



HAL
open science

Lab and pilot-scale ultrasound-assisted water extraction of polyphenols from apple pomace

Daniela Pingret, Anne-Sylvie Fabiano-Tixier, Carine Le Bourvellec-Samour, Catherine M.G.C. Renard, Farid Chemat

► To cite this version:

Daniela Pingret, Anne-Sylvie Fabiano-Tixier, Carine Le Bourvellec-Samour, Catherine M.G.C. Renard, Farid Chemat. Lab and pilot-scale ultrasound-assisted water extraction of polyphenols from apple pomace. *Journal of Food Engineering*, 2012, 111, pp.73-81. 10.1016/j.jfoodeng.2012.01.026 . hal-02650064

HAL Id: hal-02650064

<https://hal.inrae.fr/hal-02650064>

Submitted on 29 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>

Contents lists available at [SciVerse ScienceDirect](http://www.sciencedirect.com)

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Lab and pilot-scale ultrasound-assisted water extraction of polyphenols from apple pomace

Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Carine Le Bourvellec, Catherine M.G.C. Renard, Farid Chemat*

INRA, UMR408 Sécurité et Qualité des Produits d'Origine Végétale, F-84000 Avignon, France

Université d'Avignon et des Pays de Vaucluse, UMR408 Sécurité et Qualité des Produits d'Origine Végétale, F-84000 Avignon, France

ARTICLE INFO

Article history:

Received 7 August 2011

Received in revised form 23 January 2012

Accepted 26 January 2012

Available online 6 February 2012

Keywords:

Caffeoylquinic acid

Flavonols

Malus × domestica Borkh

Procyanidin

Ultrasound

Water extraction

ABSTRACT

Apple pomace, a residue from juice or cider production, shows high content of exploitable polyphenols. In this work, apple pomace was submitted to an Ultrasound-Assisted Extraction (UAE) in order to produce extracts rich in antioxidants. After a preliminary study, a solid/liquid ratio of 150 mg of dry material per mL was used, and optimized conditions obtained by response surface methodology for polyphenols water-extraction were 40 °C, 40 min and 0.764 W/cm². A comparison showed Total Phenolics Content (TPC) obtained by UAE was 30% higher than the content obtained by Conventional Extraction (CE) (555 and 420 mg of catechin equivalent per 100 g of dry weight, respectively) and both methods presented the same extraction kinetics. Furthermore, extracts obtained by ultrasound showed higher antioxidant activity, which was confirmed by HPLC analysis, that revealed main polyphenols were not degraded under the applied conditions. The large scale experiments of this ultrasound procedure showed a potential industrial application.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Apples (*Malus × domestica* Borkh.) are known to contain many types of phenolic acid derivatives and flavonoids with high nutritional value, which are present particularly at high concentrations in cider apples (Sanoner et al., 1999; Wijngaard and Brunton, 2010). Apple pomace, the solid waste resulting from industrial processing of apple juice or cider production, is rich in extractable polyphenols (Cao et al., 2009; Cetkovic et al., 2008; Kołodziejczyk et al., 2009; Virost et al., 2010). The quality and amount of pomace produced (which can represent 20–30% of the weight of processed apples) is directly related to the technology used in the apple juice extraction. The polyphenols extracted from apples present numerous biological activities, such as antiallergic activity (Akiyama et al., 2000; Kanda et al., 1998), *in vivo* anticarcinogenic activity (Yanagida et al., 2000), and *in vitro* and *in vivo* inhibitory activity against some enzymes and receptors (Shoji et al., 2000).

