intact glucosinolates

The glucosinolates of the smoothie were identified by their chromatographic behaviour, UV spectra and HPLC/MS (Supplementary material 4). Figure 4 shows a characteristic chromatogram of intact glucosinolates of the smoothie. The glucosinolates found from higher to lower amounts (mg kg⁻¹ fw) were glucobrassicin (95.25) > 4-hydroxyglucobrassicin (16.96) > glucoraphanin (4.48) > 4-methoxyglucobrassicin (1.12) (Table 5). Neoglucobrassicin and proigoitrin contents were lower than 0.01 mg kg⁻¹ fw (data not shown). The initial total glucosinolate content of CTRL samples was 128.77 mg kg⁻¹. This is in accordance with the reported glucosinolate content range of broccoli (110-340 mg kg⁻¹) since it can greatly vary (up to 3 fold) depending of seasons, cultivars and inflorescences (primary or secondary) (Hanschen, Lamy, Schreiner & Rohn, 2014; Rosa and Rodrigues, 2001).

Total glucosinolate content was not affected by any of the thermal treatments. However, different patterns were observed among individual glucosinolates. Isothiocyanates are
the biologically active breakdown products from glucosinolates which lack of those chemopreventive properties. However, the presence of the ephithiospecifier protein (ESP), among other factors, may lead to other breakdown products different from isothiocyanates. The thermal treatments applied may ensure the thermal degradation of ESP since this protein was completely inactivated in broccoli florets after 70°C for 5 min (Matusheski, Juvik, & Jeffery, 2004), being the ESP inactivation probably even enhanced in our smoothie due to better heat transmission. Regarding to structure-activity relationships, it was generally observed that glucosinolates with a hydroxyl function in the side chain are more labile compared to their corresponding non-hydroxylated relatives (Hanschen et al., 2014). Accordingly, while glucobrassicin content did not change for any of the thermal treatments, 4-hydroxyglucobrassicin was reduced by 29% after T1 treatment. Furthermore, T1 treatment induced 4-methoxyglucobrassicin decrease of 49%. T2 treatment did not induce significant changes among glucosinolates contrary to T1. Glucoraphanin content of the smoothie increased by 36% after T1 treatment. It has been widely reported that aliphatic glucosinolates (such as glucoraphanin) are more heat stable than indole glucosinolates (such as glucobrassicin 4-hydroxyglucobrassicin and 4-methoxyglucobrassicin) for temperature treatments below 110°C (Hanschen et al., 2014). Therefore, the apparent glucoraphanin increment may be owed to a better heat stability of this glucosinolate together with an enhanced extractability of this compound during T1, the longest treatment.

Glucosinolates contents of CTRL samples did not change after 7 days at 5°C, except 4-hydroxyglucobrassicin which decreased by 41%. However, storage of CTRL samples at 15°C for 7 days induced 58, 65 and 84% decreases of 4-hydroxyglucobrassicin, glucoraphanin and 4-methoxyglucobrassicin, respectively, although glucoraphanin was
preserved. Similarly, Rangkadilok et al. (2002) reported a 50% decrease in glucoraphanin in ‘Marathon’ heads after 7 days at 15°C, but no decrease after 7 days at 4°C. The breakdown of glucosinolates by myrosinase is usually a very rapid event which is greatly enhanced after mechanical homogenisation such as smoothie preparation (Verkerk, Van der Gaag, Dekker & Jongen, 1997). In agreement to our data, myrosinase activity of ‘Marathon’ heads was probably greatly reduced at 4°C while it was enhanced at 15°C. Glucosinolates levels from day 7 to day 21 did not change at 5°C except glucoraphanin that greatly decreased by 81%. Similarly, glucoraphanin showed the greatest losses among glucosinolates in broccoli florets stored at 4°C (Verkerk, Dekker & Jongen, 2001).

Regarding to thermal-treated samples, no great glucosinolates changes were observed after 11 days at 5°C except 4-hydroxyglucobrassicin of T2 samples that, similarly to CTRL samples, decreased by 43%. The observed 4-hydroxyglucobrassicin reduction may be owed to the commented higher degradation of these glucosinolates with a hydroxyl function. From day 11 to the end of storage at 5°C, low glucosinolates losses were observed in those thermal-treated samples (<26%). In the same way, no great glucosinolates losses (<30%) were observed in those T1 and T2 samples stored at 15°C for 7 days. Van Eylen, Oey, Hendrickx and Van Loey (2007) reported a residual myrosinase activity of 23% in broccoli juice treated at 60°C for 3 min. Accordingly, the low glucosinolates losses of thermal-treated samples during storage at low temperature may be owed to a complete myrosinase inactivation.

