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Effects of freezing, freeze drying and convective drying on *in vitro* gastric digestion of apples

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ABSTRACT

The influence of processing (freezing at −196 °C in liquid N_2, FN sample; freeze-drying at −50 °C and 30 Pa, FD sample; and convective drying at 60 °C 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.

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 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 °C, 5 Pa) and convective drying (2 h at 80 °C followed by 6 h at 60 °C) 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 °C to 70 °C) 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 and Rubiolo (2005) observed that slow freezing rates (<1.5 °C/min) greatly influenced tissue structure and caused water loss in strawberries (*Fragaria x ananassa*). Additionally, Chassagne-Berces 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 °C, 100 Pa) in a microwave vacuum dryer (75–300 W, 5 kPa) resulted in cell wall shrinkage in apples (var. *Red Fuji*). Also, Rodríguez, Santacatalina, et al. (2014) observed cell collapse and cell disruption in apple slices (var. *Granny Smith*) dried with hot air, with more cellular changes occurring at higher (>70 °C) drying temperatures compared to lower drying temperatures (30–60 °C).

In addition to initial composition and quality parameters, processing that results in changes in food nutrient content and cellular

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Nomenclature

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

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 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 °C, 10 min) or blanched (80 °C, 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 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 et al., 2010; Tydeman, Parker, Wickham, et al., 2010).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 °Brix). 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 °C) until the core temperature reached equilibrium with the freezing temperature (~30 s). Once frozen, samples were thawed in a cold chamber at 4 ± 1 °C 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 60 °C with an air velocity of 2 m/s (Garau et al., 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 and Singh (2013). Briefly, simulated saliva was prepared by dissolving 1 g/l mucin, 2 g/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 1 g/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 37 °C. 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, García-Pérez, Rosselló, Femenia, and Simal (2012) with minor modifications. Samples were fixed in formaldehyde (10%), followed by dehydration, embedding in paraffin (60 °C for 3 h), and sectioning into 4–5 μm 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 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 chromatography 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 and 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, AOAC, 1997) and soluble solid content (method no. 932.14C, AOAC, 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 4000 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 et al., 2013; González-Centeno, Comas-Serra, Femenia, Rosselló, & Simal, 2015; Rodríguez, Ortuño, et al., 2014; 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) (González-Centeno et al., 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.

2.8. Data and statistical analysis

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

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

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

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

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

3. Results and discussion

3.1. Cell walls

3.1.1. Microstructural changes (SEM and LM)

Scanning electron microscopy (SEM) and light microscopy (LM) were used to assess the microstructural changes in apple samples as a result of processing and after 180 min of *in vitro* gastric digestion (Fig. 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.

Fig. 1a_1 and a_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, Santacatalina, et al. (2014). After 180 min of digestion (Fig. 1a_3 and a_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, Bootten, Mishra, Monro, and Sims (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.

Fig. 1b_1 and b_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 and Rubiolo (2005). After *in vitro* gastric digestion of frozen apples (Fig. 1b_3 and b_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 (Fig. 1b_4). In addition, cell wall fragments resulting from cell lysis could be observed (Fig. 1b_4).