Some of the polyphenols in the apple pomace present a high exploitable industrial potential as dietary or food antioxidant, exhibiting 2–3 times DPPH-scavenging activity and 10–30 times superoxide scavenging activity compared to vitamins C and E (Lu and Foo, 1997; Lu, 2000). Polyphenols in apple pomace also showed antiviral properties against *Herpes simplex* virus from methanolic

extracts (Suárez et al., 2010). The safety of those polyphenols has also been evaluated and confirmed (Shoji et al., 2004). The content of phenolic compounds in the pomace is higher than the content in the juice and varies amongst different varieties of apples (Guyot et al., 1998, 2003; Cetkovic et al., 2008; Van der Sluis et al., 2002; Price, 1999; Kołodziejczyk et al., 2009). The main polyphenol class in apple pomace is procyanidins. The hydroxycinnamic acid derivatives are mainly represented by chlorogenic acid (5'-caffeoylquinic acid). Phloridzin (major constituent of dihydrochalcones) was thought to be a specific component to apples (Mangas et al., 1999), however, further studies have shown this compound is also present in strawberries (Hilt et al., 2003). Compared to the apple fruit, apple pomaces are richer in procyanidins, due to interactions with the polysaccharides (Le Bourvellec et al., 2007), and in flavonols and dihydrochalcones due to their location in the peel and pips, respectively (Guyot et al., 1998); in addition, they contain lower concentrations of hydroxycinnamic acids and catechins.

Ultrasound has been used for various processes in the chemical and food industry. The technique is fast, consumes less fossil energy and permits the reduction of solvents, thus resulting in a more pure product and higher yields. This method has been applied to extract food components such as aromas (Caldeira et al., 2004; Xia et al., 2006), antioxidants (Ma et al., 2009; Rodrigues et al., 2008; Wang et al., 2008; Virost et al., 2010), pigments (Chen et al., 2007; Barbero et al., 2008) and other organic and mineral components from a variety of matrices. Ultrasound plays an important role as real potential

* Corresponding author.

E-mail address: farid.chemat@univ-avignon.fr (F. Chemat).

sustainable technique for industrial applications for polyphenols extraction (Khan et al., 2010). The cavitation process that occurs during sonication causes the rupture of cell walls, consequently enhancing solvent contact with available extractable cell material (Vinatoru, 2001).

The purpose of the present work was to evaluate the effects of ultrasound-assisted extraction of polyphenols obtained from dried apple pomace using an aqueous buffer as extraction solvent at mild temperatures. The extraction conditions (ultrasound intensity, temperature and extraction time) were optimized in order to obtain optimum polyphenol content using a response surface methodology. Comparative studies between ultrasound and conventional maceration were done for extraction kinetics, antioxidant tests such as lipid peroxidation activity and treating large amount for large scale experimentations. Finally, ultrasound effect on polyphenols molecules was also evaluated for three isolated polyphenolic compounds in order to verify the innocuousness of ultrasound technology.

2. Materials and methods

2.1. Plant material and chemicals

Apple pomace was obtained from Val-de-Vire Bioactives (Conde-sur-Vire, France) and kept in the dark until use. Standards of chlorogenic acid, (–) epicatechin and phloridzin, were purchased from Sigma Aldrich (St. Louis, USA). Other chemicals were of analytical grade and purchased from VWR International (Darmstadt, Germany).

2.2. Extraction procedures

In all extraction procedures, a 50 mM malate buffer in a pH 3.8 was used in order to mimetize fruit's conditions. To determine the optimal extraction conditions, the solid/liquid ratio was evaluated in function of total polyphenols obtained by a conventional maceration method. The samples subjected to extraction ranged from 5 to 35 g of dry material. The experiments were performed in flasks containing 100 mL of the buffer in a RT-10 magnetic stirrer plate (IKAMAG, Germany) over 8 h in the dark. Samples were then pressed using a manual press and filtered before analysis with a 0.45 µm mesh filter. The total polyphenols content (TPC) was measured using Folin–Ciocalteu's reagent and results are expressed in mg of catechin equivalent per 100 g of dry weight. All experiments were carried out in triplicates.