Conclusively, short-time/high temperature treatment (T2) did not induce individual glucosinolates losses regarding T1 samples (<49% losses). The glucosinolates degradation observed in CTRL samples during storage was greatly reduced in both thermal-treated samples.
4. Conclusions
This study presents a green fresh vegetables smoothie with excellent nutritional, microbial and physicochemical quality during a shelf life of 49 days at 5°C. Mild thermal treatments were necessary during processing to preserve its quality achieving T2 (45 s at 90°C) better microbial reductions and health-promoting compounds preservation (related to phenolics and glucosinolates contents). Furthermore, low temperature storage at 5°C is recommended to preserve quality and safety. A 250-g portion of this green smoothie can highly cover the established recommended daily nutrient intakes for dietary fibre, minerals and vitamin C of different population groups.

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REFERENCE LIST


FIGURE AND TABLE CAPTIONS

Table 1. pH, soluble solids content (SSC), titratable acidity (TA), total colour differences ($\Delta E$) and total chlorophylls content of untreated (CTRL) and heat-treated (T1 and T2) green vegetables smoothies stored at 5 and 15ºC (n=5±SD). Different capital letters denote significant differences ($P \leq 0.05$) among treatments stored at the same temperature for the same sampling day. Different lowercase letters denote significant differences ($P \leq 0.05$) among sampling days for the same treatment stored at the same temperature.

Table 2. Estimates of Weibullian distribution parameters $\delta$ and $p$ and adjusted $R^2$ for vitamin C content changes in untreated (CTRL) and heat-treated (T1 and T2) green vegetables smoothies during storage at 5ºC.

Table 3. Phenolic contents of untreated (CTRL) and heat-treated (T1 and T2) green vegetables smoothies stored at 5 and 15ºC (n=5±SD). Different capital letters denote significant differences ($P \leq 0.05$) among treatments stored at the same temperature for the same sampling day. Different lowercase letters denote significant differences ($P \leq 0.05$) among sampling days for the same treatment stored at the same temperature.

Table 4. Total antioxidant capacity (FRAP, ABTS and DPPH methods) of untreated (CTRL) and heat-treated (T1 and T2) green vegetables smoothies stored at 5 and 15ºC (n=5±SD). Different capital letters denote significant differences ($P \leq 0.05$) among treatments stored at the same temperature for the same sampling day. Different lowercase letters denote significant differences ($P \leq 0.05$) among sampling days for the same treatment stored at the same temperature.
Table 5. Intact glucosinolates contents of untreated (CTRL) and heat-treated (T1 and T2) green vegetables smoothies stored at 5 and 15°C (n=5±SD). Different capital letters denote significant differences ($P \leq 0.05$) among treatments stored at the same temperature for the same sampling day. Different lowercase letters denote significant differences ($P \leq 0.05$) among sampling days for the same treatment stored at the same temperature.

Figure 1. Mesophilic (A), psychrophilic (B), Enterobacteria (C) and yeast and moulds (D) counts (log CFU g$^{-1}$) of untreated (CTRL) and heat-treated (T1 and T2) green vegetables smoothies stored at 5 and 15°C (n=5±SD). Different capital letters denote significant differences ($P \leq 0.05$) among treatments stored at the same temperature for the same sampling day. Different lowercase letters denote significant differences ($P \leq 0.05$) among sampling days for the same treatment stored at the same temperature.

Figure 2. Total vitamin C (logarithmic scale) in untreated (CTRL) and heat-treated (T1 and T2) green vegetables smoothies stored at 5°C (n=5±SD). Experimental (points) and fitted values are derives from the Weibullian model (lines).

Figure 3. HPLC-PAD chromatogram of phenolic acids profile of green vegetables smoothie.

Figure 4. HPLC-PAD chromatogram of intact glucosinolates profile of green vegetables smoothie.
Supplementary material 1. Nutritional content of green vegetables smoothie.

Supplementary material 2. Evolution of the storage and loss moduli with frequency (A) and viscous flow curves (B) at 25°C of green vegetables smoothie.

Supplementary material 3. Thermogravimetric and thermogravimetric-derivative curves of green vegetables smoothie.

Supplementary material 4. List of identified phenolic acids and glucosinolates of green vegetables smoothie with the corresponding retention times, maximum UV absorption (UPLC/DAD) and MS data.