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Title: EFFECTS OF FREEZING, FREEZE DRYING AND CONVECTIVE DRYING ON IN VITRO GASTRIC DIGESTION OF APPLES

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Keywords: in vitro gastric digestion; food processing; mathematical model; polyphenol content; antioxidant activity; microstructure

Corresponding Author: Dr. Susana Simal, Dr.

Corresponding Author's Institution: University of the Balearic Islands

First Author: María E Dalmau, phD

Order of Authors: María E Dalmau, phD; Gail M Bornhorst, Dr.; Valeria S Eim, Dr.; Carmen Rosselló, Dr.; Susana Simal, Dr.

Abstract: The influence of processing (freezing at -196°C in liquid N2, FN sample; freeze-drying at -50oC and 30 Pa, FD sample; and convective drying at 60oC and 2 m/s, CD sample) on apple (var. Granny Smith) behavior during in vitro gastric digestion was investigated. Dried apples (FD and CD samples) were rehydrated prior to digestion. Changes in carbohydrate composition, moisture, soluble solids, acidity, total polyphenol content (TPC), and antioxidant activity (AA) of apple samples were measured at different times during digestion. Processing resulted in disruption of the cellular structure during digestion, as observed by scanning electron microscopy, light microscopy, and changes in carbohydrate composition. Moisture content increased (6-11% dmo), while soluble solids (55-78% dmo), acidity (44-72% dmo), total polyphenol content (30-61% dmo), and antioxidant activity (41-87%) decreased in all samples after digestion. Mathematical models (Weibull and exponential models) were used to better evaluate the influence of processing on apple behavior during gastric digestion.

S. Simal
Department of Chemistry
University of the Balearic Islands
Palma, 9/07/2015

Sian Astley

Editor

Food Chemistry

Paper: **Effects of freezing, freeze drying and convective drying on in vitro gastric digestion of apples** (FOODCHEM-D-15-03458)

Dear Prof. Astley,

We are in agreement with most of the comments suggested by the reviewers and editor, we hope that this new version is now in accord with your requirements.

We have carried out the modifications suggested. Detailed lists of each reviewer suggestion are added together with our comments.

Yours sincerely,

Susana Simal.

Detailed Responses to Reviewers

Reviewer 2:

Comment 1: Concerning figures and tables, they are relevant but I suggest to remove pictures of light microscopy as it is really difficult to see something.

The pictures of light microscopy have been improved in order to appreciate better the differences

Comment 2: Lines 98-99: "Samples were dried until they reached a final moisture content of 0.20±0.03 kg water/kg dm." How long?

Figure 1 show the experimental drying curve (performed in triplicate) of apple at 60 °C and 2 m/s of air flow. As it can be observed, air-drying time was 136.0 ± 0.8 min.

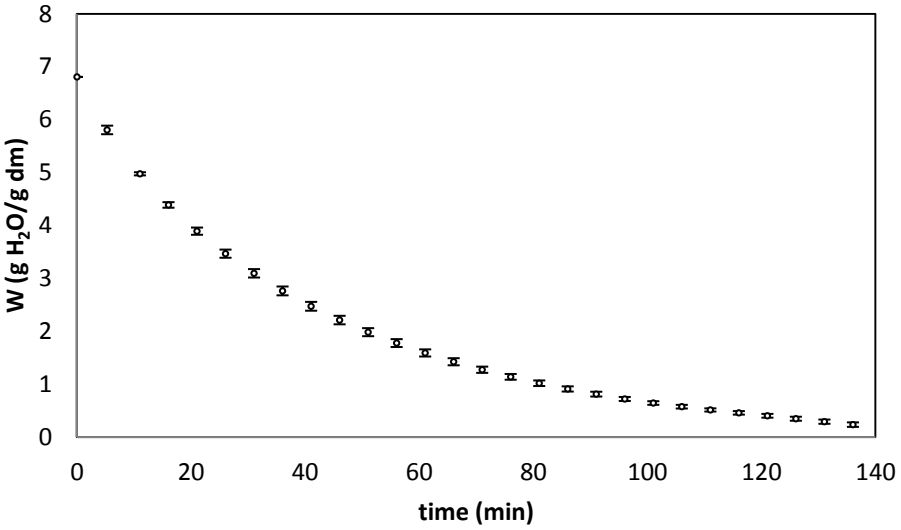

Fig. 1: Drying kinetics corresponding to the dehydration of apple

The sentence (line 99) “Samples were dried until they reached a final moisture content of 0.20±0.03 kg water/kg dm” has been changed to: “Samples were dried until they reached a final moisture content of 0.20±0.03 kg water/kg dm (136.0 ± 0.8 min).”

Comment 3: Section 2.4.4: "Uronic acids were colorimetrically determined, as total uronic acid using AIR samples hydrolyzed for 1 h at 100 °C in 1 M H_2SO_4. The results were expressed in mg of sugar per 100 g of initial dry matter (dm_o)." Please specify which assay was performed, which uronic acid was used as standard, and the related reference.

In the section “2.4.4.ANALYSIS OF CARBOHYDRATE COMPOSITION” (line 146) the sentences: “Uronic acids were colorimetrically determined, as total uronic acid using AIR samples hydrolyzed for 1 h at 100 °C in 1 M H_2SO_4. The results were expressed in mg of sugar per 100 g of initial dry matter (dm_o)”, has been changed to: “Uronic acids were colorimetrically determined as total uronic acid using AIR samples hydrolyzed for 1 h at 100 °C in 1 M H_2SO_4 as described Blumenkrantz & Asboe-Hansen (1973). Briefly, after hydrolysis of AIR samples, 1.2 ml/g sulfuric acid/tetraborate was added and the tubes were cooled in crushed ice. The cooled mixture was agitated using a Vortex mixer followed by heating at 100°C for 5 min. After heating, samples were cooled in a water-ice bath and 20 µl of m-hydroxydiphenyl reagent was added. The tubes were shaken and absorbance measurements were made at 520 nm in a Cary Bio 300 (Varian, California, USA) spectrophotometer within 5 min. Galacturonic acid dissolved in saturated benzoic acid was used as standard (0-80 µg/ml).”

Comment 4: Lines 304-306: "No significant differences ($p < 0.05$) were observed between the initial moisture content between raw and processed apples." Isn't it normal? I think you can remove this sentence as you specified in Materials and methods section (lines 100-101): "Before in vitro digestion, FD and CD samples were rehydrated by immersion in distilled water at 37 °C until they reached a final moisture content similar to raw samples (6.81 ± 0.04 g/ g dm).”

The sentence "No significant differences ($p < 0.05$) were observed between the initial moisture content between raw and processed apples" (lines 304-306) has been removed.

Comment 5: Lines 403-412: Authors observed an increase in total phenolic compounds for convective drying. This result is surprising for me, because in these drying conditions (60°C, I assume several hours), browning due to the enzymatic conversion of phenolic compounds into quinones (polyphenoloxidase reactions) may have occurred. So I attended to observe a decrease in phenolic compounds content. Do quinone are measured by the Folin-cocialteu method? Can you clarify this point?

The drying time was about two hours as we show in Figure 1, we think that this is enough so that browning due to the enzymatic conversion of phenolic compounds into quinones (polyphenoloxidase reactions) has occurred. Magalhães et. al (2010) performed a study whose purpose was the improvement of the time-consuming Folin-

Ciocalteu assay in order to attain a fast and high-throughput methodology providing Folin-Ciocalteu reducing capacity results similar to those obtained by the classical procedure. To achieve this objective, the assay was adapted to a 96-well microplate format. The concentration of the reagents and their influence in the reaction kinetics were thoroughly studied, using gallic acid as standard compound. The proposed methodology was applied to several phenolic compounds and food products and the results were compared with the classical method. This study indicated that caffeic acid, catechol, propyl gallate and pyrogallol with 2, 2, 3 and 3 free hydroxyl groups, respectively, in ortho-position gave similar reactivity to gallic acid due to the possibility to form ortho-quinone derivatives. Therefore, this suggests that the quinones are measured by the Folin-cocialteu method. In addition, we have performed a calibration curve (Figure 2) from known concentrations (between 2 and 15 µg/ml) of p-benzoquinone by Folin-cocialteu method. Therefore, we can conclude that the quinones are measured by Folin-cocialteu method.

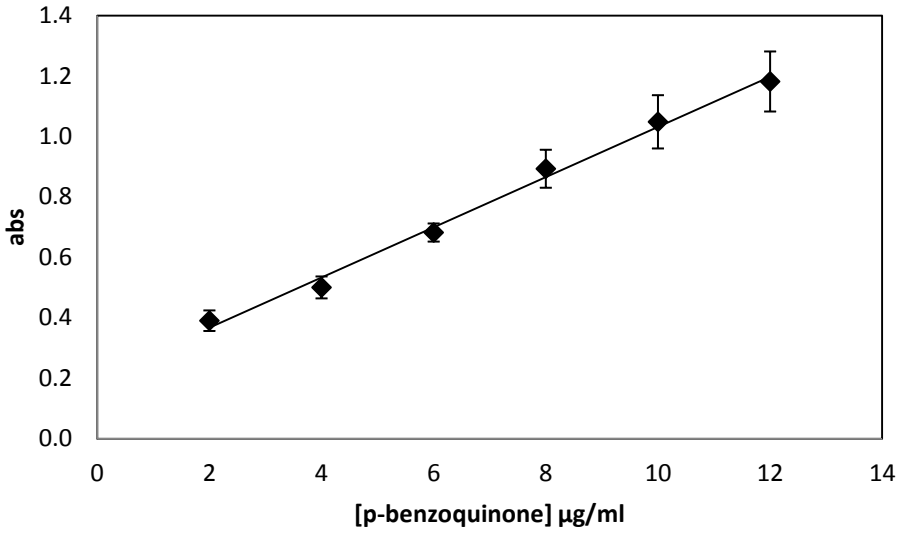

Fig. 2: Calibration curve of p-benzoquinone by Folin-cocialteu method

Receiving editor's comments:

The entire manuscript has been reviewed by a native English speaker, and all resources have been quoted as company, city, and country.