Ultrasound-assisted extractions (UAE) were performed in an ultrasonic extraction reactor PEX1 (R.E.U.S., Contes, France) with 14 × 10 cm internal dimensions and maximal capacity of 1 L, equipped with a transducer at the base of jug operating at a frequency of 25 kHz with maximum input power (output power of the generator) of 150 W. The double-layered mantle (with water circulation) allowed the control of extraction temperature by cooling/heating systems. Considering the actual input power from the device is converted to heat which is dissipated in the medium, calorimetric measurements were performed to assess actual ultrasonic power, calculated as shown in the Eq. (1) below (Toma et al., 2011).

$$P = m \cdot C_p \cdot \frac{dT}{dt} \quad (1)$$

Where C_p is the heat capacity of the solvent at constant pressure ($J g^{-1} °C^{-1}$), m is the mass of solvent (g) and dT/dt is temperature rise per second. Then, the applied ultrasonic intensity (UI) was calculated using the calculated power as shown in the Eq. (2). (Tiwari et al., 2008).

$$UI = \frac{4P}{\pi D^2} \quad (2)$$

Where UI is the ultrasonic intensity ($W cm^{-2}$), P is the ultrasound power (W) as calculated by the equation 1, and D is the internal diameter (cm) of the ultrasound reactor. To the 500 mL of malate buffer (50 mM pH 3.8), 75 g of dried apple pomace were added and submitted to extraction and the obtained extracts were filtered with a 0.45 µm mesh filter before been lyophilized (for HPLC analysis) or analyzed for TPC. Conventional extraction was performed by agitation in the same conditions for comparison. All experiments were carried out in triplicates.

2.3. Isolated compounds study

In order to verify whether antioxidants present in the extracts undergo degradation during sonication, the following isolated compounds were submitted to ultrasound treatment: (–) epicatechin, phloridzin and chlorogenic acid. These compounds (in a final concentration of 0.5 mg/mL) were diluted in 2 mL of methanol and then introduced in the ultrasonic extraction reactor with 200 mL of malate buffer (50 mM pH 3.8) followed by ultrasound treatment in the optimized conditions. The extractions were subsequently observed in the UV spectrophotometer (Spectronic Genesys 5, Thermo Fischer Scientific, France) at respective characteristic wavelengths for each molecule and then analyzed by HPLC-DAD for quantification purposes. All experiments were carried out in triplicates.

2.4. Total phenolics determination (TPC)

TPC was determined using Folin–Ciocalteu reagent (Singleton and Rossi, 1965). In a test tube, 50 µL of the filtered sample were mixed with 1 mL of a 10% Na_2CO_3 solution and 250 µL of Folin–Ciocalteu reagent. The absorbance was determined using a spectrophotometer (Spectronic Genesys 5, Thermo Fischer Scientific, France) after 1 h at 765 nm against a calibration curve. The results were expressed in mg of catechin equivalent per 100 g of dry weight.

2.5. Identification of phenolic compounds by HPLC-DAD

Polyphenols were measured by HPLC after re-dissolution of the freeze-dried extracts in acidic methanol (1% acetic acid, v/v), or after thioacidolysis as described previously (Guyot et al., 2001), followed by filtration (PTFE, 0.45 µm). A Waters HPLC apparatus (Milford, MA, USA) was used, a system 717 plus autosampler equipped with a cooling module set at 4 °C, a 600 E multisolvent system, a 996 photodiode array detector, and a Millennium 2010 Manager system. The column was a Purospher RP18 endcapped, 5 µm (Merck, Darmstadt, Germany). The solvent system was a gradient of solvent A (aqueous acetic acid, 25 mL/L) and solvent B (acetonitrile): initial, 3% B; 0–5 min, 9% B linear; 5–15 min, 16% B linear; 15–45, 50% B linear, followed by washing and reconditioning the column. HPLC peaks were identified on chromatograms according to their retention times and their UV–visible spectra by comparison with available standard compounds as described by Guyot et al. (2001). Quantification is performed by reporting the measured integration area in the calibration equation of the corresponding standard. Phloretin and phloretinxyloglucoside were calculated as phloridzin equivalent, all flavonols were quantified against quercetin (molar responses, then their respective contents of glycosides are used to calculate concentrations in g/L or g/kg). Total flavonols and total polyphenols were the sums of the corresponding compounds, quantified by HPLC. The average degree of polymerization of flavan-3-ols was calculated as the molar ratio of all the flavan-3-ols units (thioether adducts plus

terminal units) to (–)-epicatechin and (+)-catechin corresponding to terminal units.

