HEALTH-PROMOTING COMPOUNDS CHANGES OF A GREEN FRESH VEGETABLES SMOOTHIE DURING SHELF LIFE

Running title: Bioactive compounds of a green smoothie during shelf life

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Abstract

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 80ºC) and T2 (45 s at 90ºC). 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 15ºC. Colour and physicochemical changes were

reduced in thermal-treated samples throughout storage, which were better preserved at 5ºC rather than at 15ºC. Vitamin C changes during storage were fitted with a Weibullian distribution. Total vitamin C losses of T1 and T2 samples during storage at 15ºC were greatly reduced when they were stored at 5ºC. 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 5ºC 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 15ºC.

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±2ºC) with a heating rate of 30ºC/min to 80ºC, then maintained for 3 min and cooled down to a final temperature of 40ºC (heating rate of 30ºC/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

Artés-Hernández (2016). Rheological data is presented as Supplementary material 2. 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 ºBrix, respectively. Total colour differences

151 (ΔE) throughout storage compared to their respective initial values were calculated
152 according to equations previously described (Walkling-Ribeiro, Noci, Cronin, Lyng &
153 Morgan, 2010).
154
155 **2.7. Chlorophylls content**
156 Sample preparation for total chlorophyll determination was conducted according to
157 Martínez-Hernández, Gómez, Pradas, Artés and Artés-Hernández (2011). An UV-
158 visible spectrophotometer (8453, Hewlet Packard, Columbia, USA) was used to
159 registered absorbances at 662 and 644 nm. The equations developed by Wellburn
160 (1994) were used to determine chlorophyll a (Cha = 10.05×A662 – 0.766×A644) and
161 chlorophyll b (Ch b = 16.37×A644 – 3.14×A662). Then, total chlorophyll content was
162 expressed as the sum of Cha and Chb (Ca + Cb). Chlorophyll content was expressed as
163 mg kg^{-1} fw. Each of the five replicates was analysed by duplicate.
164
165 **2.8. Bioactive compounds**
166 **2.8.1. Vitamin C**
167 The ascorbic (AA) and dehydroascorbic (DHA) acids were measured according to the
168 method of Zapata and Dufour (1992) with modifications from Martínez-Hernández,
169 Artés-Hernández, Colares-Souza, Gómez, García-Gómez and Artés (2013). Derivatized
170 samples (20 μL) were injected onto a Gemini NX (250 mm×4.6 mm, 5 μm) C18 column
171 (Phenomenex, Torrance CA, USA), using an HPLC (Series 1100 Agilent Technologies,
172 Waldbronn, Germany) equipped with a G1322A degasser, G1311A quaternary pump,
173 G1313A autosampler, G1316A column heater and G1315B photodiode array detector.
174 The HPLC system was controlled by the software ChemStation Agilent, v. 08.03. AA
175 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

225 Total antioxidant capacity (TAC) extraction and analysis were conducted according to
226 Rodríguez-Verástegui et al. (2015) using three different methods: free radical
227 scavenging capacity with 2,2-diphenyl-1-picrylhydrazil (DPPH) (Brand-Williams,
228 Cuvelier & Berset, 1995), ferric reducing antioxidant power (FRAP) (Benzie & Strain,
229 1999) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (Cano,
230 Hernández-Ruíz, García-Cánovas, Acosta & Arnao, 1998). All TAC data were
231 expressed as mg of Trolox equivalents kg^{-1} fw. Each of the five replicates was analysed
232 by duplicate.
233
234 **2.9. Statistical Analysis**
235 The experiment was a two-factor (treatment×storage time) design subjected to analysis
236 of variance (ANOVA) using Statgraphics Plus software (vs. 5.1, Statpoint Technologies
237 Inc., Warrenton, USA). Statistical significance was assessed at the level P=0.05, and
238 Tukey's multiple range test was used to separate means.
239
240 **3. Results and discussion**
241 **3.1. Rheological properties**
242 The texture of a smoothie has to provide a balance between desired mechanical stability
243 (for storage and handling) and desired instability (to elicit a specific texture attribute
244 during mastication). Rheological properties are useful in determining the most
245 ingredients proportions in the product development, quality control, and correlation of
246 food texture to sensory attributes. Smoothies are viscoelastic food materials that exhibit
247 both solid-like and fluid-like behaviour. The rheological properties of the smoothie are
248 presented as Supplementary material 2. The storage modulus (G') of smoothies was
249 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 tanδ 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 tanδ 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