Comment 1: Convective drying at different temperatures from 50 ºC to 70 ºC, has also been shown to be associated with increased antioxidant activity in orange peel (…) compared to fresh samples (…). - is there an increase or does removing the water simply concentrate the antioxidants?

There is an increased concentration of antioxidants because the results are expressed on a dry matter.

Comment 2: Before in vitro digestion, FD and CD samples were rehydrated by immersion in distilled water at … - why distilled water and not, for example, saline, synthetic saliva or HCL to mimic the in vivo conditions?

This has been clarified in Lines 102-104. Water was used to rehydrate the apple samples, as this was more representative of how these products may be consumed.

Comment 3: Apple cubes (10-15 g) were mixed with 10 ml of simulated saliva for 30 s, followed by - does this refer to all samples or just those frozen in liquid nitrogen and thawed? If all, please clrify, e.g. all samples were mixed …

An additional statement in Line 111 has been added to clarify that all processed and raw apple samples were mixed with simulated saliva, etc.

Finally, the acknowledgments section (line 519) has been modified.

As a result of the corrections done in this paper the section "REFERENCES" has been modified:

<u>References added</u>

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489. http://doi.org/http://dx.doi.org/10.1016/0003-2697(73)90377-1

HIGHLIGHTS

- Processing changes the microstructure and composition of apples compared to raw apples
- The structural modifications resulted in behavioural changes in apples during *in vitro* digestion
- These changes were satisfactory simulated and better evaluated by using mathematical models

EFFECTS OF FREEZING, FREEZE DRYING AND CONVECTIVE DRYING ON *IN VITRO* GASTRIC DIGESTION OF APPLES

Maria Esperanza Dalmau[a], Gail M. Bornhorst[b], Valeria Eim[a], Carmen Rosselló[a], Susana Simal[a*]

[a]*Department of Chemistry, University of the Balearic Islands, Ctra. Valldemossa km. 7.5, 07122, Palma de Mallorca, Spain;*

[b]*Department of Biological and Agricultural Engineering, University of California, Davis, CA 95616, USA.*

* Corresponding author: Tel.: +34 971 172 757 Email: susana.simal@uib.es

Abstract: The influence of processing (freezing at -196ºC in liquid N_2, FN sample; freeze-drying at -50ºC and 30 Pa, FD sample; and convective drying at 60ºC and 2 m/s, CD sample) on apple (var. *Granny Smith*) behavior during *in vitro* gastric digestion was investigated. Dried apples (FD and CD samples) were rehydrated prior to digestion. Changes in carbohydrate composition, moisture, soluble solids, acidity, total polyphenol content (TPC), and antioxidant activity (AA) of apple samples were measured at different times during digestion. Processing resulted in disruption of the cellular structure during digestion, as observed by scanning electron microscopy, light microscopy, and changes in carbohydrate composition. Moisture content increased (6-11% dm_o), while soluble solids (55-78% dm_o), acidity (44-72% dm_o), total polyphenol content (30-61% dm_o), and antioxidant activity (41-87%) decreased in all samples after digestion. Mathematical models (Weibull and exponential models) were used to better evaluate the influence of processing on apple behavior during gastric digestion.

Keywords: *in vitro* gastric digestion; food processing; mathematical model; polyphenol content; antioxidant activity; microstructure

Nomenclature:

AA	antioxidant activity	mg trolox/g dm_o
AIR	alcohol insoluble residues	g/100 g dm_o

C	extraction yield	g/g dm_o or g/100 g dm_o
C_o	initial extraction yield	g/g dm_o or g/100 g dm_o
C_{calc}	calculated value	
C_{eq}	equilibrium extraction yield	g/g dm_o or g/100 g dm_o
C_{exp}	experimental value	
CD	convective drying	
CI	confidence intervals	
dm	dry matter	
dm_o	initial dry matter	
FD	freeze-drying	
FN	frozen with liquid nitrogen	
GAE	gallic acid equivalent	
LM	light microscopy	
MRE	mean relative error (%)	
r^2	coefficient of determination	
S_{calc}	standard deviation of the calculated values	
S_{exp}	standard deviation of the experimental values	
SE	standard error of the estimated parameters	
SEM	scanning electron microscopy	
SSE	summed square of residuals statistics	
TPC	total polyphenol content	mg GAE/g dm_o
VAR	percentage of explained variance (%)	
α	kinetic reaction constant of the Weibull model	s
β	shape parameter of the Weibull model	

1. INTRODUCTION

Food processing results in modifications of food properties. These properties include initial chemical and nutritional composition, physical properties and structure, stability of nutrients during storage, as well as release and absorption of beneficial compounds (MacEvilly & Peltola, 2008).

Commonly used processing operations for fruits and vegetables include freezing, freeze drying, and convective drying.

Previous studies have shown that freezing modifies fruit initial properties and composition of fruits. For example, freezing of apples (var. *Granny Smith* and *Golden*) and mangos (var. *Kent*) has been shown to modify the fruit texture, color, and physico-chemical (water content, soluble solids content, and pH) parameters (Chassagne-Berces, Fonseca, Citeau, & Marin, 2010; Chassagne-Berces, Poirier, Devaux, Fonseca, Lahaye, Pigorini, et al., 2009). Mazzeo et al. (2015) observed different color values between frozen asparagus, green beans, and zucchini compared to their fresh counterparts. In contrast, phytochemicals, in particular lutein and flavonoids, were similar in fresh and frozen asparagus, green beans, and zucchini.

In addition to freezing, freeze and convective drying may also influence initial food properties and composition. Freeze drying of apples has been shown to cause a reduction in the reducing sugar content, total sugar content, and total phenol content (Huang, Zhang, Wang, Mujumdar, & Sun, 2012). Both freeze drying (−50 ºC, 5 Pa) and convective drying (2 h at 80 ºC followed by 6 h at 60 ºC) have been shown to increase the antioxidant activity of tomatoes (*Lycopersicon esculentum Mill*) (Chang, Lin, Chang, & Liu, 2006). Convective drying at different temperatures (from 50 ºC to 70 ºC) has also been shown to cause increases in antioxidant activity in dried orange peel (*Citrus aurantium* v. Canoneta) compared to fresh samples (Garau, Simal, Rosselló, & Femenia, 2007).

The influence of processing on initial food properties might be the result of cellular and structural changes that occur during processing. For example, Delgado & Rubiolo (2005) observed that slow freezing rates (<1.5 ºC/min) greatly influenced tissue structure and caused water loss in strawberries (*Fragaria x ananassa*). Additionally, Chassagne-Bercesa et al. (2009) observed that freezing caused cell membrane breakage, which resulted in cell wall collapse and tissue breakage in *Granny Smith* apples. Freeze drying and convective drying of apples have also been shown to cause cellular changes in the food matrix. Huang et al. (2012) found that freeze drying (-40 ºC, 100 Pa) in a microwave vacuum dryer (75-300 W, 5 kPa) resulted in cell wall shrinkage in apples (var. *Red Fuji*). Also, Rodríguez et al. (2014b) observed cell collapse and cell disruption in apple slices (var. *Granny Smith*) dried with hot air, with more cellular changes occurring at higher (> 70ºC) drying temperatures compared to lower drying temperatures (30-60ºC).

In addition to initial composition and quality parameters, processing that results in changes in food nutrient content and cellular structure may also influence the release, bioaccessibility, and bioavailability of nutrients from the food matrix (Parada & Aguilera, 2007). Previous studies have shown that both processing conditions and cellular structure of foods influence the release and absorption of their constituent nutrients. Ellis et al. (2004) showed (*in vivo*) the role of cell walls on the bioavailability of lipids in almond seeds and concluded that intact cell walls prevented the release of intracellular lipids. Furthermore, a theoretical model has been developed relating the bioaccessibility of lipids in almonds to the rupture properties of almond cell walls. This model has been related to the breakdown and size reduction of almond particles during digestion (Grassby, Picout, Mandalari, Faulks, Kendall, Rich, et al., 2014).

In fruit and vegetable products, Netzel et al. (2011) found that the liberation of carotenoids, evaluated using an *in vitro* gastric and intestinal digestion model, was higher in a puree of cooked (100 ºC, 10 min) or blanched (80 ºC, 10 min) carrots compared to fresh carrot puree. Similarly, blanching of carrots (in both water and acidified water (45 g/l citric acid, pH 1.3 at 100 °C for 4 min)) has been shown to promote the release of β-carotene, most likely as a result of cell wall breakdown (Jabbar, Abid, Hu, Wu, Hashim, Lei, et al., 2014). Bioaccessibility and bioavailability of β-carotene in carrots has been shown to be influenced by the degree of particle size reduction, heat treatment, and cell wall rupture. Of these factors, cell wall rupture was found to be necessary, prior to release and absorption of β-carotene in carrots. This indicates that cell wall rupture may play a crucial role in nutrient release and absorption in other fruit and vegetable products as well (Tydeman, Parker, Faulks, Cross, Fillery-Travis, Gidley, et al., 2010a; Tydeman, Parker, Wickham, Rich, Faulks, Gidley, et al., 2010b).