2.6. Antioxidant activity: inhibition of linoleic acid peroxidation

A freshly prepared 2.55 mM solution of linoleic acid (2 mL) in a pH 7.4 phosphate buffer with 100 mM of NaCl containing 10 mM SDS (sodium dodecyl sulfate) were placed at 37 °C in the spectrometer cell. At time zero, 25 µL of a freshly prepared 80 mM solution of AAPH (2,2'-azobis(2-amidinopropane)) in the same buffer was added (Roche et al., 2005). After 15 min, 25 µL of an antioxidant solution were added in MeOH. The experiments were repeated with different phenol concentrations (1 mM and lower). The initial level of hydroperoxides (molar absorption coefficient at 234 nm = 26 100 M⁻¹ cm⁻¹) were below 2% in all experiments. The uninhibited and inhibited peroxidation rates were calculated from the slope of the absorbance at 234 nm versus time before and after antioxidant addition using fixed time intervals. All experiments were carried out in triplicates. Standard deviations were lower than 10%.

2.7. Experimental design

Results of preliminary investigations showed the volume of solvent to be used in the extraction (thus, the solid:liquid ratio) affect the extraction of polyphenols due to an insufficient interaction between the solvent and the matrix. This parameter had an influence on the applied ultrasonic intensity, since a minimum of free liquid is necessary to the functioning of the apparatus. In addition, the temperature and sonication duration have an interaction in the experiment since the ultrasonic energy input tends to increase the temperature of the medium, and both parameters have a direct influence in the yield of extracted polyphenols. Therefore, results of preliminary studies showed polyphenols yield is mainly dependent on the ratio of solvent to sample, the extraction time, the temperature and the ultrasonic intensity.

In order to investigate the influence and relevance of the operating parameters required during extractions, a Central Composite Design (CCD) was used to analyze total polyphenol content (TPC) and extract main polyphenols. Three independent factors (namely temperature (*T*), sonication duration (*t*) and Ultrasonic intensity (*UI*)) were evaluated, as well as eventual interaction between these variables.

The full uniformly routable CCD presents the following characteristics (Bezerra et al., 2008): (1) total number of experiments (*N*) are given $N = k^2 + 2k + cp$, where *k* is the number of variables and *cp* is the number of replicates of the central point; (2) The star points are at a distance α from the center of the design and α -values are calculated by $\alpha = 2(k-p)/4$; and (3) all factors are studied in five levels ($-\alpha, -1, 0, +1, +\alpha$). Therefore, in the case of three variables, the number of experiences is 20, the number of replicates of the central point in 6 and the α -value is 1.68.

Preliminary experiments allowed us to distinguish the variables implied in the model at five separated coded levels: $-\alpha$ ($= -1.68$), -1 , $0, +1, +\alpha$ ($= +1.68$). The limit values of each variable range were chosen as function of limitations of ultrasonic apparatus (minimum and maximum power available in the device), temperature of extraction for polyphenols (which might degrade above 40 °C) and time of sonication. Values are presented on Table 1 and involved a total of 20 experiments; including six replications at the centre point to evaluate experimental error measurement, and randomized to avoid effects of extraneous variables. Variables were coded according to the following Eq. (3), where X_i is the coded value, x_i , the real value of a variable, \bar{X}_i , the real value of a variable at the center point, and Δx_i , the step change:

Table 1

Variables involved in the Central Composite Design (CCD) and response obtained for TPC.

