The effects of sous-vide cooking parameters on texture and cell wall modifications in two apple cultivars: a response surface methodology approach

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Abstract

Cooking ready-to-serve fruits in heat-stable, vacuumized pouches improves their shelf-life and that's the reason the early-2010s have seen a huge increase in the use of vacuum cooking in catering houses, restaurants and homes. One of the major concerns with ready-to-serve cooked fruits is preservation of their textural attributes as texture is an essential factor in the consumers' perception of the quality of agrofood products et more particularly apples. This research work aimed to evaluate the effects of different vacuum cooking parameters on fruit texture of two different apple cultivars ('Mondial Gala' and 'Granny Smith'). Mechanical properties related to fruit resistance were measured throughout an experimental design aiming to evaluate the effects of time and temperature and their interaction on preservation of these attributes. Additionally, since texture modifications during thermal processing of plant-based products arise in part as a result of cell walls disruption, cell wall materials were extracted and analysed from vacuum cooked apples. The regression models showed that both the linear and quadratic effects of the setting temperature of cooking had a significant influence in the mechanical parameters and cell wall properties herein studied, as well as the time at setting temperature. 'Mondial Gala' apples seemed to be more suitable to vacuum cooking, as they displayed a better aptitude to preserve textural properties after high temperatures processing conditions than 'Granny Smith' fruit. This dissimilarity was apparently related to differences in pectin methylesterase-mediated cell wall modifications.

Keywords

Apple; cell-walls; texture; vacuum; cooking;

Introduction

The total world apple production for 2012 was about 75 million tons and its annual growth rate during the last 20 years has been estimated at 3%. Moreover, apples remain the most consumed fruit in Europe (both in fresh and processed forms), thus highlighting its great economic and nutritional relevance. From the point of view of consumer's preference, instead, apple texture represents a major trait (Daillant-Spinnler et al. 1996; Harker et al. 2008). However, this attribute is susceptible to be altered by industrial processing, and particularly after thermal treatments. Several studies have established that thermal treatments modify the structural, mechanical and surface properties of apple products (Anantheswaran et al. 1985; Kim et al. 1993). Anantheswaran et al. (1985) studied the kinetics of thermal degradation of apple texture and showed that both time and temperature significantly changed fruit hardness, cohesiveness and chewiness, and the degree of these modifications was dependent on apple cultivar.

In order to preserve fruit textural attributes during and after thermal processing, several strategies such as the selection of cooking-resistant raw material, the optimisation of cooking parameters or the use of soft cooking techniques can be envisaged. Sous-vide cooking represents one these alternatives, and consists on cooking the raw material under controlled conditions of temperature and time inside heat-stable vacuum pouches (Baldwin 2012), which are subsequently preserved under chill conditions (0-3 °C) until further processing or consumption. Until the late 60's, vacuum was only used to seal and pasteurise commodities to extend its shelf-life. Through the last 10 years, though, sous-vide cooking has emerged as a popular technique and it has been extensively adopted by food industrials and catering services to provide healthy prepared, ready-to-serve meals with preserved organoleptic quality, enhanced safety, improved health-promoting properties and extended

shelf-life because of the mild conditions during processing and the absence of oxygen in the pack, if compared to conventional processing technologies (Gormley and Tamsey 2011; Baldwin 2012).

Some studies have focused on the beneficial effects of sous-vide cooking on the overall final quality and perishability of meat- and fish-based foodstuffs (Picouet et al. 2011; Roldan et al. 2014). Other works have also addressed the impact of this cooking technique on the physico-chemical properties of vegetable-based commodities such as green bean pods (*Phaseoulus vulgaris* L.), carrots (*Daucus carota* L.), and Brussels sprouts (*Brassica oleracea* var. *gemmifera* L.) (Iborra-Bernad et al. 2013; Chiavaro et al. 2012), but a limited number have dealt with the effect of vacuum cooking on physico-chemical characteristics of fruit products (Bourles et al. 2009; Keenan et al. 2012). As regards the use of sous-vide cooking applied to fruits, to our knowledge, no previous works have focused on the combined effects of time and temperature of treatment on the textural attributes of the final products.

Apple fruit texture is remarkably altered during thermal process mainly through the breakdown of cellular membranes and cell walls disassembly resulting from both enzymatic and non-enzymatic modifications in pectin structure and composition (Waldron et al. 2003; De Roeck et al. 2010). A main structural component of pectin in plant cell walls is homogalacturonan, a linear chain of α-(1-4)-linked galacturonic acid residues, which is partially methyl esterified (Brummell and Harpster, 2001; Goulao and Oliveira, 2008). At high temperatures, softening of plant tissues has been mainly attributable to the β-elimination-mediated hydrolysis of uronic acid polymers such as homogalacturonan. This chemical reaction is highly dependent on the degree of methylation (DM), being pectin with high DM more susceptible to β-elimination than pectin with a low DM (Krall and Mcfeeters 1998; Kunzek et al. 1999). For apples, the degree of texture modifications during sous-vide

cooking has been demonstrated to be cultivar-dependent (Bourles et al. 2009). This observation was suggested to arise from dissimilarities in the mechanisms underlying cell wall modifications during the cooking process, but no further research has been carried out to confirm this hypothesis. On the other hand, the complexity of plant tissues makes it difficult to identify with confidence these mechanisms and no conclusive elucidations are agreed to date. Therefore, more research employing models which include texture and cell wall-related modifications are worthwhile to extend knowledge in this area.

In this work, two different apple cultivars ('Mondial Gala' and 'Granny Smith') were subjected to sous-vide cooking under different conditions in order to monitor texture modifications during processing. In that order the response surface methodology (RSM) was used to describe the relationship between the response of the system (fruit mechanical resistance) and a set of independent factors (time, temperature). Additionally, changes in the cell wall composition during the process were also studied with the aim to deepen in the knowledge of the main cell wall-related mechanisms having a role on the textural modifications during sous-vide cooking of apples.