Fig. 1c_1 and c_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

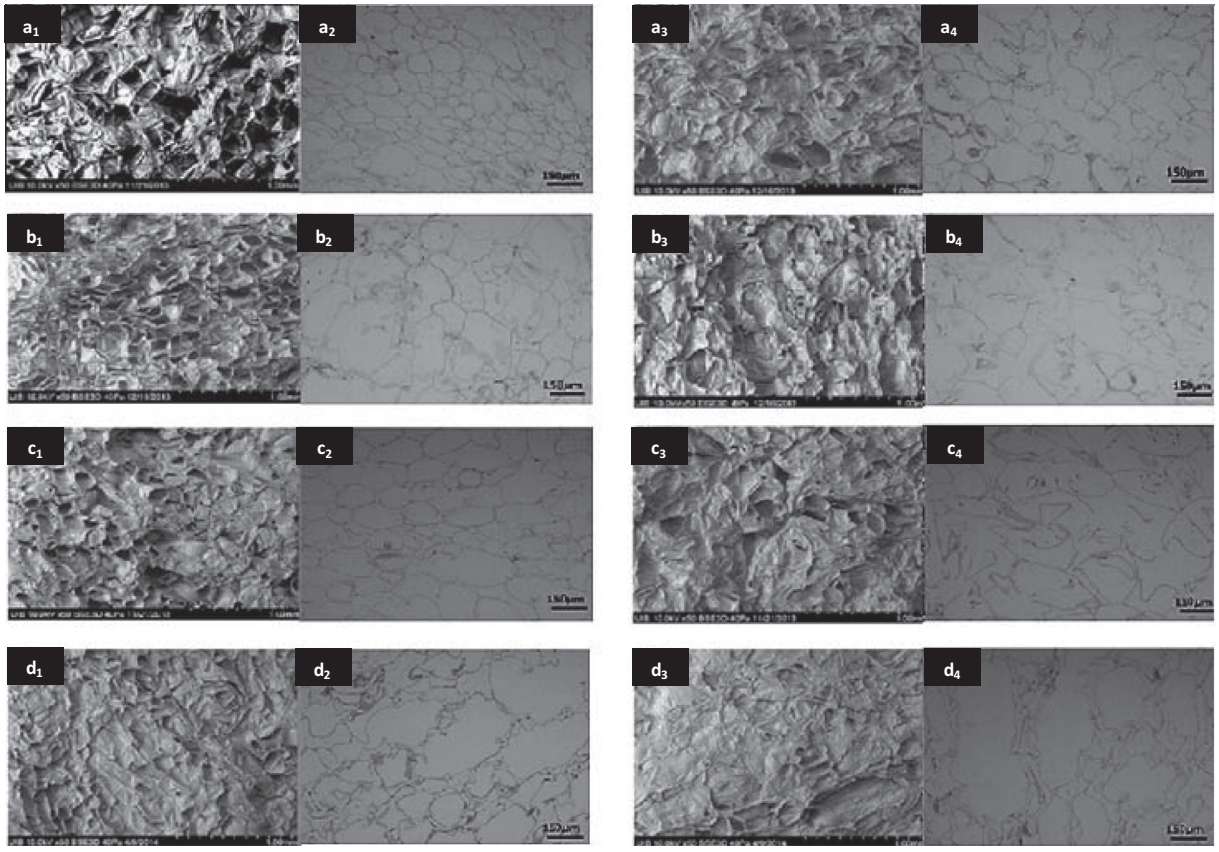

Fig. 1. Microphotographs of the samples of apple: a-Raw, b-FN, c-FD and d-CD. 1-SEM and 2-LM of initial apple samples (prior to *in vitro* digestion). 3-SEM and 4-LM after 180 min of *in vitro* gastric digestion.

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 and 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 (Fig. 1c_3 and c_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 Fig. 1d_1 and d_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 the microstructure of *Granny Smith* apple have been previously studied by several authors (Rodríguez, Santacatalina, et al., 2014; Vega-Gálvez et al., 2012). These authors agreed that during drying, one of the most important phenomena is cell shrinkage, which leads to a major modification of the apple structure and allows the release of water. In addition to the changes that occurring during convective drying, additional cell collapse was visible in CD samples after *in vitro* gastric digestion (Fig. 1d_3 and d_4). Structural modifications that occurred during digestion eliminated the open pores present on the surface and ruptured many of the interior cell walls.

3.1.2. Alcohol insoluble residues (AIRs)

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

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

3.1.3. Analysis of carbohydrate composition

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

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

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

The carbohydrate composition of all processed apples significantly ($p < 0.05$) changed after digestion for most neutral sugars, expect mannose. Additionally, the total sugars significantly decreased after digestion for all processed apples. Frozen and freeze dried apples had similar decreases in total sugars (13–17%

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/100 g dm_o)									
	AIRs (g/100 g 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*	11,500 ± 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*	11,700 ± 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*	11,000 ± 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*

of initial value), and convective dried apples had the greatest decrease in total sugars (38% decrease from initial value) during 180 min of *in vitro* gastric digestion. These decreases in total sugars follow a similar trend as the magnitude of microstructural damage observed in the SEM and LM analyses (Fig. 1), where convective drying resulted in the largest changes in microstructure, and freezing and freeze drying both resulted in significant, but similar, microstructural modifications.

3.2. Chemical characteristics

Fig. 2 shows the changes of chemical characteristics (moisture content, soluble solid content, and titratable acidity) of raw and processed apples during *in vitro* gastric digestion. The initial moisture content (Fig. 2a) of raw apples was of 6.81 ± 0.04 g water/g dm. This is similar to previously reported values for *Granny Smith* apples (6.8–7.7 g water/g dm (Chassagne-Berces et al., 2010)).