No	UI (W/cm ²) ^a	Temperature (°C)	Sonication time (min)	TPC ^b
1	0.431	16	45	370
2	0.575	10	30	315
3	0.719	16	45	381
4	0.719	16	15	306
5	0.431	16	15	288
6	0.335	25	30	360
7	0.575	25	55	384
8	0.575	25	30	368
9	0.431	34	45	384
10	0.575	25	30	393
11	0.575	25	5	257
12	0.719	34	15	370
13	0.719	34	45	448
14	0.431	34	15	382
15	0.575	25	30	380
16	0.575	25	30	383
17	0.575	25	30	379
18	0.575	25	30	367
19	0.575	40	30	460
20	0.764	25	30	393

^a UI: ultrasonic intensity.

^b mg catechin eq/100 g MS.

$$X_i = \frac{x_i - \bar{x}_i}{\Delta x_i} \quad (3)$$

Experimental data for predicting TPC have then been represented using a second order polynomial Eq. (4) as follows:

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n \beta_{ij} X_i X_j, \quad (4)$$

Where: *Y* is the response variable TPC (mg of catechin equivalent per 100 g of dried apple pomace sample), β_0 is the average response obtained during replicated experiments of the CCD, β_i ; β_{ii} ; β_{ij} are the linear, quadratic and cross-product effects, respectively, X_i and X_j are the independent coded variables. The results were analyzed using the Statgraphics XV[®] software.

2.8. Kinetics studies

The extracts obtained were analyzed with a mathematical model derived from Fick's second law (Herodez et al., 2003). The extraction of polyphenols from apple pomace follows first-order kinetics (Spiro and Jago, 1982), which can be represented as follows:

$$C_t = C_\infty (1 - e^{-kt}) \quad (5)$$

Where C_t is the concentration of total polyphenols at time *t*, C_∞ is the final concentration of total polyphenols and *k* is the apparent first-order rate constant of extraction.

When $\ln(C_\infty/[C_\infty - C])$ is plotted against time, the points fall on two intersecting straight lines, the first with a relatively steep slope and the second with a relatively shallow one. The points of intersection of $\ln(C_\infty/[C_\infty - C])$ vs. *t* plots for the fast and the slow stages are termed transition points.

3. Results and discussion

3.1. Solid–liquid ratio

To determine the optimum solid/liquid ratio, total polyphenol compounds and the liquid absorbing capacity of the apple pomace were considered, as represented in Fig. 1. From this figure it is

Table 2
ANOVA for TPC in the CCD.

Source	Sum of squares	Df	Mean squares	f-ratio	p-value
U: ultrasonic intensity	2966.43	1	2966.43	5.84	0.0362
T: temperature	38446.5	1	38446.5	75.72	0.0000
t: sonication time	33210.4	1	33210.4	65.41	0.0000
U ²	14.83	1	14.83	0.03	0.8677
U.T	224.084	1	224.084	0.44	0.5215
U.t	1225.13	1	1225.13	2.41	0.1514
T ²	653.003	1	653.003	1.29	0.2832
T.t	1653.12	1	1653.12	3.26	0.1013
t ²	11470.2	1	11470.2	22.59	0.0008
Total error	5077.52	10	507.752		
Total (corr.)	95724.2	19			

$R^2 = 0.947$; R^2_{adj} (adjusted for Df) = 0.899.

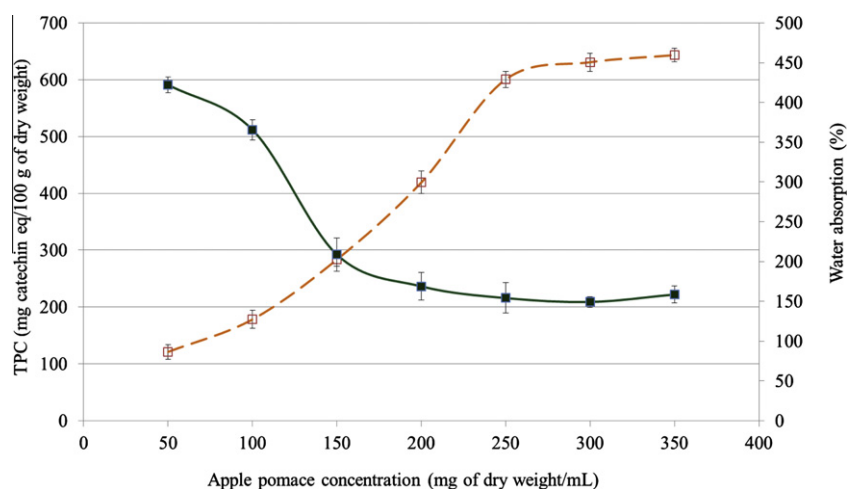


Fig. 1. Optimization of solid/liquid ratio for apple pomace extraction by water: polyphenol concentration in the extract (TPC) (□) and water absorption (■).