Materials and Methods

Plant material

Apples (*Malus* × *domestica* Borkh.) from the cultivars 'Mondial Gala' and 'Granny Smith' were harvested at commercial maturity (23rd of August and 29th of September 2011, respectively) according to the usual standards for each cultivar (based on colour, starch regression, soluble solids content and titratable acidity) in the experimental station 'La Morinière' (Indre and Loire, France). At harvest, average firmness (N), soluble solids

content (SSC; %) and titratable acidity (TA; g malic acid L^{-1}) values for 'Mondial Gala' apples were 76.5 ± 6.14, 11.5 ± 0.5 and 3.9 ± 0.3, correspondingly. For 'Granny Smith', these values were 71.6 ± 5.69, 11.2 ± 0.3 and 7.2 ± 0.4. The size of apples ranged from 70 to 75 mm diameter in both cultivars. Before further processing and owing to logistics reasons, raw material was cold-stored (4 °C) for 7 days at most. During this storage period, though, no significant changes were observed in firmness, SSC and TA values if compared to harvest (data not shown). Prior to vacuum cooking, defect-free fruits were washed, peeled, cored, vacuum-sealed (-0.1 MPa) into thermo resistant pouches (polyamide/polyethylene; 30 × 40 cm; 80 µm thickness) and cooked at atmospheric pressure. Cooking was carried out by immersion in water in an Auriol type A-5B-E-V (model 50 I 3BE) autoclave. Each pouch contained 10 fruit placed so that optimal heat exchange was allowed. For each cultivar, three pouches were set apart of cooking and served as control. At the end of cooking, pouches were immediately cooled to ambient temperature by submersion in ice water prior to further analysis.

Experimental design

Response surface methodology (RSM) and central composite rotatable design (CCRD) were used to define the experimental conditions during vacuum cooking and to evaluate the impact of cooking conditions on texture and cell wall modifications in apples. RSM was chosen in order to diminish the number of experimental trials needed to assess multiple parameters and their interactions. The factors considered in this work were the temperature of water at the start of cooking (when apples were dipped in the autoclave) (T*i*, X_1), the maximum temperature (setting temperature) of water during cooking (T*m*, X_2) and the time which fruit remained at maximum temperature (setting time) during the final phase of cooking (*t*, X_3). The independent variables levels were chosen on the basis of the standard

cooking procedures carried out by industry (Table 1). In addition, setting temperatures lower than 65 °C, which are not usual in the industry, were also included in order to deepen in the knowledge of the effects of these heating/transient temperatures on texture and cell wall modifications in fruits. The rate of water heating from T*i* to T*m* values was established at 1 ^{o}C min^{-1}.

For a given trial, three pouches containing 10 fruits each were vacuum cooked.

Texture analysis

In order to analyse instrumentally the evolution of textural characteristics of fruits, the compression test was used as this test showed its efficiency to follow thermal degradations of fruits (Anantheswaran et al. 1985) and more precisely vacuum cooked apples (Bourles et al. 2009)

Thus after each cooking/cooling trial, the mechanical properties of apples were analysed by double compression test performed with an universal testing machine (MTS Synergy 200 H; MTS Systems, Créteil, France). Ten fruits randomly selected from the three cooked pouches were analysed for each trial. Briefly, apple slices (2-cm height) obtained from a transversal cut of cooked fruit were compressed twice with a 20% deformation of their height at 20 mm min^{-1} with a 2.5 mm diameter round flat tip. Two measures were made on each fruit slice, and different force-deformation curves were obtained. For each force/deformation curve, two parameters were calculated from the first compression cycle: the hardness, which is related to the first compression maximum force (H1; expressed in N), and the energy (N mm) associated (WH1; expressed in N mm), which corresponded to the positive area obtained in the curve. The results related to the second compression will not be presented as under the most extreme cooking parameters of temperature some trials collapsed and results of second compression cycle were in exploitable (Figure 1).

Comentario [*1]: Je mettrais bien ici une figure présentant un fruit qui est resté entier et un fruit qui s'est destructuré sous la sseconde compression.

Cell wall materials

Samples of flesh tissue were taken from apples cooked in different pouches (five fruits per pouch), stored at -80 °C, freeze-dried, mixed and powdered before analyses. The extraction of cell wall materials (CWM), as alcohol insoluble materials, was carried out according to Renard (2005a), with some modifications. In brief, 3 g of freeze-dried tissue were suspended in 20 mL ethanol (70% v/v) in a 75-mL column equipped with a 20 μm pore size filter. After agitation for 10 min, the mix was vacuum-filtered. The retentate was rinsed twice with ethanol (80% v/v), and 8-10 times with ethanol (60% v/v), until no soluble sugars were detected in the filtrate by the phenol-sulphurique acid procedure (Dubois et al. 1956). The resulting retentate was washed thrice with 20 mL ethanol (96% v/v) and thrice with 10 mL acetone. Afterwards, the residues were freeze-dried for 24 h and weighed. The yield of CWM was expressed as % (w/w) of fresh weight (FW). All extractions were done in duplicate.

For further fractionation, CWM (200 mg) from each replicate were extracted sequentially with 0.05 M ammonium oxalate (pH 5) and 0.05 M NaOH as described previously (Renard 2005b), and the two fractions obtained (Oxal-sf and NaOH-sf, subsequently) were considered to be representative loosely-bound and covalently-bound pectin, respectively. Each fraction was intensively dialysed (mol.wt. cut-off 12,000 Dalton) against distilled water, lyophilised and weighed. Yields are expressed in % (w/w) of CWM.