Previous studies have shown that processing (freezing, freeze drying and convective drying) may influence both the initial properties and composition as well as the cellular structure of food products. Consequently, modifications in cellular structure of food matrices may result in modification of the release, bioaccessibility, and bioavailability of nutrients from foods. As such, the objective of this study was to evaluate the effects of different processing techniques (freezing, (FN), freeze drying, (FD), and convective drying, (CD)) on the microstructure, chemical characteristics, and release of bioactive compounds from *Granny Smith* apples during *in vitro* gastric digestion.

2. MATERIALS AND METHODS

2.1. Samples

Apples (*Malus domestica* var. *Granny Smith*) were purchased from a local supermarket (initial moisture content of 6.81±0.04 g/g dm and total soluble solids of 12.1±0.5 ºBrix). Apples were stored at 4 °C for a maximum of one week. Cubes were cut (0.01 m edge) from the center regions of the apple tissue, not including the peel or core, and immediately processed after cutting.

2.2. Freezing, freeze drying and convective drying processes

Apple cubes were frozen by immersion in liquid nitrogen (FN) (boiling point = -196 ºC) until the core temperature reached equilibrium with the freezing temperature (~30 s). Once frozen, samples were thawed in a cold chamber at 4 ± 1 ºC for aprox. 45 min prior to *in vitro* digestion.

Freeze drying (FD) was completed using a freeze-drier (Telstar LyoQuest, Barcelona, Spain) operating at −50 °C and (vacuum pressure of 30 Pa) until a final moisture content of 0.05±0.01 kg water/kg dm.

Convective drying (CD) was completed in a laboratory-scale hot air dryer operating at 60ºC with an air velocity of 2 m/s (Garau, Simal, Rosselló, & Femenia, 2007). Samples were dried until they reached a final moisture content of 0.20±0.03 kg water/kg dm (136.0 ± 0.8 min).

Before *in vitro* digestion, FD and CD samples were rehydrated by immersion in distilled water at 37 °C until they reached a final moisture content similar to raw samples (6.81 ± 0.04 g/ g dm). Distilled water was used to rehydrate the apple samples, as this is similar to what may be done prior to consumption of certain dried products.

2.3. *In vitro* digestion procedure

Apple samples were digested following the *in vitro* gastric digestion method reported by Bornhorst & Singh (2013). Briefly, simulated saliva was prepared by dissolving 1g/l mucin, 2g/l α-amylase, 0.117 g/l NaCl, 0.149 g/l KCl, and 2.10 g/l $NaHCO_3$ in deionized water at pH 7.0. Simulated gastric juice was prepared by dissolving 1g/l pepsin, 1.50 g/l mucin, 8.78 g/l NaCl in deionized water at pH 1.8–2.0. All solutions were prepared daily.

For all processed and raw apples cubes, samples (10-15 g) were mixed with 10 ml of simulated saliva for 30 s, followed by immersion in 100 ml of simulated gastric juice pre-heated to 37ºC. The mixture was incubated in a shaking water bath (Unitronic 320 OR, Barcelona, Spain) at 37 °C and 100 rpm for up to 3 h. Samples were taken initially (no digestion), after mixing with saliva, and after 10, 20, 30, 45, 60, 90, 120, and 180 min of gastric digestion for moisture, acidity, and soluble solid analyses. Samples were taken initially (no digestion), and after 60, 120, and 180 min of gastric digestion for total polyphenol content, and antioxidant activity analyses. Samples were taken initially (no digestion) and after 180 min of gastric digestion for carbohydrate composition and microstructural analyses. All digestion experiments were performed at least in triplicate, and results were expressed in initial dry matter basis to facilitate comparison between the different treatments.

2.4. Cell walls

Cell walls were observed by scanning electron microscopy (SEM) and light microscopy (LM). Alcohol insoluble residues (AIRs) were prepared to analyze the carbohydrate composition of raw and processed apple samples before and after digestion.

2.4.1. Scanning electron microscopy (SEM)

Apple cubes were soaked in liquid nitrogen in order to be fractured with a sharp razor blade, and freeze dried for observation in a scanning electron microscope (SEM): HITACHI S-3400N (Sysmex, Krefeld, Germany), accelerated at 15 kV and under vacuum pressure of 40 Pa.

2.4.2. Light microscopy (LM).

Apple samples were prepared for light microscopy as described by Eim et al. (2012) with minor modifications. Samples were fixed in formaldehyde (10%), followed by dehydration, embedding in paraffin (60 ºC for 3 h), and sectioning into 4-5µm slices with a microtome (model Finesse 325, Thermo Shandon, Cheshire, UK). The slices were stained with acid Schiff and haematoxylin to visualize cell walls (Paciulli, Ganino, Pellegrini, Rinaldi, Zaupa, Fabbri, et al., 2014). Images were obtained using a light microscope (Olympus BX60FS, Japan) at 100× magnification.

2.4.3. Alcohol insoluble residues (AIRs)

AIRs were obtained by immersing apple samples in boiling ethanol (85% (v/v) aq.) as described by Garau et al. (2007). Prior to further analysis, the AIRs were milled using a laboratory grain mill and

passed through a 0.5 mm sieve. Results were expressed in gram of AIR per 100 g of initial dry matter (dm_o).

2.4.4. Analysis of carbohydrate composition

Sugars were released from cell wall polysaccharides by acid hydrolysis as described by Garau et al. (2007). AIR samples (~5 mg) were dispersed in 72% H_2SO_4 for 3 h followed by dilution to 1 M and hydrolyzed at 100 °C for 2.5 h (Saeman hydrolysis conditions). A 1 M H_2SO_4 hydrolysis (100 °C for 2.5 h) was also included to determine the cellulose content by difference. Neutral sugars were derivatized as their alditol acetates and isothermally separated by gas chromotography at 220 °C on a 3% OV225 Chromosorb WHP 100/120 mesh column (Hewlett-Packard 5890A, Waldbronn, Germany) with Argon as the carrier gas flowing at 20 ml/min. Injector and FID detector temperatures were 230°C and 240 °C, respectively. Uronic acids were colorimetrically determined as total uronic acid using AIR samples hydrolyzed for 1 h at 100 °C in 1 M H_2SO_4 as described Blumenkrantz & Asboe-Hansen (1973). Briefly, after hydrolysis of AIR samples, 1.2 ml/g sulfuric acid/tetraborate was added and the tubes were cooled in crushed ice. The cooled mixture was agitated using a Vortex mixer followed by heating at 100°C for 5 min. After heating, samples were cooled in a water-ice bath and 20 µl of m-hydroxydiphenyl reagent was added. The tubes were shaken and absorbance measurements were made at 520 nm in a Cary Bio 300 (Varian, California, USA) spectrophotometer within 5 min. Galacturonic acid dissolved in saturated benzoic acid was used as standard (0-80 µg/ml). Results were expressed in mg of sugar per 100 g of initial dry matter (dm_o).

2.5. Chemical characteristics: moisture content, soluble solids content, and titratable acidity

Moisture content (method no. 934.06, 1997) and soluble solid content (method no. 932.14C, 1990) of all samples were determined according to AOAC official methods and expressed in gram of water or saccharose/g initial dry matter (dm_o), respectively.

Prior to measurement of titratable acidity, a known mass of apple sample (~5 g) was mixed with 20 ml of distilled water with an Ultra-turrax (T25 Digital IKA, Staufen, Germany). Titratable acidity was measured via titration of this sample with 0.1 NaOH to an end point of pH 8.1 using a pH meter (Crison, pH 25, Barcelona, Spain). Results were expressed as gram-equivalents of malic acid per 100 g of initial dry matter (dm_o).

2.6. Release of bioactive compounds: total polyphenol content (TPC) and antioxidant activity (AA)

Methanol extracts from all samples were prepared according to the methodology described by Eim et al. (2013) with minor modifications. Samples were weighed (~1.0 g), and 20 ml of methanol (MeOH) extraction solvent was added. Mixtures were homogenized using an Ultra-Turrax T25 Digital (IKA, Staufen, Germany) at 13,000 rpm for 1 min at 4 °C, and these solutions were refrigerated overnight. The mixtures were then centrifuged at 4,000 rpm for 10 min followed by filtration to obtain the methanol extract. The extracts were refrigerated at 4 °C until analysis.

Total polyphenol content (TPC) was determined using the Folin-Ciocalteu assay as described by Eim et al. (2013). The antioxidant activity (AA) was determined using the ABTS, FRAP, and CUPRAC assays as described by González-Centeno et al. (2012). In all assays, absorbance measurements were carried out at 25 °C in an UV/Vis/NIR spectrophotometer (Thermo Scientific MultiSkan Spectrum, Vantaa, Finland). Absorbance measurements for all the assays were correlated with standard curves. The TPC was expressed as mg gallic acid equivalent (GAE)/g initial dry matter (dm_o). The AA was expressed as mg Trolox/g initial dry matter (dm_o).