possible to observe that the optimum ratio was 150 mg of dry material/mL. For concentrations above 200 mg/mL the dry pomace absorbed all of the available liquid, increasing in volume. Since the ultrasound apparatus requires a minimum amount of free solvent for extraction procedures, a combination of high TPC yields and higher amount of available solvent was chosen. The 150 mg/mL ratio results are corroborated by the values achieved by earlier studies such as Viroit et al. (2010) who used ethanol as extraction solvent of dry apple pomace.

3.2. Experimental design studies

Three key variables that affect extraction of phenolic compounds were studied in a central composite design: namely, ultrasonic intensity, temperature and sonication duration. Ultrasonic intensity ranged from 0.335 to 0.764 W/cm². The chosen ultrasonic intensity limits were function of regulation limitations in the ultrasonic apparatus. Since appropriate temperature setting is necessary to avoid destruction of organic compounds as well as provide an efficient application of ultrasound (ultrasound effects are known to decrease with temperatures higher than 40–50 °C), moderate temperatures were chosen with a range of 9.9–40 °C. Also, the increase in cavitation phenomena is directly proportional to the increase in the system temperature. However, at too high temperatures a decrease in shock waves is observed, diminishing the effect of ultrasounds (Lorimer and Mason, 1987). At last, polyphenols might undergo degradation at temperatures higher than 40 °C, especially when combined to ultrasounds (Kyi et al., 2005;

Svitelska et al., 2004); therefore, a maximum temperature of 40 °C was chosen. Finally, the sonication time range chosen (from 5 to 55 min) was relatively short yet competitive with conventional extraction, showing a potential future industrial application. Since after a certain time cavitation bubbles do not continue to absorb energy to grow and collapse (Ozcan, 2006), and the usual time used for ultrasound-assisted extraction in the industry are usually not longer than 60 min (Chemat et al., 2011), 55 min was chosen as maximum limit. These three controlled variables were studied in a multivariate study with 20 experiments as shown in the Table 1.

3.2.1. Results for TPC

Coded experiments and responses obtained for each run of the central composite design are presented on Table 2. The responses varied widely in function of parameters settings of experiments (from 257 to 460 mg of catechin equivalent per 100 g of dry weight). Significance and suitability of the design were then studied using a variance analysis (ANOVA, Table 2). Statistical significance of each effect (including interaction terms, linear and quadratic T² effects) was tested by comparing the mean square against an estimate of the experimental error. Depending upon the degree of freedom (Df) involved, F-ratio can be calculated (ratio of the mean squared error to the pure error). With a confidence level of 95%, F-ratio significance can be evaluated using the p-value column (significant effects have been typed in bold). Four effects were found significant at a 95% confidence level in the experimental domain studied. This observation can also be pointed out on a Pareto chart of standardized effects, presented

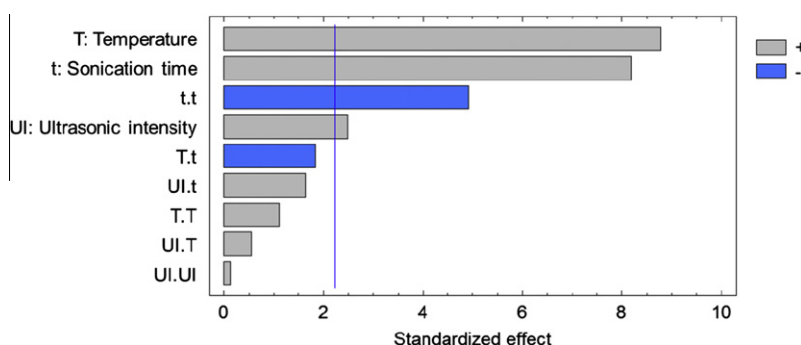


Fig. 2. Standardized Pareto chart of optimization multivariate study.

on Fig. 2. Linear effects of the three key variables (*UI*, *T*, *t*) appear to be highly significant, as well as the quadratic effect of the sonication time (t^2).