For further analysis, CWM, ammonium oxalate- and NaOH-soluble fractions were hydrolysed with sulphuric acid as previously explained (Ortiz et al. 2011a). Uronic acid content in the hydrolysate was measured by the *m*-hydroxydiphenyl method (Blummenkrantz and Asboe-Hansen 1973) using galacturonic acid as a standard. The degree of methylation (DM) was determined in the CWM fraction. For this, methanol was

195 determined according to Klavons and Bennett (1986) and DM was calculated as molar ratio
196 of methanol to uronic acid content.
197
198 *Pectin methylesterase activity*
199 A 10% (w/v) tissue homogenate was prepared by homogenising 100 mg of freeze-dried
200 apple flesh in an extraction buffer prepared according to Lohani et al. (2004). Pectin
201 methylesterase (PME: EC 3.1.1.11) activity was measured according to Hagerman and
202 Austin (1986). For the assay, the reaction mixture contained enzyme extract, apple pectin
203 and bromothymol blue prepared as described previously (Alonso et al. 1997). One unit (U)
204 of PME activity was defined as the decrease of one unit of A_{620} min^{-1}. Total protein content
205 in the crude extracts was determined with the Bradford (1976) method, using BSA as a
206 standard. All analyses were done in triplicate, and results were expressed as specific activity
207 (U mg^{-1} protein).
208
209 *Statistical analysis*
210 Results were fitted to second-order polynomial equations (1) using "Statgraphics
211 Centurion XVI" software (StatPoint Technologies Inc., Warrenton, VA, US):
212
213 $$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{22}X_2^2 + b_{23}X_2X_3 + b_{33}X_3^2 \quad (1)$$
214
215 where b_n are the regression coefficients and X_1 (T*i*), X_2 (T*m*) and X_3 (t) the considered
216 independent variables. Analysis of variance (ANOVA) was performed to determine the lack
217 of fit and the significance of the effects of the independent variables. The least significant
218 difference (LSD) Fisher's test ($p \leq 0.05$) was performed for comparison of means calculated
219 for each experimental trial.

Results and Discussion

Influence of multivariate factors on textural attributes of vacuum cooked apples

In order to determine their mechanical properties, slices taken from cooked apples were subjected to a double compression test as exposed above. The related experimental values obtained are shown in Table 2, and significant modifications in the texture of fruits after cooking were observed. According to hardness (H1) values, 'Granny Smith' apples were mechanically more resistant (235 – 281 N) than 'Mondial Gala' (144 – 192 N) at T*m* values between 36 and 48 ºC. However, the inverse was observed at higher setting temperatures, and at T*m* ≥ 82 °C, H1 values in 'Mondial Gala' apples were at least two-fold higher than in 'Granny Smith', as previously reported (Bourles et al. 2009). When setting temperature of cooking was 65 °C, though, little differences were observed in terms of H1 values between cultivars. Similar observations were extracted after the analysis of WH1 values (data not shown), thus suggesting that 'Mondial Gala' apples reveal a better aptitude to vacuum cooking process at cooking conditions normally used by industry.

To better estimate the textural changes occurring during vacuum cooking of fruit, data obtained from compression tests were used to develop regression models by means of RSM. The regression coefficients for the second order polynomial equations corresponding to H1 are shown in Table 3. The statistical analysis indicates that the suggested models were adequate, displaying R^2 values of 90.33 and 93.60 for 'Mondial Gala' and 'Granny Smith' apples, respectively. Actually, it is commonly established that the goodness of models is acceptable if R^2 > 70% (Granato et al. 2014). Moreover, the values of the adjusted regression coefficients (R^2 = 77.90% for 'Mondial Gala' and R^2 = 85.33% for 'Granny

Comentario [*2]: Abel, il faut préciser dans les résultats à quelles conditions experimentales la texture des fruits n'a pas tenue. On perd cette information alors qu'elle est très utile.
Il faut églement que dans le tableau 2 figure l'energie sous la courbe de première compression .

Comentario [*3]: Pourquoi alors qu'on les annonce dans le metareil et methode?

Smith') were also satisfactory, and the *p* values associated to lack of fit, which were higher than 0.05, reinforced the models' goodness. In these models, the linear effects of T*m* (X_2) and time of cooking (X_3) were significant and negative, indicating a better preservation of fruit hardness with lower values of temperature and time during processing, as it was expected. Furthermore, the quadratic effect of T*m* was also significant for both 'Mondial Gala' and 'Granny Smith' cultivars, and accordingly, at high T*m* values, the rate of fruit softening declined progressively (Fig. 1A and 1E). For 'Granny Smith', in addition, the quadratic effects of T*i* and setting time (*t*; X_3) were also significant (Table 3).

Optimization of heat processing

Abel je pense que nous avons oublié un point important dans cet article: presenter les conditions optimales de caisson des deux variétés. Peux tu regarder dans la publie de Iborra-Bernad comment ça a été fait et refaire la même analyse ?

Influence of multivariate factors on cell wall properties of vacuum cooked apples

Since texture modifications during thermal processing of plant-based products arise in part as a result of cell walls disruption (Waldron et al. 2003; Christiaens et al. 2011), cell wall materials (CWM) were extracted from *vacuum* cooked apples. As shown in Table 2, yields of CWM from flesh tissue of *vacuum* cooked 'Mondial Gala' fruit were generally lower than from 'Granny Smith'. As to 'Mondial Gala' apples, the highest CWM yields were obtained in fruit cooked at T*m* = 82 °C, whereas for 'Granny Smith' they corresponded mainly to apples cooked at T*m* = 65 °C. For both cultivars, the CWM related regression models showed adequate fitting parameters, being R^2-adj = 69.64 and 73.52 in 'Mondial Gala' and 'Granny Smith', respectively (Table 3). Similarly to H1, the linear effect of T*m* was significant in the CWM related models for both cultivars. For 'Granny Smith', the

quadratic effect of T*m* (X_2) appeared also to be significant. Besides, neither the linear nor the quadratic effects of setting time for both cultivars were significant in the CWM models, contrarily to H1 (Table 3).