2.7. Mathematical model

The kinetics of moisture content, soluble solid content, titratable acidity, total polyphenol content, and antioxidant activity (ABTS, FRAP, and CUPRAC assays) in raw and processed apples during *in vitro* digestion were described using the Weibull model (Eq. (1)). This model has previously been used to describe microbial, enzymatic, and chemical degradation, as well as hydration/dehydration kinetics (Eim, Urrea, Rosselló, García-Pérez, Femenia, & Simal, 2013; M. R. González-Centeno, Comas-Serra, Femenia, Rosselló, & Simal, 2015; Rodríguez, Ortuño, Simal, Benedito, Femenia, & Rosselló, 2014a; Zura-Bravo, Vega-Gálvez, Lemus-Mondaca, Ah-Hen, & Di Scala, 2013).

$$\frac{C - C_{eq}}{C_o - C_{eq}} = e^{\left[-\left(\frac{t}{\alpha}\right)^{\beta}\right]} \quad (1)$$

where α is related to the inverse of the change/input rate (s), β is a characteristic shape parameter, and C_{eq} is the equilibrium concentration (g/100 g dm_o) (M. R. González-Centeno, Comas-Serra, Femenia, Rosselló, & Simal, 2015). When β equals 1, the equation corresponds to first order kinetics. The terms α, β and C_{eq} were identified for each experimental treatment.

193 **2.8. Data and statistical analysis**

194 Results are presented as mean values with their corresponding standard deviations. Statistical
195 analyses were performed using R 3.1.0 software. Parametric ANOVA and Tukey tests were used to
196 evaluate the existence and the degree of significant differences, respectively. The statistical analyses were
197 replaced by Kruskal-Wallis and pairwise-Wilcox (BH corrected) when data were not normally distributed
198 and/or showed heterogeneity of variances. Significance was assessed at a level of $p < 0.05$.

199 The identification of the Weibull model parameters α, β and C_{eq}, was carried out using the
200 'nlinfit' function of the optimization toolbox of Matlab 7.5 (The MathWorks Inc., Natick, USA) which
201 estimates the coefficients of a nonlinear regression function and the residuals using least squares. To
202 determine the 95% confidence intervals (CI) and the standard error of the estimated parameters (SE), the
203 'nlparci' function and the covariance matrix were used, respectively.

204 Linear regression analyses were carried out by using "Curve Fitting" Toolbox of Matlab 7.5 (The
205 MathWorks Inc., Natick, USA), to determine the coefficient of determination (square of the correlation
206 between the response values and the predicted response values, r^2) and the summed square of residuals
207 (sum of squares due to error of the fit, SSE) statistics.

208 The mean relative error (MRE) (Eq. 2), estimated by the comparison of experimental and
209 simulated data, was calculated to statistically evaluate the accuracy of the proposed mathematical model
210 to simulate change kinetics.

$$MRE = \frac{\sum_{i=1}^{n}\left(\frac{\left|C_{exp} - C_{calc}\right|}{C_{exp}}\right)}{n} \cdot 100 \quad (2)$$

211

212 **3. RESULTS AND DISCUSSION**

213 **3.1. Cell walls**

214 **3.1.1. Microstructural changes (SEM and LM)**

215 Scanning electron microscopy (SEM) and light microscopy (LM) were used to assess the
216 microstructural changes in apple samples as a result of processing and after 180 min of *in vitro* gastric
217 digestion (Figure 1). Microstructural differences were observed both as a result of processing and as a

result of *in vitro* gastric digestion when compared to raw, undigested apple samples. Compared to raw apples, freeze dried and convective dried apples exhibited the greatest changes, both before and after digestion. This trend can be observed in both surface (SEM) and cellular (LM) structures.

Figure $1a_1$ and $1a_2$ shows the microstructure of raw apples. Raw apples are composed of many well-arranged pores in a heterogeneous and anisotropic pattern, as was previously observed by Rodríguez et al. (2014b). After 180 min of digestion (Figure $1a_3$ and $1a_4$) a significant cell lysis was observed, resulting in a smaller number of cells per unit area, along with increases in the intercellular space between remaining cells. Carnachan et al. (2012) studied the microstructure of kiwi pulp after 30 min *in vitro* gastric digestion (adjusting the pH to 2.5 and adding pepsin), followed by 120 min *in vitro* intestinal digestion (adding pancreatin solution (5% in maleate buffer pH 6.5, 20 mL followed by amyloglucosidase)). Similar to the current study, these authors observed an increase in the intercellular spaces after *in vitro* digestion.

Figure $1b_1$ and $1b_2$ shows the microstructure of frozen apple samples before *in vitro* digestion. These micrographs indicate that freezing by immersion in liquid nitrogen caused irregular shapes, cellular damage, and more intercellular spaces. Similar effects were observed in frozen strawberries by Delgado & Rubiolo (2005). After *in vitro* gastric digestion of frozen apples (Figure $1b_3$ and $1b_4$), cell collapse was evident. The structure became less porous, resulting in an irregular surface where cell walls were less visible, and larger, more irregular shapes were present (Figure $1b_4$). In addition, cell wall fragments resulting from cell lysis could be observed (Figure $1b_4$).

Figure $1c_1$ and $1c_2$ shows the microstructure of freeze dried samples before *in vitro* digestion. The heterogeneity of the pore structure was similar to that of the raw apples. However, a collapse of cell membranes was observed. A similar effect was observed in freeze-dried *Red Fuji* apples by Huang et al. (2012) and in freeze-dried *Idared* apples by Lewicki & Pawlak (2003). An additional increase in cell collapse and destruction of cell wall material can be observed as a result of *in vitro* gastric digestion (Figure $1c_3$ and $1c_4$). The changes during digestion eliminated a majority of the pore structure observed in undigested, raw apples.

Cells exhibited shrinkage during convective drying, as can be observed in Figure $1d_1$ and $1d_2$. In addition, there was a reduction in the number and size of pores as well as cellular collapse that was observed in convective dried apples when compared to raw apples. The influence of convective drying on

247 the microstructure of *Granny Smith* apple have been previously studied by several authors (Rodríguez,
248 Santacatalina, Simal, Garcia-Perez, Femenia, & Rosselló, 2014b; Vega-Gálvez, Ah-Hen, Chacana,
249 Vergara, Martínez-Monzó, García-Segovia, et al., 2012). These authors agreed that during drying, one of
250 the most important phenomena is cell shrinkage, which leads to a major modification of the apple
251 structure and allows the release of water. In addition to the changes that occurring during convective
252 drying, additional cell collapse was visible in CD samples after *in vitro* gastric digestion (Figures $1d_3$ and
253 $1d_4$). Structural modifications that occurred during digestion eliminated the open pores present on the
254 surface and ruptured many of the interior cell walls.

255 **3.1.2. Alcohol insoluble residues (AIRs)**

256 The alcohol insoluble residue (AIR) values from raw and processed apples before (initial) and
257 after 180 min of *in vitro* gastric digestion are shown in Table 1. The initial AIR content of raw apples
258 (17.5±0.1 g AIR/100g dm_o) was similar to previously reported values (17.0 g AIR/100 g dm, (Christensen
259 2009). All processing methods resulted in a significant decrease in AIR ($p < 0.05$), with raw apples
260 having the greatest AIR, followed by frozen (16.8±0.5 g AIR/100g dm_o), freeze dried (15.2±0.1 g
261 AIR/100g dm_o), and convective dried (13.1±0.6 g AIR/100g dm_o) apple samples. Convective dried apples
262 had the greatest decrease in AIR of all processing treatments (~25%). This finding is similar to previous
263 studies that have also observed significant decreases in AIR after convective drying (60 ^oC, 2 m/s), with
264 up to 15 or 20% AIR losses being reported in orange pulp or skin, respectively (Garau, Simal, Rosselló,
265 & Femenia, 2007).

266 Apple samples from all processing methods had significant decreases in AIR content after 180
267 min of *in vitro* gastric digestion ($p < 0.05$), compared to their initial values. After 180 min of *in vitro*
268 gastric digestion, the AIR content of the raw, FN, FD and CD samples decreased to 16.7±0.1, 15.9±0.1,
269 12.6±0.1 and 10.8±0.7 % dm_o, respectively. FD and CD samples had similar decreases in AIR as a result
270 of digestion (~17% decrease); these decreases were greater than those observed in Raw or FN samples
271 (~5% decrease). The greater changes in AIRs in FD and CD apple samples might be due to the
272 breakdown of cell walls in the FD and CD samples that was observed in the microstructural analysis,
273 allowing the components to be more accessible to hydrolysis by gastric acid and enzymes during *in vitro*
274 digestion. Yuliarti et al. (2008) also reported decreases in AIR content (32% decrease) as a result of *in*
275 *vitro* digestion (with commercial enzyme preparations (Cellulyve, NS33048, Celluclast, and Cytolase) at
276 25 °C for 30 min) of golden kiwifruit.

277 **3.1.3. Analysis of carbohydrate composition**

278 Cell wall polysaccharides of raw and processed apples before and after 180 min of *in vitro*
279 gastric digestion are shown in Table 1. The most abundant neutral sugar of the AIRs in all the samples
280 was glucose, both before and after *in vitro* gastric digestion. Glucose was followed by arabinose, xylose,
281 and galactose; rhamnose, fucose and mannose were minority sugars. The results obtained for the raw
282 apples in this study were in the range of those previously described for *Granny Smith* apples by
283 Chassagne-Berces et al. (2009).