The lack of significance of the cross-product terms (*UI.T*, *UI.t*, *T.t*) suggests the absence of interactions between variables. The experimental data obtained from the CCD allowed us to determine an empirical relationship linking response studied (TPC) and key variables involved in the model (in coded units). Thus, a second order polynomial equation was obtained:

$$Y = 154.0 + 0.98 UI + 5.98T + 10.99t - 0.12t^2$$

Where, *Y* represents TPC (expressed in mg of catechin equivalent per 100 g of dry weight), *UI* represents the applied ultrasonic intensity, *t* is the sonication time and *T*, the temperature in coded units. Only significant variables were shown ($p < 0.05$). The applied model appears to be adequate for our experimental results at the 95% confidence level. More than 94% of the variability of responses was explained (R^2 statistics > 0.94), asserting a good accuracy and ability of the established model within the limits of the range used (Mirhosseini et al., 2008). R^2_{adj} is a regression coefficient adjusted for the number of coefficient involved in the model; it allows comparison between models with different numbers of independent variables and allows testing the level of suitability to the regression coefficient. Its value (> 0.89) indicates a high degree of correlation between observed and predicted data.

3.2.2. Optimization of ultrasound-assisted extraction

A graphical representation can be introduced in order to visualize the significant relationship linking levels of variables and response studied (TPC). Fig. 3 depicts three-dimensional plots, each plot highlighting the response behavior function of two variables with the third variable fixed to its central point. The most influential variables are the linear terms of sonication duration (*t*) and temperature (*T*): TPC increases linearly as sonication time and temperature increase. The same effect has been noticed with ultrasonic intensity (*UI*) but with a less predominant influence as observed in the Pareto chart. A slight influence of quadratic effect of sonication time (t^2) is also illustrated on these surfaces (presence of weak surface curvature when sonication time increases), Fig. 3a and c optimal settings for TPC maximization were 0.764 W/cm² for ultrasonic intensity, 40 °C for temperature and 40 min for sonication duration. The TPC yield (555 mg of catechin equivalent per 100 g of dry weight) predicted by the model was verified experimentally using the optimized settings. Similar results were obtained by Viriot et al. (2010) for ultrasound-assisted extraction optimization using ethanol 50% (0.142 W/g, 40 °C and 45 min), while other techniques like pressurized fluid extraction and manual maceration resulted in optimized conditions of ethanol 60% and 102 °C (Wijngaard and Brunton, 2009) and ethanol 56% at 80 °C for 31 min or acetone 65% at 25 °C for 60 min (Wijngaard and Brunton,

2010), respectively. This shows the importance of optimizing parameters when a modification is done in one or various parameters such as solid/liquid ratio, temperature or solvent. Our extracts were obtained and optimized for water extraction, showing the viability of this procedure with great yields using water as solvent.