As regard the response surface plots obtained, a lack of parallelism between H1 and CWM regression models was observed in each cultivar (Fig. 1). These plots showed that, contrarily to H1, the yields of CWM did not dropped with increasing setting temperatures (T*m*). Therefore, during *vacuum* cooking of apples, the modifications in the composition and linkages between cell wall polysaccharides, rather than the total amount of CWM, may be a critical factor influencing texture alterations, as also suggested in the case of cold-stored, intact apples (Ortiz et al. 2011b) and pears (*Pyrus communis* L.) (Murayama et al. 2002). So far, in previous works focusing on other thermally processed plant products such as carrots, broccoli (*Brassica oleracea* L.) or peaches (*Prunus persica* L. Batsch), similar conclusions were obtained (De Roeck et al. 2010; Christiaens et al. 2011; Zhang et al. 2012). Then, CWM from cooked apples were further fractionated, and the yields of the pectin-enriched fractions are shown in Table 2. In almost all cases, the yields of Oxal-sf, which represent the fraction enriched in non-covalently (loosely) bound pectins, were higher in 'Mondial Gala' than in 'Granny Smith' apples. The only exception was detected in the case of T*m* = 94 °C, where the contrary was observed. The yields of NaOH-sf, which denote the content of covalently-bound pectins in the walls, were similar among cultivars when the setting temperature was 65 °C or higher, with only a few exceptions. For the rest of the samples (Tm ≤ 48 °C), the yields of NaOH-sf in 'Mondial Gala' apples were lower than in 'Granny Smith', thus indicating that the content of NaOH-sf might be a key factor influencing H1 values in samples cooked at low-mild temperatures (T*m* ≤ 48 °C).

Yields of NaOH-sf in both 'Mondial Gala' and 'Granny Smith' apples were generally lower with increasing T*m* values, and the inverse was observed for Oxal-sf yields (Table 2).

295 In fact, it is generally known that heating normally increases the water- and oxalate-soluble
296 CWM fractions and decreases the acid- and alkaly-soluble fractions (Kunzek et al., 1999).
297 Moreover, these trends were also observed in the response surface plots obtained (Fig. 1).
298 The regression models corresponding to Oxal-sf content ('Mondial Gala' and 'Granny
299 Smith') and NaOH-sf ('Granny Smith') showed that both the linear and quadratic effects of
300 T*m* (X_2) and the linear effect of time at T*m* were significant (Table 3). In the case of NaOH-
301 sf from 'Mondial Gala', in contrast, only the linear effect of T*m* was significant. These
302 findings, together with the cultivar-to-cultivar differences in terms of mechanical properties
303 of *vacuum* cooked apples (Table 2), uphold the above described hypothesis and suggest that
304 texture changes in plant-based produces might have arisen from modifications in cell wall
305 structure and composition, rather than from changes in total amount of CWM, but through
306 different mechanisms depending on the cultivar.
307 The cell wall structural and chemical changes that lead to textural modifications in
308 vegetable tissues include the depolymerisation and solubilisation of pectic polymers
309 involved in cell-to-cell adhesion, as well as readjustments of their associations (Goulao and
310 Oliveira 2008). The depolymerisation of pectic polysaccharides may arise in part from the
311 rupture of the linkages between the neutral sugar-rich side-chains attached to rhamnosyl
312 residues in the rhamnogalacturonan backbones (Brummell and Harpster 2001). Given done
313 that they help to connect covalently the rhamnogalacturonan backbone to other cell wall
314 polymers such as cellulose and xyloglucans (Caffall and Mohnen 2009; Agoda-Tandjawa et
315 al. 2012), the removal of these side-chains might facilitate pectin solubilisation. According
316 to the obtained data (Table 2) and the response surface plots associated to the models (Fig.
317 1), depolymerisation of covalently-bound pectic polysaccharides, as suggested by lowered
318 NaOH-sf yields, augmented with increasing cooking temperatures (T*m*) and may thus have
319 contributed not only to the loss of firmness (Table 2), but also to the general increase in the

yields of Oxal-sf. The yields of Oxal-sf were higher in 'Mondial Gala' than in 'Granny Smith' apples if cooking at T*m* values ≥ 65 °C. At these setting temperatures conditions, in turn, a better retention of textural attributes, as reflected from H1 values, was observed in 'Mondial Gala' fruit (Table 2). Therefore, changes in both the content and properties of Oxal-sf might represent a key factor for texture properties preservation of apples submitted to *vacuum* cooking process at T*m* ≥ 65 °C.

Since they are pectin-enriched fractions, D-galacturonic acid is a main constituent of both NaOH-sf and Oxal-sf. In this work, uronic acids content in the NaOH-sf was consistently higher in 'Mondial Gala' than in 'Granny Smith' apples, while little differences among cultivars were found regarding uronic acids content in the Oxal-sf (data not shown). These uronic acids are partially esterified with methanol at C6 carboxyl group, and the corresponding degree of methylation (DM), together with the degree of polymerisation, has an influence on the mechanical properties of pectins. Further, the DM of pectins can be modified by endogenous pectin methylesterase (PME) activity, which catalyse the enzymatic demethylation of pectin (Brummell 2006) and thus render non-covalently bound pectins not only less vulnerable to depolymerisation through the β-elimination reaction but also more susceptible to be cross-linked by calcium ions naturally present in the tissues. So, this enzyme activity can promote the strengthening of the cell walls (Krall and McFeeters 1998; Brummell and Harpster 2001; De Roeck et al. 2010).