284 Processed apple samples (before digestion) had significantly lower ($p < 0.05$) total cell wall
285 polysaccharides compared to raw apples. Frozen samples showed the least change from raw apples, with
286 only ~5% decrease in total sugars compared to raw apples. Chassagne-Berces et al. (2009) also reported a
287 significant decrease of total cell wall polysaccharides in *Granny Smith* apples (~9%) after freezing in
288 liquid nitrogen. However, freeze dried and convective dried apples showed much greater decreases in
289 total sugars compared to raw apples (17 and 45% decrease from the raw value, for freeze and convective
290 dried, respectively). Frozen apples showed a significant increase ($p < 0.05$) in glucose and decrease
291 ($p<0.05$) in uronic acids from raw apples. Freeze dried apples also had a statistically significant decrease
292 in uronic acids, as well as arabinose and xylose. Convective dried apples had significantly lower values
293 for all individual neutral sugars and uronic acids compared to raw apples ($p < 0.05$). Significant decreases
294 in cell wall carbohydrate composition due to convective drying (60 ºC at 2 m/s) of kiwifruit were also
295 reported by Femenia et al. (2009).

296 No significant differences were observed between the specific carbohydrate composition of the
297 raw apples before and after digestion, except a slight significant ($p<0.05$) increase in both mannose and
298 glucose. In addition, total sugars did not change ($p > 0.05$) in raw apples as a result of *in vitro* gastric
299 digestion. Similar results were reported by Carnachan et al. (2012), who also observed no significant
300 differences between the carbohydrate composition of fresh kiwifruit before and after *in vitro* digestion.

301 The carbohydrate composition of all processed apples significantly ($p<0.05$) changed after
302 digestion for most neutral sugars, expect mannose. Additionally, the total sugars significantly decreased
303 after digestion for all processed apples. Frozen and freeze dried apples had similar decreases in total
304 sugars (13-17% of initial value), and convective dried apples had the greatest decrease in total sugars
305 (38% decrease from initial value) during 180 min of *in vitro* gastric digestion. These decreases in total

306 sugars follow a similar trend as the magnitude of microstructural damage observed in the SEM and LM
307 analyses (Figure 1), where convective drying resulted in the largest changes in microstructure, and
308 freezing and freeze drying both resulted in significant, but similar, microstructural modifications.

309 **3.2. Chemical characteristics**

310 Figure 2 shows the changes of chemical characteristics (moisture content, soluble solid content,
311 and titratable acidity) of raw and processed apples during *in vitro* gastric digestion. The initial moisture
312 content (Figure 2a) of raw apples was of 6.81±0.04 g water/g dm. This is similar to previously reported
313 values for *Granny Smith* apples (6.8-7.7 g water/g dm (Chassagne-Berces, Fonseca, Citeau, & Marin,
314 2010)).

315 The moisture content (Figure 2a) increased in all samples during *in vitro* gastric digestion, with
316 raw and processed apples following a similar trend. The percentage of moisture content increase of raw,
317 FN, FD and CD samples after 3 h of *in vitro* digestion were of 6±1, 10±1, 11±2 and 8±1 % dm_o,
318 respectively. All processed apples had a greater capacity to absorb water compared to raw apples. This
319 was most likely due to the damage of the cellular structure caused by processing, which facilitated water
320 transport within the food matrix. Previous *in vitro* and *in vivo* studies have reported increases in food
321 moisture content during gastric digestion. For example, Bornhorst et al. (2013) observed moisture content
322 increases of 75 and 23% dm_o in white and brown rice after 180 min of *in vivo* digestion in pigs, and
323 Bornhorst et al. (2014) observed moisture content increases of 79 and 95 % dm_o in raw and roasted
324 almonds after 120 min of *in vitro* digestion.

325 Figure 2b shows the evolution of the soluble solid content of raw and processed apples during *in*
326 *vitro* gastric digestion. The initial soluble solid content of raw apples in this study (0.94 ± 0.04 g /g dm_o)
327 was similar but slightly higher than previously reported values for *Granny Smith* apples (0.88-0.90 g/g
328 dm_o (Chassagne-Berces, Fonseca, Citeau, & Marin, 2010). No significant differences were observed
329 between the soluble solid content of raw and FN samples before *in vitro* digestion. In contrast, FD and
330 CD samples exhibited significant ($p < 0.05$) soluble solid losses (6 and 40% dm_o, respectively) in
331 comparison to raw apples, most likely due to the rehydration step before digestion. These trends are
332 similar to previously reported studies. Chassagne-Berces et al. (2010) also observed no significant effect
333 of freezing on the soluble solid content of *Granny Smith* apples. Schulze et al. (2014) reported soluble

334 solid losses (19 % dm_o) due to freeze-drying of *Granny Smith* apples. Maldonado et al. (2010) reported
335 that the soluble solid content of dried mango decreased by 70% after 100 min of rehydration in water.

336 As can be observed in Figure 2b, significant decreases in soluble solid content were observed in
337 all samples after 3 h of *in vitro* digestion (55±4, 70±1, 78±5, and 66±1 % dm_o in raw, FN, FD and CD
338 apples, respectively). Soluble solid losses were higher in processed samples than in raw samples, the
339 opposite trend as seen in the moisture absorption curves (Figure 2a).

340 Figure 2c shows the evolution of the titratable acidity in raw and processed samples during *in*
341 *vitro* gastric digestion. The acidity of raw apples (5.1±0.2 g/100g dm) was similar to previously reported
342 values for *Granny Smith* apples (4.2-5.4 g/100g dm (Chassagne-Berces, Fonseca, Citeau, & Marin,
343 2010)). Frozen samples did not have significantly different titratable acidity compared to raw apples.
344 However, the titratable acidity of FD and CD samples was significantly lower ($p<0.05$; 9.5 and 37.5 %
345 dm_o, respectively) compared to raw and frozen apples. This decrease was mostly likely due to acid losses
346 during the rehydration step of the dried apples prior to digestion.

347 The titratable acidity of all samples significantly decreased during *in vitro* digestion (Figure 4),
348 with the greatest decreases occurring during the first 90 min. After 180 min of digestion, the acidity losses
349 were of 44±3, 67±5, 72±4 and 70±3 % dm_o, in raw, FN, FD and CD samples, respectively. Similar to the
350 trend observed in soluble solid content decrease during digestion, all processed samples showed
351 significantly ($p<0.05$) greater acidity losses during digestion compared to raw samples. The trends
352 observed in titratable acidity and soluble solid loss were the same as the decreases observed in total cell
353 wall polysaccharides (Table 1) and the microstructural analysis, where raw < frozen < freeze dried <
354 convective dried in terms of total changes from the initial state. These results suggest that microstructural
355 changes during processing and digestion that prompt damage of cell walls result in losses of intercellular
356 material, such as soluble solids and acid.

357 The Weibull model was used to mathematically describe the kinetics of moisture, soluble solid,
358 and titratable acidity content in raw and processed apples during *in vitro* digestion. Table 2 shows the
359 Weibull model parameters (α, β, Ceq) with the corresponding confidence intervals and the standard error
360 associated with each estimated parameter. As can be observed, some confidence intervals and standard
361 errors were high as a result of the simultaneous identification of a high number of parameters.

Simulations obtained by disregarding any of the parameters were not satisfactory; therefore, all parameters were retained in the model.

To statistically evaluate the accuracy of the proposed mathematical models and their capacity to simulate the experimental results and predict variation within the system, the mean relative error (MRE, Eq. 2) was calculated for all samples by comparing experimental and predicted values. If the MRE is lower, the model provides a better fit to the experimental data (M. R. González-Centeno, Comas-Serra, Femenia, Rosselló, & Simal, 2015). The MRE (Table 2), was lower than 5.3% for all parameters of each model with average values of 0.1±0.1 %, 3.2±1.5 % and 3.5±0.9 % for the simulation of the moisture, soluble solid, and titratable acidity kinetics, respectively. From these results, it can be seen that the proposed model successfully simulated the changes in moisture, soluble solid, and acidity during *in vitro* gastric digestion in raw and processed apples. The simulated curves of moisture (Figure 2a), soluble solids (Figure 2b) and titratable acidity (Figure 2c) contents are shown with the observed values for all apple samples.

The α parameter of the Weibull model is related to the inverse of the change rate. As such, a lower α indicates a faster rate of change of a given quantity. As can be seen in Table 2, α decreased from 11225.3 s in raw sample to ~3000 s in processed apples for moisture content. This indicates that all of the processed samples had a faster rate of change in moisture content compared to the raw apple samples. Differences in α values for soluble solids and acidity kinetics between apple processing treatments were less evident, indicating all apples had similar rates of change of soluble solids and acidity. In the case of the acidity change kinetics, the CD sample had a greater α value, which was nearly 50% higher than that of the raw sample.

The shape parameter β represents a behavior index of the material during the process (M. R. González-Centeno, Comas-Serra, Femenia, Rosselló, & Simal, 2015). When β is equal to 1, the model corresponds to first order kinetics; with a constant input rate (Eim, Urrea, Rosselló, García-Pérez, Femenia, & Simal, 2013). However, when β has a value above or below 1, this parameter denotes the concavity (increasing change rate over time) or convexity (decreasing change rate over time) of the curve, respectively (M. R. González-Centeno, Comas-Serra, Femenia, Rosselló, & Simal, 2015). For all treatments and response variables, β was less than 1, indicating convexity in all of the curves and thus, decreasing change rate over time. For moisture content, convective dried apples had a higher β value

(0.864) compared to the raw, frozen and freeze dried samples (β values of 0.595, 0.505 and 0.594, respectively). This may indicate that convective drying hampers hydration during *in vitro* digestion. Similar trends were observed in the soluble solid kinetics, with all samples exhibiting low but similar β values (0.364, 0.444 and 0.505 in raw, FN and FD samples, respectively) except the CD sample, with a β value of 0.920. A similar trend was not observed in the β values of the acidity kinetics, which may indicate that although graphical trends are similar between acidity and soluble solid losses, their fundamental mechanisms of mass transport may be different.