The moisture content (Fig. 2a) increased in all samples during *in vitro* gastric digestion, with raw and processed apples following a similar trend. The percentage of moisture content increase of raw, 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, respectively. All processed apples had a greater capacity to absorb water compared to raw apples. This was most likely due to the damage of the cellular structure caused by processing, which facilitated water transport within the food matrix. Previous *in vitro* and *in vivo* studies have reported increases in food moisture content during gastric digestion. For example, Bornhorst, Chang, Rutherfurd, Moughan, and Singh (2013) observed moisture content increases of 75 and 23% dm_o in white and brown rice after 180 min of *in vivo* digestion in pigs, and Bornhorst, Roman, Dreschler, and Singh (2014) observed moisture content increases of 79 and 95% dm_o in raw and roasted almonds after 120 min of *in vitro* digestion.

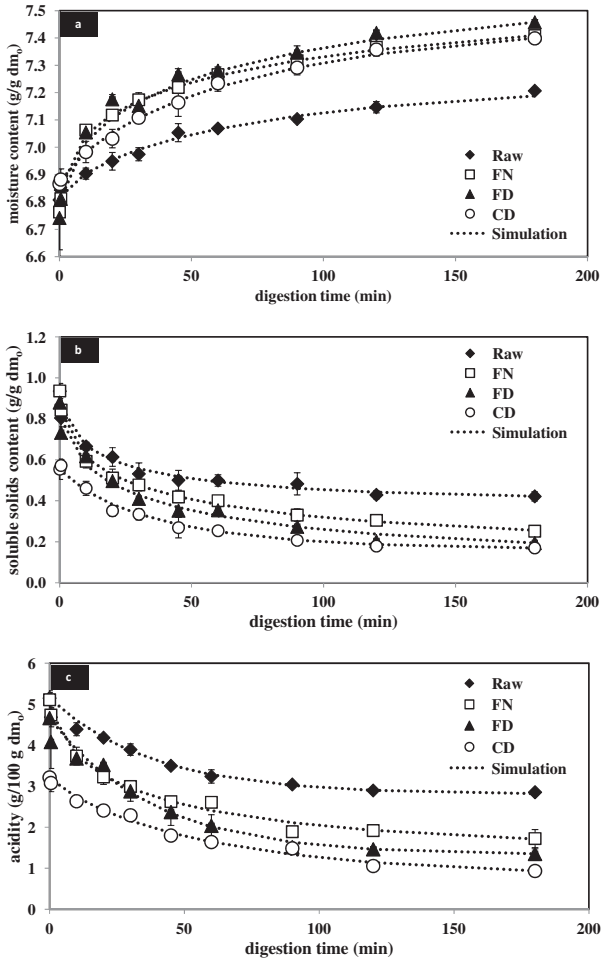

Fig. 2. Evolution of chemical characteristics of raw and processed apple samples during *in vitro* digestion: a-moisture content, b-soluble solids content and c-acidity.

Fig. 2b shows the evolution of the soluble solid content of raw and processed apples during *in vitro* gastric digestion. The initial soluble solid content of raw apples in this study (0.94 ± 0.04 g/g dm_o) was similar but slightly higher than previously reported values for *Granny Smith* apples (0.88–0.90 g/g dm_o (Chassagne-Berces et al., 2010). No significant differences were observed between the soluble solid content of raw and FN samples before *in vitro* digestion. In contrast, FD and CD samples exhibited significant ($p < 0.05$) soluble solid losses (6 and 40% dm_o, respectively) in comparison to raw apples, most likely due to the rehydration step before digestion. These trends are similar to previously reported studies. Chassagne-Berces et al. (2010) also observed no significant effect of freezing on the soluble solid content of *Granny Smith* apples. Schulze, Hubbermann, and Schwarz (2014) reported soluble solid losses (19% dm_o) due to freeze-drying of *Granny Smith* apples. Maldonado, Arnau, and Bertuzzi (2010) reported that the soluble solid content of dried mango decreased by 70% after 100 min of rehydration in water.

As can be observed in Fig. 2b, significant decreases in soluble solid content were observed in 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 apples, respectively). Soluble solid losses were higher in processed samples than in raw samples, the opposite trend as seen in the moisture absorption curves (Fig. 2a).