In this work, PME activity in the flesh tissue of *vacuum* cooked 'Mondial Gala' and 'Granny Smith' apples was assessed in all samples, and results showed that if cooked at intermediate setting temperatures (T*m* = 48 – 65 ºC), PME activity in 'Mondial Gala' was up to two-fold higher than in 'Granny Smith' apples (Table 4), while no differences were observed between cultivars when cooked at higher temperatures (Tm ≥ 82 °C). According to the developed regression models (R^2-adj = 84.31 and 85.66 for 'Mondial Gala' and 'Granny

345 Smith', respectively), T*m* was the only variable having significant effects on PME activity
346 (Table 2). As shown in the corresponding response surface plots (Fig. 2), PME activity in
347 'Mondial Gala' apples was optimal at around 65 °C, whereas for 'Granny Smith', this
348 enzyme activity progressively decreased with increasing cooking temperatures. Therefore,
349 the observed lower DM of pectins in 'Mondial Gala' apples cooked at T*m* ≥ 65 °C might
350 partly arise from an enhanced PME activity, if compared to 'Granny Smith' fruit. Along
351 with a higher amount of Oxal-sf in this cultivar (Table 2), it might explain the better texture
352 preservation in *vacuum* cooked 'Mondial Gala' apples at setting temperatures analogous to
353 those applied in the industry (T*m* ≥ 65 °C) (Table 2). Actually, thermal treatments of some
354 plant-based products at mild conditions (generally from 50 to 70 ºC) have also proved to
355 stimulate the catalytic action of pectin methylesterase (PME) and in consequence to prevent
356 an excessive softening of tissues (Ni et al 2005; Anthon and Barrett 2006; Guillemin et al.
357 2008).
358
359
360 **4. Conclusion**
361
362 Response surface methodology has proved a suitable tool for the evaluation of changes
363 in textural parameters and in related cell wall modifications during *vacuum* cooking of
364 'Mondial Gala' and 'Granny Smith' apples. In both cultivars, an overall texture degradation
365 may have arisen from depolymerisation of covalently-bound pectins present in the cell
366 walls. Though, texture degradation in 'Granny Smith' apples resulted more dramatic than in
367 'Mondial Gala' when the setting temperature of cooking was higher than 65 °C, which
368 appoint this latter cultivar more appropriate to *vacuum* cooking than 'Granny Smith'.
369 Contrarily to 'Granny Smith', PME activity in 'Mondial Gala' apples was enhanced at mild

cooking temperatures and as a result pectin in this cultivar displayed a lower degree of methylation. Therefore, the establishment of calcium-mediated linkages between cell wall polymers might have been favoured in 'Mondial Gala' apples, thus reinforcing tissues and improving the preservation textural attributes, if compared to 'Granny Smith' apples. However, in addition to PME-mediated cell wall alterations, other mechanisms underlying cell wall modifications might be also dependent on cultivar, so more research aimed to deepen in the knowledge of texture modifications during thermal processing of fruits should involve both other enzyme activities and more detailed changes occurring in the cell wall.

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FIGURE CAPTIONS

Fig. 1. Response surface plots showing the effect of the maximum temperature of cooking (T_m; X_2) and related setting time (time; X_3) on maximum force associated to first compression (H1) and yields of CWM, Oxal-sf and NaOH-sf obtained from the flesh tissue of *vacuum* cooked 'Mondial Gala' (A-D, respectively) and 'Granny Smith' (E-H, respectively) apples. Initial temperature of water (T*i*; X_1) is fixed at the central point (30 ºC)

Fig. 2. Response surface plots showing the effect of the maximum temperature of cooking (T_m; X_2) and related setting time (time; X_3) on the degree of methylation (DM) of CWM and PME activity in the flesh tissue of *vacuum* cooked 'Mondial Gala' (A-B, respectively) and 'Granny Smith' (C-D, respectively) apples. Initial temperature of water (T*i*; X_1) is fixed at the central point (30 ºC)

Table 1

Three-factor[a], five-level central composite design used for RSM.

Trial[b]	Coded level			Uncoded level		
	X_1	X_2	X_3	T*i*	T*m*	t
1	-1	-1	-1	27	48	24
2	1	-1	-1	33	48	24
3	-1	1	-1	27	82	24
4	1	1	-1	33	82	24
5	-1	-1	1	27	48	36
6	1	-1	1	33	48	36
7	-1	1	1	27	82	36
8	1	1	1	33	82	36
9	-1.68	0	0	25	65	30
10	1.68	0	0	35	65	30
11	0	-1.68	0	30	36	30
12	0	1.68	0	30	94	30
13	0	0	-1.68	30	65	20
14	0	0	1.68	30	65	40
15	0	0	0	30	65	30
16	0	0	0	30	65	30
17	0	0	0	30	65	30

[a] Independent variables: X_1 and T_i, initial temperature of water (°C); X_2 and T_m, maximum temperature of water or setting temperature (°C); X_3 and t, time of remaining at maximum (set) temperature (min).

[b] Experimental trials were carried out randomly.

Table 2

Maximum force (H1) values from compression test and yields of insoluble cell wall materials (CWM) and of pectin-enriched CWM fractions isolated from the flesh tissue of *vacuum* cooked apples.