The equilibrium concentration (C_{eq}) was similar in all samples for the moisture content change (7.49±0.04 g/g dm_o). However, in soluble solid and acidity kinetics, raw samples had almost double the Ceq values compared to all of the processed samples, although not statistically significant. This trend may indicate that all processing treatments altered the cellular structure such that the processed apples lost a greater amount of soluble solids or acidity during digestion.

3.3. Release of bioactive compounds

The total polyphenol content and antioxidant activity (ABTS, CUPRAC, and FRAP methods) in raw and processed samples before (0 min) and after 60, 120 and 180 min of *in vitro* gastric digestion are given in Table 3. The TPC of raw apples (4.4±0.2 mg GAE/g dm) was similar to previously reported values for *Granny Smith* apples by Francini & Sebastiani (2013) (4.9.±0.5 mg GAE/g dm). Prior to *in vitro* digestion, TPC in CD apples significantly increased ($p<0.05$) ~16% dm_o while the TPC significantly decreased ($p<0.05$) in FN and FD samples (45 and 34 % dm_o, respectively) compared to raw apples.

The decrease in TPC in frozen and freeze dried apples was similar to previously reported values. Loncaric et al. (2014) observed decreases in the TPC of *Fuji* apples due to freezing and freeze-drying (~48% dm). However, both increases and decreases in TPC have been seen as a result of convective drying. Some authors have reported TPC losses due to convective drying (Garau, Simal, Rosselló, & Femenia, 2007), concluding that the long drying times necessary with low process temperatures may promote the degradation of antioxidant compounds. However, in other cases, the TPC of samples dried by hot air increased, exhibiting a similar behavior to that observed in this study. Chang et al. (2006) reported TPC increases of 13-29% dm after convective drying of tomatoes compared to raw samples. The increase

418 in TPC was most likely due to the release of polyphenolic compounds from the food matrix during
419 drying.

420 As can be seen in Table 3, after 3 h of *in vitro* digestion, the TPC of the raw and FN, FD and CD
421 samples significantly decreased by 32±3, 58±4, 55±4 and 47±3 % dm_o, respectively. Similar decreases in
422 TPC (from 44.6 to 62.7 %) were reported by Bouayed et al. (2011) in *Jonaprinz*, *Jonagold*, *Golden* and
423 *Mutza* apples during *in vitro* gastric digestion. After 180 min of digestion, raw apples retained the greatest
424 amount of polyphenols, with the highest TPC (3.0 mg GAE/g dm_o). Interestingly, although CD samples
425 had the highest initial TPC value (5.1 mg GAE/g dm_o), they did not have the highest TPC value after 180
426 min of gastric digestion (2.7 mg GAE/g dm_o). These results indicate that not only the initial TPC value,
427 but also the structure of the food matrix, may be important in the release of nutrients from food matrices
428 during digestion. Although bioaccessibility was not directly measured in the current study, these results
429 highlight the influence of processing on nutrient release during *in vitro* gastric digestion, and may lead us
430 to hypothesize that differences in bioaccessibility would be obtained as well. However, bioaccessibility
431 determinations were outside the scope of this work, but are an area that merits future investigation.

432 In order to provide a more complete view of the antioxidant activity (AA) of the samples, three
433 methods were used to evaluate the AA: ABTS, CUPRAC, and FRAP analyses. Due to the fact that each
434 method is based on a different chemical system and/or reaction, different AA results could be expected
435 depending on the specific analysis performed (María Reyes González-Centeno, Jourdes, Femenia, Simal,
436 Rosselló, & Teissedre, 2012). The selection of different methods allows a better understanding of the
437 wide variety and range of action of antioxidant compounds present in apples (María Reyes González-
438 Centeno, Jourdes, Femenia, Simal, Rosselló, & Teissedre, 2012). The average values for the AA of raw
439 apples were of 7.6±0.3, 12.5±0.6 and 4.7±0.2 mg trolox/g dm_o from the ABTS, CUPRAC and FRAP
440 methods, respectively.

441 As can be observed in Table 3, raw and CD apples had the highest AA values before digestion (0
442 min), from the CUPRAC and FRAP assays, with no significant differences between them. However, by
443 using the ABTS method, the AA of CD sample was 24% dm higher than that of the raw apples. FN and
444 FD samples exhibited significant decreases ($p < 0.05$) in AA of 57 and 45% dm (CUPRAC assay) and 54
445 and 47% dm (ABTS assay), respectively, compared to raw apples. However, by using the FRAP method,

446 no significant differences were observed between FN and FD samples, although both FN and FD samples
447 exhibited significant decreases ($p < 0.05$; ~44% dm) compared to raw apples.

448 The results obtained for the AA of FN and FD samples prior to digestion were similar to those
449 previously described by Loncaric et al. (2014) for frozen (60 % dm loss) and freeze-dried (64 % dm loss)
450 *Fuji* apples. However, antioxidant activity of CD samples did not follow the same trend. In the current
451 study, CD samples had an increase in AA compared to the raw samples. This is different from several
452 previous studies that have reported that convective drying processes may promote a decrease in
453 antioxidant activity (Eim, Urrea, Rosselló, García-Pérez, Femenia, & Simal, 2013; Rodríguez,
454 Santacatalina, Simal, Garcia-Perez, Femenia, & Rosselló, 2014b). The differences observed in CD
455 samples in this study are most likely related to the generation and accumulation of different antioxidant
456 compounds having a varying degree of antioxidant activity and developing antagonistic or synergistic
457 effects with themselves or with other constituents of the apple extract. Although a different AA method
458 was used, a similar increase in AA after convective drying was observed by Vega-Gálvez et al. (2012).
459 They found that the AA (using the DPPH method) of *Granny Smith* apples dried at 60ºC and 1.5 m/s in a
460 convective drier increased by 87% after convective drying compared to raw apples. Other authors have
461 also reported that processing either caused no change in the antioxidant activity of fruit and vegetables or
462 enhanced it due to the improvement of antioxidant properties of naturally occurring compounds or
463 formation of novel compounds such as Maillard reaction products with antioxidant activity (Amarowicz,
464 2009). Maillard-derived melanoidins, responsible for color change during the drying process, may be
465 associated with increased antioxidant activity of the dried apples observed in the current study.

466 Raw apples had the least decrease in AA during *in vitro* gastric digestion (Table 3). The AA, as
467 measured by the ABTS method, did not significantly change over 180 min in raw apples ($p < 0.05$).
468 However, the AA, as measured by the CUPRAC and FRAP methods, significantly decreased between 0
469 and 60 min of digestion, but did not decrease significantly after longer digestion times ($p < 0.05$). These
470 results indicate that although slight decreases in AA of raw apples might occur in the initial stages of
471 digestion, the raw apple AA remained relatively stable throughout the gastric digestion process. These
472 results are similar to those previously described by Bouayed et al. (2011) for the *in vitro* digestion of
473 *Jonaprinz*, *Golden* and *Mutza* apples.

However, similar behavior was not observed in any of the processed apples. Both frozen and freeze dried apples showed significant decreases ($p < 0.05$) in AA across the entire *in vitro* digestion period for all measurement methods. Frozen apples had decreases of 74±6, 57±4 and 76±4% dm_o in AA after 180 min of gastric digestion as measured by the ABTS, CUPRAC, and FRAP methods, respectively. Similarly, freeze dried apples had decreases of 73±7, 41±3 and 88±3% dm_o after 180 min of gastric digestion as measured by the ABTS, CUPRAC and FRAP assays, respectively. Convective dried apples had similar results with AA measured by the CUPRAC and FRAP assays, where values significantly decreased ($p < 0.05$) with each increasing digestion time. However, in the AA measurements from the ABTS method, convective dried apples did not show a significant decrease in AA after 60 min of digestion compared to the initial value, but showed a decrease after 120 min of digestion that then stayed constant to 180 min of digestion. These results indicate that processing promoted the release of antioxidant compounds from the apple matrix during *in vitro* gastric digestion, most likely due to structural changes induced by processing. Future studies may be completed to determine if the release of antioxidant compounds during gastric digestion promotes or decreases their bioaccessibility and bioavailability.

With the aim of better evaluating the influence of the processing method on the TPC and AA changes in samples during *in vitro* digestion, the inverse of the input rate was estimated from the slope of the natural logarithm of the ratio of concentration/initial concentration vs the gastric digestion time (assuming a first order reaction with equilibrium concentration equal to zero). Table 4 shows the results obtained from these fittings together with the coefficients of determination (r^2) and the summed square of residuals (SSE). As can be seen in Table 4, r^2 and SSE had average values of 0.96±0.03 and 0.02±0.03, respectively; as such, the model was considered satisfactory to describe the data. In general, it can be seen that α decreased with processing, indicating increases in the rate of change of TPC or AA during digestion. For example, α decreased 93, 91 and 90 % for TPC in FN, FD and CD apples compared to raw apples. Similarly, α decreased in frozen, freeze dried, and convective dried apples ~87-95%, 34-59 % and 85-93% for AA measured by ABTS, CUPRAC and FRAP methods, respectively. These results further confirm the finding that processing of apples promoted the release of polyphenols and antioxidant compounds from the apple matrix during *in vitro* gastric digestion.