275 (data not shown). A 250 g portion of this smoothie provides 29-34, 9-15, 7-12 and 15-
276 21% of the RNIs for Mg, Ca, Fe and Zn, respectively, covering population groups with
277 special nutritional requirements such as elders, pregnant women or adolescents
278 (FAO/WHO, 2004).
279
280 **3.4. Microbial analysis**
281 Microbial load is a major quality criterion to determine the shelf life of food products.
282 The unit operations of the smoothie processing, which includes several injury stresses
283 such as peeling, cutting, shredding or blending greatly increase the risk of microbial
284 development. The mesophilic load of CTRL smoothie on processing day was 4.4 log
285 CFU g^{-1} (Figure 1A). Thermal treatments reduced mesophilic load by 1.7-1.8 log units
286 without differences among them. Zhao et al. (2014) reported 2 log units mesophilic
287 reductions in cucumber juice heat treated at 85ºC for 15 s. The similar microbial
288 reduction in the juice with shorter treatment time may be owed to the fibres and other
289 particles contained in the smoothie which may difficult the heat transmission contrary to
290 the juice. However, Walkling-Ribero et al. (2010) reported higher mesophilic reductions
291 (3.5 log units) in a fruit smoothie treated with a milder heat treatment (70ºC for 15 s).
292 The dynamic system used by Walkling-Ribero et al. (2010) during heat treatment
293 compared to our static system may explain the better microbial reductions achieved by
294 those authors. Great mesophilic increases of 4.7 and 5.2 log units were observed in
295 CTRL and thermal-treated smoothies after 7 days at 15ºC, respectively. However,
296 storage at 5ºC greatly reduced mesophilic growth of CTRL and heat-treated samples
297 compared to those stored at 15ºC since increments of 3.6-3.8 and 0.7 log units were
298 registered after 21 days at 5ºC. Heat-treated samples stored at 5ºC showed a great
299 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.

325 Initial *Enterobacteriaceae* counts of 3.8 log CFU g^{-1} were only significantly reduced
326 after T2 treatment by 1.6 log units (Figure 1C). The *Enterobacteriaceae* levels of CTRL
327 and T1 samples stored at 15ºC increased by 2.2-2.3 after 7 days while T2 samples
328 increased in a greater extend with 3.5 log units increments after 7 days. CTRL and
329 thermal-treated samples registered *Enterobacteriaceae* counts of 5.0 and 4.6-4.8 log
330 CFU g^{-1} after 21 and 49 days of at 5ºC, respectively.
331 Yeasts and moulds counts of CTRL smoothie of 3.9 log CFU g^{-1} were reduced by 1.3-
332 1.4 log units after heat treatments without significant differences among them (Figure
333 1D). Similarly to mesophiles, dynamic heating system during a milder heat treatment
334 (70ºC for 15 s) induced greater yeasts and moulds reductions (3.7 log units) in a fruit
335 smoothie (Walkling-Ribero et al., 2010). Furthermore, data from Zhao et al. (2014)
336 showed better heat transmission in cucumber juice compared to our smoothie since
337 initial yeasts and moulds (4 log CFU g^{-1}) were greatly reduced below the detection limit
338 (1 log CFU g^{-1}). Yeasts and moulds counts of CTRL and T2 samples were incremented
339 by 3.6 and 3.4 log units, respectively, after 7 days at 15ºC while T1 samples only
340 increased in 0.5 log units. Similarly to mesophilic and psychrophilic, yeasts and moulds
341 counts greatly augmented by 2.5 and 2.8 log units after 11 days followed by a 1.3-1.4
342 log units increment in the last 38 days of storage at 5ºC. CTRL and thermal-treated
343 samples showed final yeasts and moulds counts of 5.4 and 6.5-6.6 log CFU g^{-1} after 21
344 and 49 days at 5ºC, respectively.
345 Conclusively, thermal treatments, with better initial microbial reductions achieved by
346 T2, combined with low temperature storage, kept microbial loads below 6 log units after
347 39 days at 5ºC. Although CTRL samples showed a similar microbial behaviour to heat-
348 treated samples during low temperature storage, thermal treatment is needed to
349 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 ºBrix (Table 1). Di Cagno et al. (2011) reported a higher SSC of 10.8 ºBrix 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 15ºC. Contrary, CTRL smoothie showed a SSC increase of 0.7 ºBrix after 7 days at 5ºC followed by a decrease. Accordingly, CTRL smoothie registered 4.6 ºBrix after 21 days at 5ºC with no differences regarding its respective initial level. SSC levels of T1 and T2 smoothies remained stable up to 35 days of storage at 5ºC followed by a decrease of 0.9 and 0.7 ºBrix, 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 15ºC 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 15ºC while T2 did not