Trial	Processing parameters[a]			H1[b] (N)	Yields[b] of cell wall fractions		
	T*i* (ºC)	T*m* (ºC)	t (min)		CWM (% FW)	Oxal-sf (% CWM)	NaOH-sf (% CWM)
'Mondial Gala'							
	Control			277.8 ± 21.4 a	1.92 ± 0.09 b	9.75 ± 0.31 a	24.83 ± 0.63 b
1	27	48	24	192.6 ± 15.5 b	1.35 ± 0.10 b	9.98 ± 1.35 a	20.83 ± 3.13 b
2	33	48	24	182.9 ± 19.3 b	1.37 ± 0.08 b	9.41 ± 1.23 a	21.00 ± 2.88 b
3	27	82	24	35.5 ± 4.1 a	1.90 ± 0.21 a	12.42 ± 1.35 a	17.97 ± 2.67 a
4	33	82	24	36.1 ± 5.2 a	1.92 ± 0.18 b	12.54 ± 1.41 a	19.36 ± 2.23 a
5	27	48	36	144.6 ± 13.0 b	1.52 ± 0.13 b	11.38 ± 1.22 a	20.50 ± 1.70 b
6	33	48	36	138.5 ± 13.2 b	1.56 ± 0.16 b	10.95 ± 0.99 a	20.62 ± 2.02 b
7	27	82	36	28.9 ± 3.4 a	1.89 ± 0.20 a	14.57 ± 1.60 a	17.67 ± 1.38 a
8	33	82	36	29.5 ± 4.5 a	1.86 ± 0.23 b	12.59 ± 1.39 a	16.88 ± 1.42 a
9	25	65	30	35.7 ± 2.9 a	1.76 ± 0.15 b	10.58 ± 0.98 a	21.92 ± 1.91 a
10	35	65	30	35.8 ± 3.4 a	1.71 ± 0.17 b	11.36 ± 1.03 a	20.99 ± 1.28 a
11	30	36	30	184.5 ± 20.1 b	1.48 ± 0.19 a	9.09 ± 0.79 a	24.80 ± 0.87 b
12	30	94	30	19.2 ± 2.8 a	1.76 ± 0.08 b	13.58 ± 0.88 b	14.83 ± 1.05 a
13	30	65	20	41.8 ± 3.8 b	1.81 ± 0.20 b	9.69 ± 0.87 a	21.21 ± 1.57 b
14	30	65	40	25.8 ± 4.6 a	1.69 ± 0.24 b	14.05 ± 1.37 a	20.59 ± 1.92 a
15	30	65	30	45.8 ± 4.0 a	1.70 ± 0.11 b	10.03 ± 1.01 a	22.20 ± 1.68 a
16	30	65	30	40.2 ± 2.9 a	1.66 ± 0.14 b	8.99 ± 0.75 a	20.39 ± 2.00 a
17	30	65	30	29.6 ± 3.0 a	1.61 ± 0.13 b	9.24 ± 10.5 a	20.14 ± 2.04 a
'Granny Smith'							
	Control			269.0 ± 18.2 a	2.19 ± 0.11 a	3.28 ± 0.20 b	28.66 ± 0.91 a
1	27	48	24	281.4 ± 25.7 a	1.73 ± 0.14 a	3.68 ± 0.45 b	24.56 ± 1.80 a
2	33	48	24	247.6 ± 19.2 a	1.95 ± 0.22 a	4.21 ± 0.39 b	25.17 ± 2.36 a
3	27	82	24	14.5 ± 2.0 b	1.93 ± 0.17 a	10.55 ± 1.25 b	20.51 ± 1.87 a
4	33	82	24	15.0 ± 1.8 b	2.17 ± 0.23 a	10.75 ± 1.68 b	19.42 ± 1.51 a
5	27	48	36	235.9 ± 22.7 a	1.95 ± 0.21 a	4.91 ± 0.59 b	24.33 ± 2.50 a
6	33	48	36	192.1 ± 21.3 a	2.02 ± 0.16 a	4.25 ± 0.48 b	24.32 ± 2.65 a
7	27	82	36	13.3 ± 2.0 b	1.98 ± 0.16 a	12.41 ± 1.13 b	19.70 ± 2.05 a
8	33	82	36	12.3 ± 1.6 b	2.01 ± 0.18 a	12.38 ± 1.07 b	19.63 ± 1.98 a
9	25	65	30	30.5 ± 3.5 a	2.08 ± 0.13 a	5.21 ± 0.58 b	23.56 ± 1.38 a
10	35	65	30	38.8 ± 4.1 a	2.06 ± 0.12 a	5.29 ± 0.31 b	23.04 ± 1.23 a
11	30	36	30	279.0 ± 25.9 a	1.60 ± 0.09 a	4.31 ± 0.29 b	28.28 ± 1.99 a
12	30	94	30	4.0 ± 0.8 b	2.01 ± 0.07 a	20.70 ± 1.60 a	14.05 ± 1.85 a
13	30	65	20	77.5 ± 8.6 a	2.00 ± 0.21 a	5.77 ± 0.44 b	24.81 ± 3.10 a
14	30	65	40	15.2 ± 2.5 a	2.07 ± 0.24 a	9.54 ± 0.90 b	20.78 ± 2.63 a
15	30	65	30	37.5 ± 4.0 a	2.07 ± 0.19 a	5.38 ± 0.69 b	23.47 ± 1.74 a
16	30	65	30	22.3 ± 3.1 b	2.16 ± 0.19 a	6.17 ± 0.71 b	22.61 ± 1.53 a
17	30	65	30	15.7 ± 2.3 b	2.09 ± 0.22 a	5.67 ± 0.55 b	22.99 ± 1.61 a

[a] Independent variables: X_1 and T_i, initial temperature of water (°C); X_2 and T_m, maximum temperature of water or setting temperature (°C); X_3 and t, time of remaining at maximum (set) temperature (min).

[b] Values represent means ± SD of twenty (H1) or two (yields of fractions) replicates. Data followed by different letters within a column for a given trial are significantly different at $p \leq 0.05$ (LSD test).

Table 3

Estimated regression coefficients of the fitted second-order polynomial equation for selected compression test parameters, yields of insoluble cell wall materials (CWM) and of pectin-enriched CWM fractions in the flesh tissue of *vacuum* cooked apples.