4. CONCLUSIONS

Processing (freezing, freeze drying, and convective drying) modified the microstructure and initial composition of *Granny smith* apples compared to raw apples. Microstructural analyses, both SEM, LM, and carbohydrate composition indicated significant cellular destruction and changes in cell wall composition both as a result of processing and during 180 min of *in vitro* gastric digestion. These structural modifications resulted in behavioral changes in apples during *in vitro* gastric digestion. Processed apples showed faster decreases in soluble solids and titratable acidity compared to raw apples, while moisture content increases were greatest in processed samples during *in vitro* gastric digestion. Freezing and freeze drying resulted in decreases in total polyphenol content and antioxidant activity in apples, both before and during *in vitro* gastric digestion. Convective drying increased initial total polyphenol content and antioxidant activity of apples, but these values decreased during *in vitro* gastric digestion. In contrast, raw apples showed minor decreases in total polyphenol content and antioxidant activity during *in vitro* gastric digestion, and exhibited the greatest retention of polyphenolic and antioxidant compounds. Given the limited knowledge that is available on this subject at present, it would be interesting to deeply investigate in this area to better understand how processing can modify the structural characteristics of the ingested food to modulate the bioaccessibility and bioavailability of active compounds in food matrices.

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Table(s)

Table 1 Composition of the cell wall polysaccharides of raw and processed apple samples before and after 180 min of in vitro gastric digestion. Different lowercase letters indicate significant differences ($p < 0.05$) for each sugar in a sample before (initial) and after (180 min) in vitro gastric digestion. Different capital letters indicate significant differences between the different method of processing (Tukey's test, $p < 0.05$).

	Carbohydrate analysis (mg/100g dm_o)									
	AIRs (g/100g dm_o)	Neutral sugars							Uronic Acids	Total
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc		
Raw Initial	17.5 ± 0.1 *a A*	190 ± 50 *a A*	190 ± 30 *a A*	1400 ± 200 *a A*	1300 ± 200 *a A*	200 ± 20 *a A*	1180 ± 40 *a A*	4100 ± 70 *a A*	2900 ± 500 *a A*	11500 ± 200 *a A*
Raw 180 min	16.7 ± 0.1 *b*	181 ± 4 *a*	170 ± 10 *a*	1300 ± 20 *a*	1100 ± 90 *a*	241 ± 7 *b*	1140 ± 40 *a*	4400 ± 200 *b*	3190 ± 80 *a*	11700 ± 200 *a*
FN initial	16.8 ± 0.5 *a B*	200 ± 40 *a A*	160 ± 7 *a A*	1200 ± 100 *a A*	1200 ± 100 *a A*	240 ± 20 *a A*	1300 ± 200 *a A*	4700 ± 200 *a B*	1960 ± 40 *a B*	11000 ± 200 *a B*
FN 180 min	15.9 ± 0.1 *b*	140 ± 30 *a*	140 ± 10 *b*	990 ± 20 *b*	900 ± 50 *b*	220 ± 20 *a*	1200 ± 200 *a*	3900 ± 300 *b*	1640 ± 30 *b*	9100 ± 300 *b*
FD Initial	15.2 ± 0.1 *a C*	140 ± 20 *a A*	150 ± 10 *a A*	1020 ± 70 *a B*	940 ± 90 *a B*	220 ± 30 *a A*	1120 ± 90 *a A*	4200 ± 400 *a AB*	1700 ± 100 *a C*	9490 ± 40 *a C*
FD 180 min	12.6 ± 0.1 *b*	100 ± 10 *b*	130 ± 10 *a*	810 ± 60 *b*	740 ± 50 *b*	200 ± 20 *a*	1150 ± 90 *a*	3700 ± 200 *b*	1300 ± 100 *b*	8100 ± 700 *b*
CD Initial	13.1 ± 0.6 *a D*	50 ± 5 *a B*	53 ± 7 *a B*	360 ± 20 *a C*	450 ± 25 *a C*	130 ± 30 *a B*	560 ± 40 *a B*	2600 ± 100 *a C*	2000 ± 300 *a BC*	6200 ± 400 *a D*
CD 180 min	10.8 ± 0.7 *b*	21 ± 2 *b*	28 ± 4 *b*	260 ± 30 *b*	310 ± 40 *b*	120 ± 50 *a*	360 ± 50 *b*	1600 ± 90 *b*	1100 ± 200 *b*	3800 ± 200 *b*

Table 2 Parameters of the Weibull model and the corresponding confidence interval (CI) and standard error (SE) associated with each parameter. Change kinetics of moisture content, soluble solid content and titratable acidity for raw and processed apples during *in vitro* digestion.

		α (s)	CI (s)	SE	β	CI	SE	C_{eq}*	CI*	SE	MRE
Moisture	Raw	**11225.3**	[-33258.1, 55708.7]	12711.4	**0.595**	[0.240, 0.950]	0.101	**7.45**	[6.52, 8.37]	0.26	0.1
	FN	**2779.8**	[-106.3, 5665.9]	824.7	**0.505**	[0.348, 0.663]	0.045	**7.51**	[7.30, 7.71]	0.06	0.1
	FD	**2990.2**	[-5146.5, 12516.9]	2523.7	**0.594**	[0.214, 0.730]	0.074	**7.54**	[7.08, 8.17]	0.16	0.2
	CD	**3871.6**	[2300.9, 5442.2]	448.8	**0.864**	[0.676, 1.051]	0.054	**7.46**	[7.36, 7.55]	0.03	0.1
Soluble solids	Raw	**2269.7**	[-4592.3, 9131.7]	1960.9	**0.364**	[0.148, 0.581]	0.062	**0.31**	[-0.07, 0.68]	0.11	2.6
	FN	**2300.8**	[333.6, 4268.0]	562.1	**0.444**	[0.342, 0.546]	0.029	**0.15**	[0.00, 0.31]	0.04	1.6
	FD	**2990.2**	[1636.6, 2922.5]	197.8	**0.505**	[0.204, 0.740]	0.093	**0.11**	[0.00, 0.23]	0.52	5.3
	CD	**2043.3**	[1175.8, 2910.8]	247.9	**0.920**	[0.515, 1.325]	0.116	**0.17**	[0.11, 0.23]	0.02	3.1
Acidity	Raw	**3115.9**	[-3699.1, 9930.8]	1947.4	**0.646**	[0.063, 1.228]	0.166	**2.46**	[0.56, 4.37]	0.54	2.6
	FN	**2859.1**	[-1345.8, 7064.1]	1201.6	**0.535**	[0.280, 0.791]	0.073	**1.15**	[-0.47, 2.76]	0.46	2.7
	FD	**2556.8**	[255.7, 4858.0]	657.6	**0.885**	[0.233, 1.537]	0.186	**1.17**	[-0.04, 2.37]	0.34	4.5
	CD	**4805.9**	[-2633.7, 12245.5]	1201.6	**0.744**	[0.336, 1.152]	0.073	**0.45**	[-1.22, 2.12]	0.46	4.1

*g/g dm_o for moisture and soluble solids contents and g/100 g dm_o for acidit

Table 3 Total polyphenol content[a] and antioxidant activity[b] (ABTS, CUPRAC and FRAP methods) of raw and processed apple samples during *in vitro* digestion.

	TPC	**ABTS**	**CUPRAC**	**FRAP**
Raw				
0 min	4.4 ± 0.2 *a A*	7.6 ± 0.3 *a A*	12.5 ± 0.6 *a A*	4.7 ± 0.2 *a A*
60 min	3.7 ± 0.2 *b*	7.4 ± 0.3 *a*	10.7 ± 0.2 *b*	4.4 ± 0.1 *b*
120 min	3.4 ± 0.1 *c*	7.3 ± 0.1 *a*	10.1 ± 0.3 *b*	4.3 ± 0.1 *b*
180 min	3.0 ± 0.1 *d*	7.1 ± 0.2 *a*	9.1 ± 0.3 *b*	4.2 ± 0.1 *b*
FN				
0 min	2.4 ± 0.2 *a B*	3.5 ± 0.1 *a B*	5.4 ± 0.2 *a B*	2.8 ± 0.2 *a B*
60 min	1.9 ± 0.1 *b*	2.5 ± 0.2 *b*	4.9 ± 0.2 *b*	2.1 ± 0.1 *b*
120 min	1.4 ± 0.1 *c*	1.7 ± 0.1 *c*	3.7 ± 0.1 *c*	1.3 ± 0.1 *c*
180 min	1.0± 0.1 *d*	0.9 ± 0.1 *d*	2.3 ± 0.1 *d*	0.6 ± 0.1 *d*
FD				
0 min	2.9 ± 0.2 *a B*	4.0 ± 0.3 *a C*	6.9 ± 0.2 *a C*	2.5 ± 0.1 *a B*
60 min	2.4 ± 0.2 *a*	3.3 ± 0.3 *b*	6.4 ± 0.2 *b*	1.3 ± 0.1 *b*
120 min	1.9 ± 0.1 *b*	2.2 ± 0.2 *c*	4.9 ± 0.3 *c*	0.7 ± 0.1 *c*
180 min	1.3 ± 0.1 *c*	1.1 ± 0.1 *d*	4.1 ± 0.2 *d*	0.4 ± 0.1 *d*
CD				
0 min	5.1 ± 0.4 *a C*	9.4 ± 0.4 *a D*	14.5 ± 0.9 *a A*	5.3 ± 0.4 *a A*
60 min	3.8 ± 0.2 *b*	8.7 ± 0.4 *a*	11.5 ± 0.5 *b*	4.3 ± 0.2 *b*
120 min	3.2 ± 0.2 *c*	6.5 ± 0.3 *b*	8.6 ± 0.8 *c*	3.1 ± 0.3 *c*
180 min	2.7 ± 0.2 *d*	5.5 ± 0.3 *b*	6.3 ± 0.2 *d*	2.3 ± 0.1 *d*