375 register differences during this period. No great pH and TA changes in CTRL samples
376 were observed after 21 days at 5ºC. The pH of thermal-treated samples was constant
377 throughout storage at both storage temperatures. Similarly, no pH changes were
378 observed in untreated and heat treated (100ºC for 60 s) spinach puree after 43 days at
379 4ºC (Wang et al., 2013). Correspondingly, TA values of T1 and T2 did not change for
380 35 days at 5ºC followed by an increase from day 35 to day 49 registering values 0.38
381 and 0.20 mg citric acid 100^{-1} g fw higher, respectively, compared to their respective
382 initial levels. As previously observed, microbial growth may be greatly reduced by
383 thermal treatment and subsequent low temperature storage. Microorganisms consume
384 sugars and other soluble solids during growth producing metabolic acidic products.
385 Accordingly, SSC and pH decreased, and TA increased during storage of the smoothie
386 being these changes greatly reduced in thermal-treated samples and during cold storage.
387

388 **3.6. Colour differences and total chlorophylls and carotenoids contents**

389 Colour is one of the most important quality parameters of smoothies to evaluate its
390 storage quality. It also influences, to a great extent, whether or not consumers prefer the
391 stored product. Green colour of vegetables is mainly due to chlorophylls which may be
392 degraded due to certain degrading-enzymes (chlorophyllase, Mg-dechelatase and POD).
393 The initial L*, a* and b* values of CTRL smoothie were 41.9, -14.0 and 22.7,
394 respectively (data not shown). The initial total chlorophylls content of CTRL smoothie
395 was 58.9 mg kg^{-1} fw (Table 1) accounting chlorophyll a 82% of total content (data not
396 shown). Meng, Zhang, Zhan and Adhikari (2014) found approximately 75 mg kg^{-1} fw
397 total chlorophylls content in fresh-cut cucumber. The higher total chlorophylls content
398 of our smoothie may be owed to the spinach contribution which has high chlorophylls
399 content. Total chlorophyll content decreased by 68-64% after heat treatments without

differences among them (Table 1). Accordingly, an initial ΔE of 4.97-5.02 was observed after thermal treatments without significant differences among them (Table 1). However, milder thermal treatment (72ºC for 15 s) reported lower ΔE 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 100ºC for 60 s (Wang et al., 2013).

Storage of CTRL samples either at 15ºC or 5ºC induced total chlorophyll losses of approximately 26 and 53% after 7 and 21 days at 5ºC, respectively (Table 1). Although T1 and T2 samples stored at 5ºC showed higher total chlorophylls degradation trends (67-71%), these differences were not significant after 7 days at 5ºC regarding CTRL samples. In general, thermally treated samples did not show total chlorophylls changes during storage at 5ºC registering similar levels after 49 days compared to their respective initial levels. Accordingly, thermal treatments reduced colour changes during storage since CTRL smoothie registered ΔE of 9.44 and 9.83 after 7 days at 15ºC and 21 days at 5ºC, respectively, while T1/T2 registered ΔE values of 6.47-6.20/7.66-7.46 after 49 days at 5ºC. Similarly, spinach puree heat treated at 100ºC for 60 s only showed a* changes of 2.3 while untreated spinach puree reported a* changes of 6.5 after 43 days at 4ºC (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 did 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 thermolabile 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