Regression coefficients[a]	H1 (N)	Yields of cell wall fractions			DM (%)	PME (U mg prot^{-1})
		CWM (% FW)	Oxal-sf (% CWM)	NaOH-sf (% CWM)		
'Mondial Gala'						
b_0	2050.17	2.01336	76.059	-7.94099	239.506	-303.289
b_1	-56.9034	-0.127163	-3.47128	1.0127	-4.49773	11.013
b_2	-21.7323**	0.0384657**	-0.15916*	-0.100438*	-1.86828**	5.32452**
b_3	-20.7972*	-0.00122672	-0.909142*	1.42564	-2.27645	3.91456
b_{11}	0.884929	0.00236925	0.0663209	-0.0245579	0.0730263	-0.206986
b_{12}	0.0417647	-0.000144619	-0.00212037	0.015484	-0.000245098	0.0182598
b_{13}	0.0247222	-0.000211676	-0.0136048	-0.0154704	0.00402778	0.00798611
b_{22}	0.108423**	-0.0000649108	0.00251422*	-0.00276884	0.00958381*	-0.0489213**
b_{23}	0.0970098	-0.000535884	-0.000892439	-0.00499658	0.00362745	-0.0158456
b_{33}	0.202229	0.000726442	0.0254557*	-0.0115305	0.0282738	-0.051943
R^2 (%)	90.33	82.34	92.50	84.47	95.93	93.14
R^2-adj (%)	77.90	69.64	82.85	64.50	90.70	84.31
p-lack of fit	0.0511	0.1155	0.3332	0.3045	0.27	0.08
'Granny Smith'						
b_0	3710.93	-5.55125	-4.68574	30.7515	185.613	238.832
b_1	-106.921	0.182677	2.50033	-0.480849	-2.34134	-11.1774
b_2	-38.5352***	0.0690462*	-0.862082***	0.342695***	-1.38246**	-0.319003**
b_3	-38.6569*	0.151502	-0.60167*	-0.209446*	-1.90509	0.443674
b_{11}	1.59124*	-0.00149929	-0.03755	0.00952056	0.0166338	0.184605
b_{12}	0.188897	-0.0000560732	0.000759733	-0.00678086	0.00321078	0.0114461
b_{13}	-0.0799306	-0.00254827	-0.00977209	0.00970221	0.038125	-0.0197917
b_{22}	0.180198**	-0.000367591**	0.00770235**	-0.00231679*	0.00774811*	-0.00369538
b_{23}	0.118836	-0.000491787	0.00272661	-0.0006386	0.00121324	-0.0148897
b_{33}	0.51286*	-0.000654951	0.0142191	-0.00259299	0.00970722	0.0133497
R^2 (%)	93.60	88.42	97.43	89.68	93.78	93.72
R^2-adj (%)	85.37	73.52	94.12	76.42	85.79	85.66
p-lack of fit	0.0520	0.2748	0.0951	0.0557	0.46	0.11

[a] Subscripts: 1 = initial temperature of water (°C); 2 = maximum temperature of water or setting temperature (°C); 3 = time of remaining at maximum temperature (min).

*Significant at 0.05 level. **Significant at 0.01 level. ***Significant at 0.001 level.

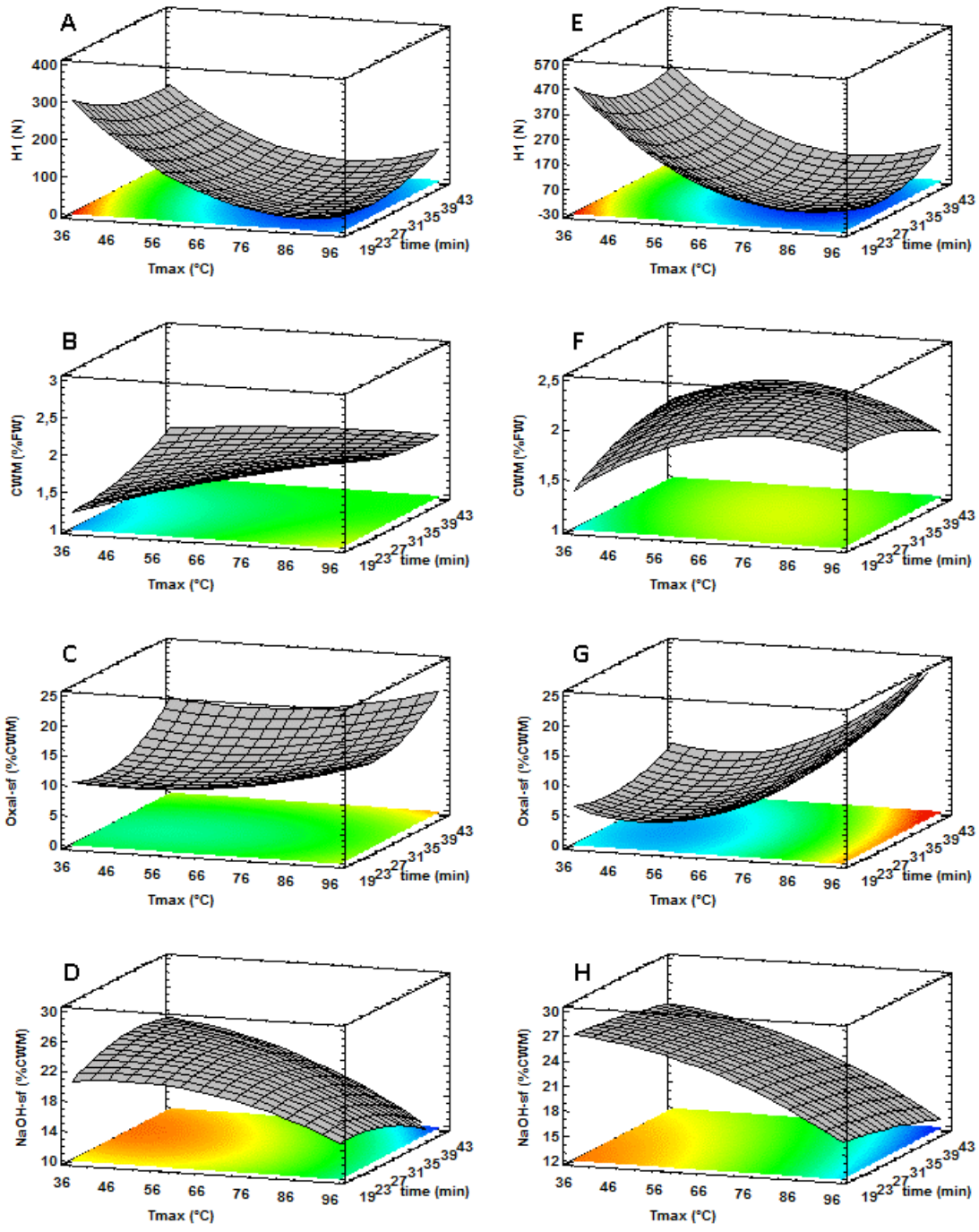
Table 4

Degree of methylation (DM) of insoluble cell wall materials (CWM) fraction and pectin methylesterase (PME) specific activities in the flesh tissue of *vacuum* cooked apples.