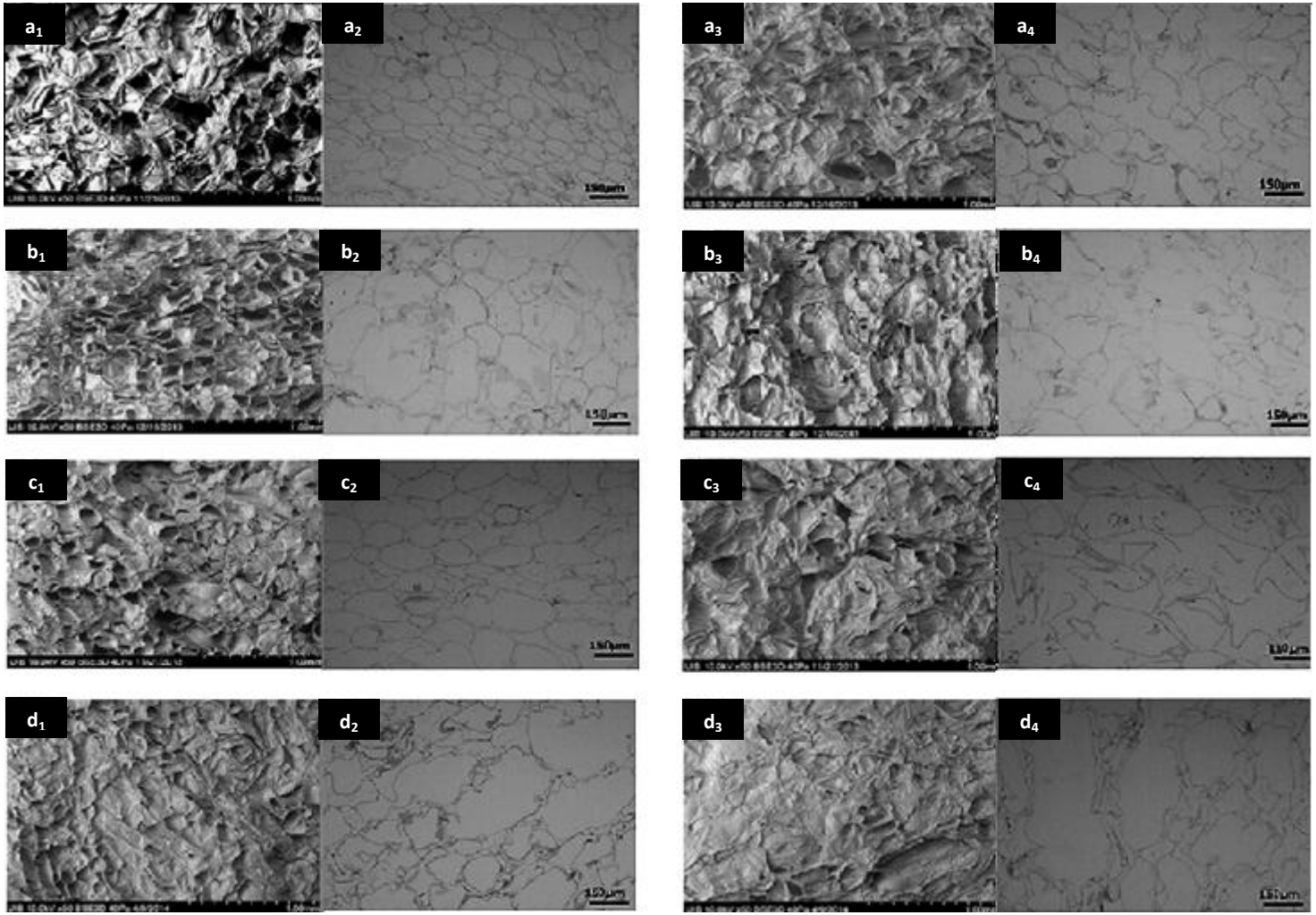
[a]Results expressed in mg GAE/g dm_o. [b]Results expressed in mg trolox/g dm_o. For each analytical method and sample, different lowercase letters indicate significant differences ($p < 0.05$) between different digestion times, and different capital letters indicate significant differences between different processing methods according to pairwise-Wilcox test ($p < 0.05$).

Table 4 Kinetic reaction constants (exponential model), coefficient of determinations (r2) and summed square of residuals (SSE) for TPC and AA (ABTS, CUPRAC and FRAP methods) changes during *in vitro* digestion of raw and processed apple samples.

	TPC			**AA(ABTS)**			**AA(CUPRAC)**			**AA(FRAP)**		
	α (s)	**r^2**	**SSE**	**α (s)**	**r^2**	**SSE**	**α (s)**	**r^2**	**SSE**	**α (s)**	**r^2**	**SSE**
Raw	161800	0.98	0.004	161900	0.98	0.004	32750	0.96	0.002	86700	0.90	0.001
FN	12090	0.99	0.006	8608	0.97	0.030	14760	0.91	0.048	7918	0.94	0.083
FD	14870	0.98	0.008	9580	0.91	0.084	21710	0.95	0.009	5761	0.97	0.067
CD	16020	0.96	0.008	20800	0.95	0.010	13340	0.99	0.002	13310	0.99	0.004

Figure(s)

Fig. 1



Figure(s)

Fig. 2

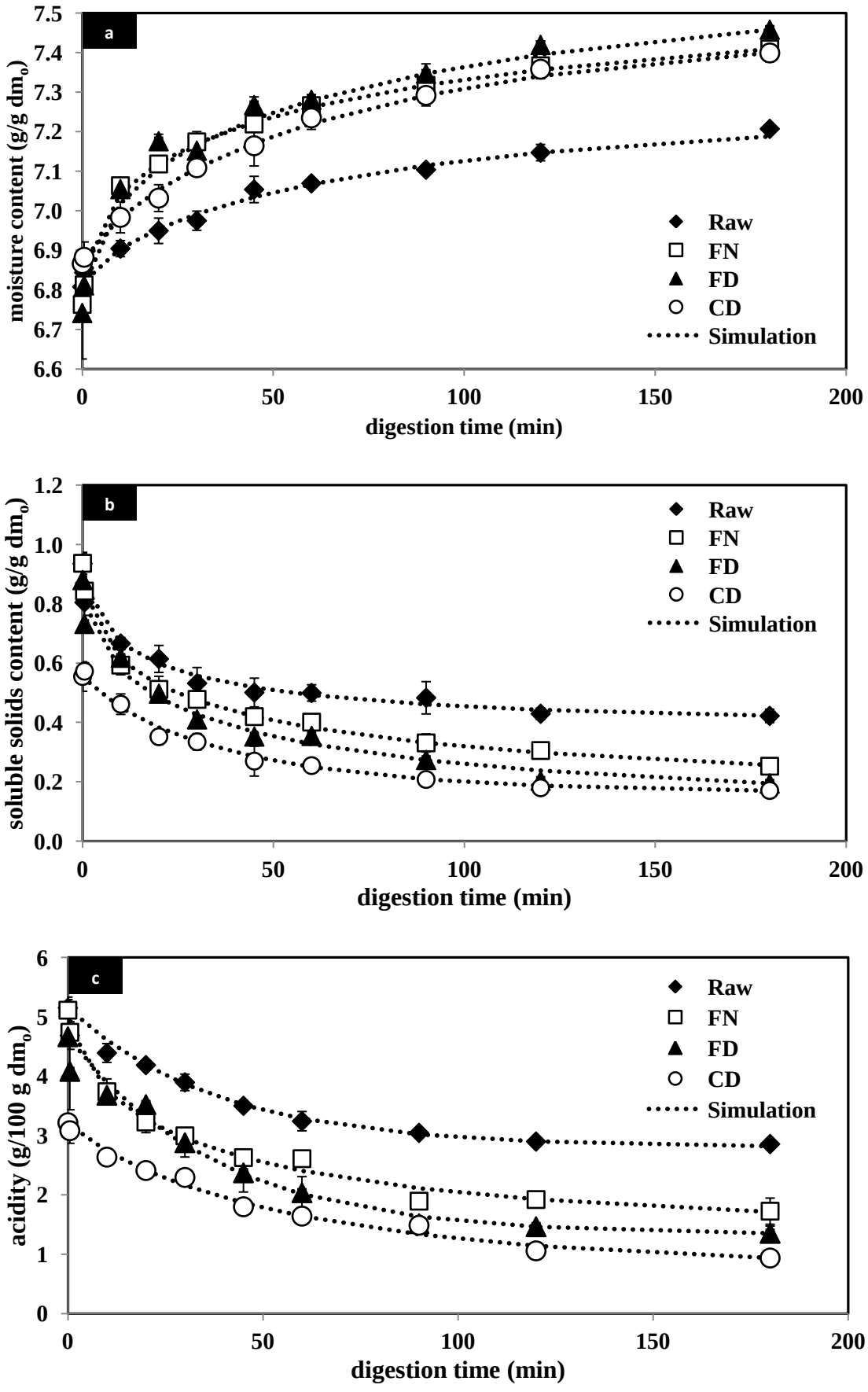


FIGURE CAPTIONS

Figure. 1 Microphotographs of the samples of apple: a-Raw, b-FN, c-FD and d-CD. 1-SEM and 2-LM of initial apple samples (prior to in *vitro* digestion). 3- SEM and 4-LM after 180 min of in *vitro* gastric digestion.

Figure. 2 Evolution of chemical characteristics of raw and processed apple samples during *in vitro* digestion: a-moisture content, b-soluble solids content and c-acidity.