450 the RNIs for vitamin C for adults and 260% for lactating women which is the
451 population group with the highest RNIs for vitamin C (FAO/WHO, 2004). However,
452 vitamin C of fruit and vegetables beverages may greatly decrease during storage due to
453 oxidative and enzymatic degradative processes, among others (Lee & Kader, 2000).
454 Accordingly, it is important to predict the vitamin C degradation during the smoothie
455 storage to know the maximum storage time that ensures the minimum vitamin C RNIs.
456 Experimental data related to total vitamin C changes during storage at 5ºC were well
457 fitted (R^2_{ADJ} > 95%; Table 2) with the cumulative form of the Weibull distribution (Eq.
458 1). Calculations were estimated with the GInaFiT application (version 1.6) for
459 Microsoft Excel (Geeraerd, Valdramidis, & Van Impe, 2005). However

460 $$log_{10}X = log_{10}X_0 - \left(\frac{t}{\delta}\right)^p \quad \text{Eq. 1}$$

461

462 Where X is the vitamin C content, X_0 is the initial vitamin C content, *t* is the storage
463 time (days), δ represents the time needed for the first decimal reduction (days) and *p* is
464 the shape parameter. Table 2 shows the calculated parameters δ and *p* for the vitamin C
465 curves determined with the Weibull model. While vitamin C curves of CTRL smoothie
466 stored at 5ºC showed downward concavity ($p>1$), T1 and T2 samples showed upward
467 concavity ($p<1$). Since total vitamin C content did not significantly change during
468 storage at 15ºC and no intermediate data were analyzed between processing and 7th day
469 of storage these data were not modelled. The maximum storage time at 5ºC of a
470 smoothie portion of 250 g that ensured the minimum vitamin C RNI (45 mg day^{-1}) for
471 CTRL T1 and T2 samples was 15.2, 10.7 and 10.8 days, respectively. At the end of
472 CTRL and T1-T2 smoothies shelf lives the vitamin C contents still represented 30 and
473 10% of the RNIs, respectively. As observed, total vitamin C degradation of thermal-
474 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).

500 The initial total phenolic content of CTRL was increased by 44 and 36% after T1 and
501 T2 treatments, respectively. The apparent increases of these phenolic compounds could
502 be primarily due to the cell membrane and wall ruptures of plant material, releasing
503 phytochemicals from the insoluble portion of the smoothie. That breakdown of plant
504 cell structures increases the pool of phenolics, making them more accessible in the
505 extraction procedure (Martínez-Hernández et al., 2013). The lower phenolic increment
506 of T2 samples may be owed to the lower treatment time which did not produce great
507 cell disruption as observed in T1 samples. Accordingly, the content of the main
508 phenolic acids (3-p-coumaroyl quinic, chlorogenic and sinapic acids) remained
509 unchanged after T2 treatment. The greatest phenolic acids increments after T1/T2
510 treatments were those corresponding to 1-sinapoyl-2-feruloylgentiobioside, 1,2,2'-
511 trisinapoylgentiobioside, 3-p-coumaroyl quinic acid (1) and 1,2'-disinapoyl-2-
512 feruloylgentiobioside with 153/98, 95/110, 82/94 and 82/83% compared to the
513 respective initial contents of CTRL samples.
514 Attending to phenolic acids changes during storage, the levels of 3-p-coumaroyl quinic
515 acid (1), 1-sinapoyl-2-feruloylgentiobioside, 1,2,2'-trisinapoylgentiobioside and 1,2'-
516 disinapoyl-2-feruloylgentiobioside smoothies decreased throughout storage for both
517 treatments and storage temperatures registering the greatest losses in the first 7 days of
518 storage. Storage at 5ºC of CTRL smoothies greatly reduced the 3-p-coumaroyl quinic
519 acid (2) and 1-sinapoyl-2-feruloylgentiobioside losses of 57 and 65% at 15ºC to 16 and
520 27%, respectively. However, 3-p-coumaroyl quinic acid (1) and 1,2'-disinapoyl-2-
521 feruloylgentiobioside showed the opposite behaviour with losses of 18-57 and 72-70%
522 in those CTRL smoothies stored for 7 days at 15 and 5ºC, respectively. On the other
523 side, 1,2,2'-trisinapoylgentiobioside and 1,2-disinapoylgentiobioside levels of CTRL
524 samples decreased by approximately 85 and 33%, respectively, after 7 days