Trial	Processing parameters[a]			DM (%)[b]	PME act. (U mg^{-1} protein)[b]
	T*i* (ºC)	T*m* (ºC)	t (min)		
'Mondial Gala'					
	Control			75.40 ± 3.18 a	40.15 ± 2.60 a
1	27	48	24	73.15 ± 5.94 a	63.98 ± 4.31 a
2	33	48	24	72.24 ± 5.50 a	61.35 ± 3.59 a
3	27	82	24	53.21 ± 3.31 b	24.36 ± 2.66 a
4	33	82	24	51.43 ± 4.12 b	25.20 ± 3.14 a
5	27	48	36	71.42 ± 4.82 a	65.56 ± 5.10 a
6	33	48	36	69.98 ± 3.99 a	63.25 ± 4.56 a
7	27	82	36	52.14 ± 5.00 b	19.22 ± 1.28 a
8	33	82	36	51.47 ± 4.66 b	20.89 ± 1.79 a
9	25	65	30	56.29 ± 4.31 b	59.32 ± 3.61 a
10	35	65	30	58.87 ± 3.46 b	61.26 ± 4.05 a
11	30	36	30	76.16 ± 7.25 a	43.13 ± 3.63 a
12	30	94	30	50.95 ± 3.84 b	8.01 ± 0.76 a
13	30	65	20	62.55 ± 5.40 b	58.31 ± 5.14 a
14	30	65	40	54.65 ± 4.33 b	62.23 ± 3.52 a
15	30	65	30	57.60 ± 3.91 b	61.69 ± 3.11 a
16	30	65	30	58.04 ± 4.51 b	60.21 ± 2.80 a
17	30	65	30	54.88 ± 3.97 b	65.55 ± 3.48 a
'Granny Smith'					
	Control			79.17 ± 4.51 a	42.52 ± 3.71 a
1	27	48	24	76.14 ± 4.30 a	53.94 ± 4.20 b
2	33	48	24	75.25 ± 3.47 a	54.55 ± 3.43 b
3	27	82	24	67.90 ± 5.57 a	19.87 ± 2.04 a
4	33	82	24	65.24 ± 4.89 a	21.01 ± 1.85 a
5	27	48	36	75.47 ± 4.68 a	56.25 ± 3.13 b
6	33	48	36	74.90 ± 5.03 a	53.63 ± 3.96 b
7	27	82	36	65.30 ± 4.41 a	14.30 ± 1.41 a
8	33	82	36	67.81 ± 4.04 a	15.82 ± 1.32 a
9	25	65	30	66.84 ± 3.70 a	36.99 ± 2.63 b
10	35	65	30	68.02 ± 4.12 a	37.80 ± 2.98 b
11	30	36	30	79.52 ± 8.13 a	48.17 ± 2.40 a
12	30	94	30	67.16 ± 4.66 a	11.18 ± 0.91 a
13	30	65	20	70.12 ± 3.10 a	38.99 ± 2.85 b
14	30	65	40	65.87 ± 4.20 a	29.12 ± 2.17 b
15	30	65	30	67.31 ± 3.79 a	36.58 ± 2.42 b
16	30	65	30	69.50 ± 4.52 a	34.05 ± 3.03 b
17	30	65	30	66.67 ± 4.23 a	32.16 ± 2.54 b

[a] Independent variables: X_1 and T_i, initial temperature of water (°C); X_2 and T_m, maximum temperature of water or setting temperature (°C); X_3 and t, time of remaining at maximum (set) temperature (min).

[b] Values represent means ± SD of three replicates. Data followed by different letters within a column for a given trial are significantly different at $p \leq 0.05$ (LSD test).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H1 (N)
400
300
200
100
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36 46 56 66 76 86 96
Tmax (°C)
19 23 27 31 35 39 43
time (min)
E
H1 (N)
570
470
370
270
170
70
-30
B
CWM (%FW)
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CWM (%FW)
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Figure 1

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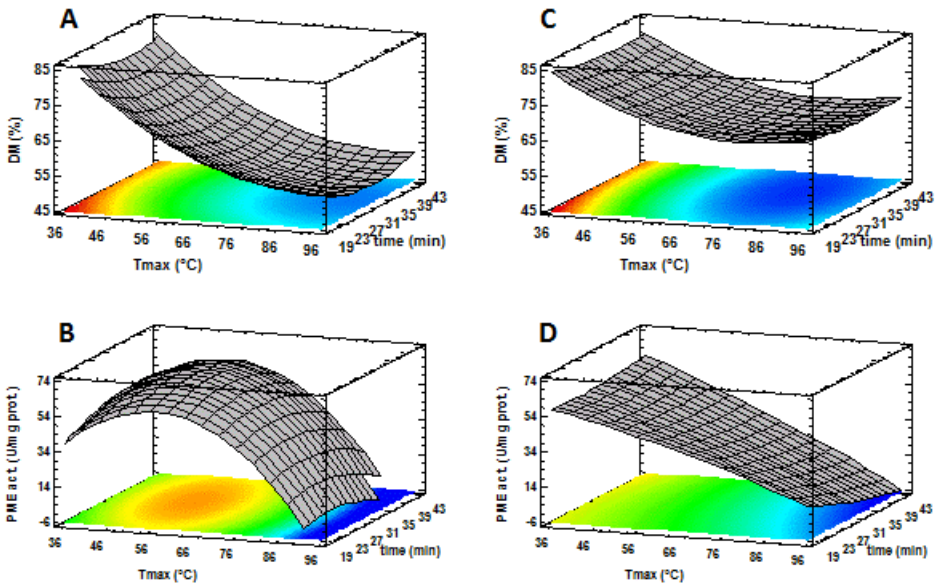

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538 **Figure 2**

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