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

The titratable acidity of all samples significantly decreased during *in vitro* digestion (Fig. 2c), with the greatest decreases occurring during the first 90 min. After 180 min of digestion, the acidity losses were of 44 ± 3, 67 ± 5, 72 ± 4 and 70 ± 3% dm_o, in raw, FN, FD and CD samples, respectively. Similar to the trend observed in soluble solid content decrease during digestion, all processed samples showed significantly ($p < 0.05$) greater acidity losses during digestion compared to raw samples. The trends observed in titratable acidity and soluble solid loss were the same as the decreases observed in total cell wall polysaccharides (Table 1) and the microstructural analysis, where raw < frozen < freeze dried < convective dried in terms of total changes from the initial state. These results suggest that microstructural changes during processing and digestion that prompt damage of cell walls result in losses of intercellular material, such as soluble solids and acid.

The Weibull model was used to mathematically describe the kinetics of moisture, soluble solid, and titratable acidity content in raw and processed apples during *in vitro* digestion. Table 2 shows the Weibull model parameters (α, β, Ceq) with the corresponding confidence intervals and the standard error associated with each estimated parameter. As can be observed, some confidence intervals and standard 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

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}[a]	CI[a]	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

The bold values correspond to the parameters identified with the Weibull model.
[a] g/g dm_o for moisture and soluble solids contents and g/100 g dm_o for acidit.

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 (González-Centeno et al., 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 (Fig. 2a), soluble solids (Fig. 2b) and titratable acidity (Fig. 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 (González-Centeno et al., 2015). When β is equal to 1, the model corresponds to first order kinetics; with a constant input rate (Eim et al., 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 (González-Centeno et al., 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 and 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, Dugalic, Mihaljevic, Jakobek, and Pilizota (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 et al., 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 in TPC was most likely due to the release of polyphenolic compounds from the food matrix during drying.

As can be seen in Table 3, after 3 h of *in vitro* digestion, the TPC of the raw and FN, FD and CD samples significantly decreased by 32 ± 3, 58 ± 4, 55 ± 4 and 47 ± 3% dm_o, respectively. Similar decreases in TPC (from 44.6 to 62.7%) were reported by Bouayed, Hoffmann, and Bohn (2011) in *Jonaprinz, Jonagold, Golden* and *Mutza* apples during *in vitro* gastric digestion. After 180 min of digestion, raw apples retained the greatest amount of polyphenols,

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 ($p < 0.05$).

with the highest TPC (3.0 mg GAE/g dm_o). Interestingly, although CD samples had the highest initial TPC value (5.1 mg GAE/g dm_o), they did not have the highest TPC value after 180 min of gastric digestion (2.7 mg GAE/g dm_o). These results indicate that not only the initial TPC value, but also the structure of the food matrix, may be important in the release of nutrients from food matrices during digestion. Although bioaccessibility was not directly measured in the current study, these results highlight the influence of processing on nutrient release during *in vitro* gastric digestion, and may lead us to hypothesize that differences in bioaccessibility would be obtained as well. However, bioaccessibility determinations were outside the scope of this work, but are an area that merits future investigation.

In order to provide a more complete view of the antioxidant activity (AA) of the samples, three methods were used to evaluate the AA: ABTS, CUPRAC, and FRAP analyses. Due to the fact that each method is based on a different chemical system and/or reaction, different AA results could be expected depending on the specific analysis performed (González-Centeno et al., 2012). The selection of different methods allows a better understanding of the wide variety and range of action of antioxidant compounds present in apples (González-Centeno et al., 2012). The average values for the AA of raw 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 methods, respectively.

As can be observed in Table 3, raw and CD apples had the highest AA values before digestion (0 min), from the CUPRAC and FRAP assays, with no significant differences between them. However, by using the ABTS method, the AA of CD sample was 24% dm higher than that of the raw apples. FN and FD samples exhibited significant decreases ($p < 0.05$) in AA of 57 and 45% dm (CUPRAC assay) and 54 and 47% dm (ABTS assay), respectively, compared to raw apples. However, by using the FRAP method, no significant differences were observed between FN and FD samples, although both FN and FD samples exhibited significant decreases ($p < 0.05$; ~44% dm) compared to raw apples.

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

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

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	161,800	0.98	0.004	161,900	0.98	0.004	32,750	0.96	0.002	86,700	0.90	0.001
FN	12,090	0.99	0.006	8608	0.97	0.030	14,760	0.91	0.048	7918	0.94	0.083
FD	14,870	0.98	0.008	9580	0.91	0.084	21,710	0.95	0.009	5761	0.97	0.067
CD	16,020	0.96	0.008	20,800	0.95	0.010	13,340	0.99	0.002	13,310	0.99	0.004

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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