525 independently of the storage temperature. Among thermally-treated samples sinapic
526 acid contents did not changed after 49 or 7 days at 5 or 15ºC, respectively. In general,
527 T1 samples registered 7-fold lower 3-p-coumaroyl quinic acid (1) losses and 1.1-1.6
528 fold lower losses for 1-sinapoyl-2-feruloylgentiobioside, 1,2,2'-trisinapoylgentiobioside
529 and 1,2'-disinapoyl-2-feruloylgentiobioside regarding T2 samples after 7 days either at
530 5 or 15ºC. Accordingly, among thermal treatment conditions, the lower temperature
531 treatment better retained latter four phenolic acids during storage compared to higher
532 temperature treatment time. 3-p-coumaroyl quinic (2) and sinapic acids did not
533 registered great changes throughout storage in all samples at 5ºC or thermally-treated
534 samples stored at 15ºC. However, 3-p-coumaroyl quinic (2) and sinapic acids decreased
535 by 57 and 27%, respectively, in CTRL samples stored for 7 days at 15ºC. Attending to
536 chlorogenic acid, no significant changes in CTRL samples were observed for 7-11 days
537 at 15 or 5ºC. Chlorogenic acid of CTRL samples stored at 5ºC increased from day 11 to
538 day 21 by 38%. However, T1 and T2 samples stored at 15ºC registered 16 and 26%
539 chlorogenic acid losses after 7 days, respectively. PAL is the key enzyme in the phenols
540 biosynthesis pathway which is activated under abiotic stresses (Cisneros-Zevallos,
541 2003) such as the wounding produced during smoothie blending. Accordingly, PAL was
542 activated in untreated red smoothies after 10 days of storage at 5ºC being this enzyme
543 activation, retarded to 20-30 days either at 5 or 20ºC in thermally-treated samples
544 (similar conditions as T1) (Rodríguez-Verástegui et al., 2015). Furthermore, PAL
545 activation of CTRL samples was double of that from heat-treated samples (Rodríguez-
546 Verástegui et al., 2015). Accordingly, the observed chlorogenic acid increment in CTRL
547 samples may be owed to PAL activation. However, a lower PAL activation of heat-
548 treated samples may lead to the observed unchanged levels in T1 and T2 samples stored
549 at 5ºC and reduced levels in those samples stored at 15ºC 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 of 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 of 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 (70ºC 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.

575 Accordingly, the unchanged TAC may be a result of the above described vitamin C
576 reduction after thermal treatment.
577 The TAC levels of all samples remained quite constant throughout storage at both
578 temperatures, except CTRL samples stored at 5ºC which showed a TAC decrease of
579 45% after 11 days followed by an increase registering final levels of 174.4 mg Trolox
580 equivalents kg^{-1} fw. Similarly, Keenan et al. (2010) reported TAC decreases in heat-
581 treated (70ºC for 10 min) fruit smoothies after 10 days at 4ºC. Accordingly, thermal
582 treatments avoided TAC losses of the smoothie during storage at both temperatures
583 probably due to the heat inactivation of enzymes involved in the degradation of
584 antioxidant compounds.
585
586 **3.10. Intact glucosinolates**
587 The glucosinolates of the smoothie were identified by their chromatographic behaviour,
588 UV spectra and HPLC/MS (Supplementary material 4). Figure 4 shows a characteristic
589 chromatogram of intact glucosinolates of the smoothie. The glucosinolates found from
590 higher to lower amounts (mg kg^{-1} fw) were glucobrassicin (95.25) > 4-
591 hydroxyglucobrassicin (16.96) > glucoraphanin (4.48) > 4-methoxyglucobrassicin
592 (1.12) (Table 5). Neoglucobrassicin and proigoitrin contents were lower than 0.01 mg
593 kg^{-1} fw (data not shown). The initial total glucosinolate content of CTRL samples was
594 128.77 mg kg^{-1}. This is in accordance with the reported glucosinolate content range of
595 broccoli (110-340 mg kg^{-1}) since it can greatly vary (up to 3 fold) depending of seasons,
596 cultivars and inflorescences (primary or secondary) (Hanschen, Lamy, Schreiner &
597 Rohn, 2014; Rosa and Rodrigues, 2001).
598 Total glucosinolate content was not affected by any of the thermal treatments. However,
599 different patterns were observed among individual glucosinolates. Isothiocyanates are

600 the biologically active breakdown products from glucosinolates which lack of those
601 chemopreventive properties. However, the presence of the ephithiospecifier protein
602 (ESP), among other factors, may lead to other breakdown products different from
603 isothiocyanates. The thermal treatments applied may ensure the thermal degradation of
604 ESP since this protein was completely inactivated in broccoli florets after 70ºC for 5
605 min (Matusheski, Juvik, & Jeffery, 2004), being the ESP inactivation probably even
606 enhanced in our smoothie due to better heat transmission. Regarding to structure-
607 activity relationships, it was generally observed that glucosinolates with a hydroxyl
608 function in the side chain are more labile compared to their corresponding non-
609 hydroxylated relatives (Hanschen et al., 2014). Accordingly, while glucobrassicin
610 content did not change for any of the thermal treatments, 4-hydroxyglucobrassicin was
611 reduced by 29% after T1 treatment. Furthermore, T1 treatment induced 4-
612 methoxyglucobrassicin decrease of 49%. T2 treatment did not induce significant
613 changes among glucosinolates contrary to T1. Glucoraphanin content of the smoothie
614 increased by 36% after T1 treatment. It has been widely reported that aliphatic
615 glucosinolates (such as glucoraphanin) are more heat stable than indole glucosinolates
616 (such as glucobrassicin 4-hydroxyglucobrassicin and 4-methoxyglucobrassicin) for
617 temperature treatments below 110ºC (Hanschen et al., 2014). Therefore, the apparent
618 glucoraphanin increment may be owed to a better heat stability of this glucosinolate
619 together with an enhanced extractability of this compound during T1, the longest
620 treatment.
621 Glucosinolates contents of CTRL samples did not change after 7 days at 5ºC, except 4-
622 hydroxyglucobrassicin which decreased by 41%. However, storage of CTRL samples at
623 15ºC for 7 days induced 58, 65 and 84% decreases of 4-hydroxyglucobrassicin,
624 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 15ºC, but no decrease after 7 days at 4ºC. 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 4ºC while it was enhanced at 15ºC. Glucosinolates levels from day 7 to day 21 did not change at 5ºC except glucoraphanin that greatly decreased by 81%. Similarly, glucoraphanin showed the greatest losses among glucosinolates in broccoli florets stored at 4ºC (Verkerk, Dekker & Jongen, 2001).

Regarding to thermal-treated samples, no great glucosinolates changes were observed after 11 days at 5ºC 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 5ºC, 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 15ºC for 7 days. Van Eylen, Oey, Hendrickx and Van Loey (2007) reported a residual myrosinase activity of 23% in broccoli juice treated at 60ºC 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 5ºC. Mild thermal treatments were necessary during processing to preserve its quality achieving T2 (45 s at 90ºC) better microbial reductions and health-promoting compounds preservation (related to phenolics and glucosinolates contents). Furthermore, low temperature storage at 5ºC 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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FIGURE AND TABLE CAPTIONS

Table 1. pH, soluble solids content (SSC), titratable acidity (TA), total colour differences (ΔE) 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 δ 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.

867

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

874

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

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

885

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

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889 **Figure 4.** HPLC-PAD chromatogram of intact glucosinolates profile of green
890 vegetables smoothie.

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892 **SUPPLEMENTARY MATERIAL**

893 **Supplementary material 1.** Nutritional content of green vegetables smoothie.

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

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

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

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