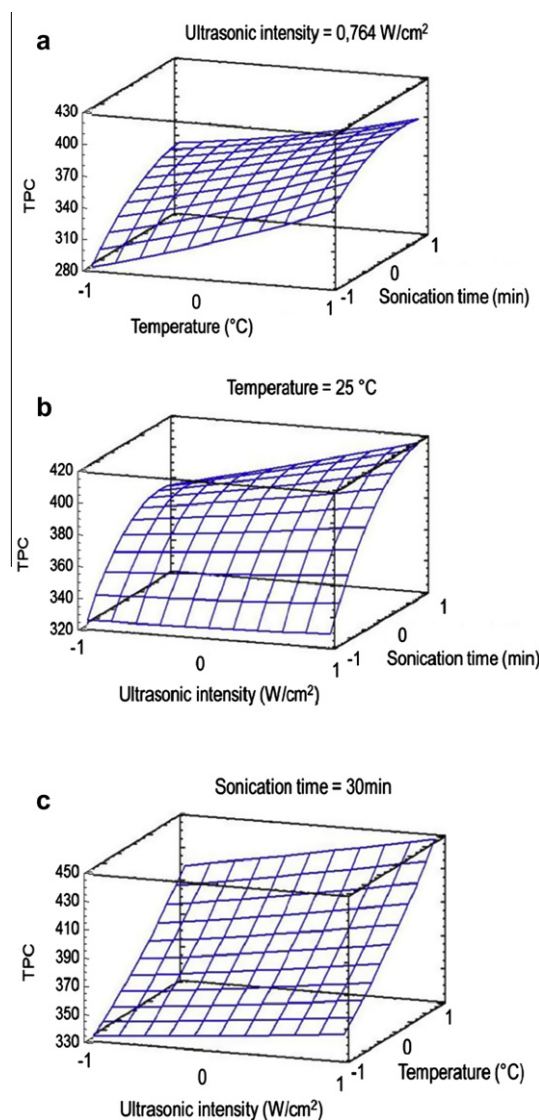


Fig. 3. Optimization of ultrasound-assisted apple pomace extraction by water: TPC investigation in the multivariate study: (A) TPC as a function of ultrasonic intensity and sonication time, (b) TPC as a function of ultrasonic intensity and temperature, and (c) TPC as a function of temperature and sonication time.

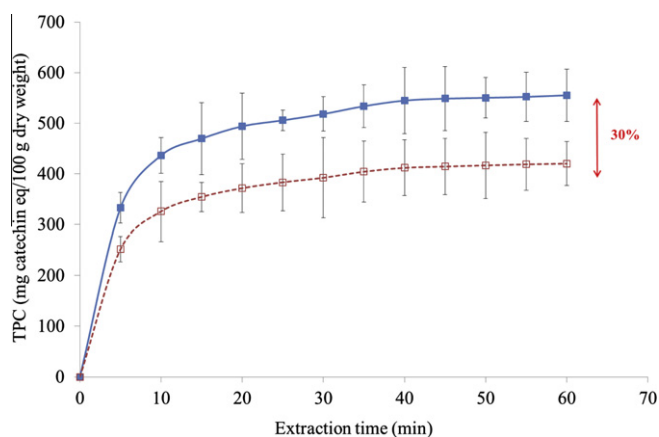


Fig. 4. Comparison between conventional (CE-□) and ultrasound-assisted extraction (US-●).

3.3. Comparison and kinetic studies

To evaluate the impact of ultrasound-assisted extraction in optimized conditions obtained from the response surface method, a comparison study was carried out between ultrasound and conventional extractions (Fig. 4; Table 3). From Fig. 4, it is possible to observe that ultrasound-assisted extraction increased in TPC yield by more than 30% (420 and 555 mg of catechin equivalent per 100 g of dry weight for conventional and ultrasound-assisted extraction, respectively). The comparison shows a clear improvement of the extraction, which is attributed to ultrasonic cavitation, since this is the only variable of treatment that differs in both experiments.

From the Table 3 it is possible to observe that extracts are rich in catechin monomers and phenolic acids, while residues are poor in phenolic acids and rich in procyanidins with a slight increase of the respective DP, which might be attributed to interactions between those compounds and the plant cell wall since the greater the DP, stronger the interaction (Le Bourvellec et al., 2007).

The ultrasound extracts are richer in phenolic acids than the conventional extracts, mainly for PCQ. Also, monomers were better extracted than polymers, which was expected since the extraction was performed in an aqueous medium. In the case of the dihydrochalcones, since they are more present in the seeds, the grinding has a lot of influence. In our work, no grinding was used, which explains the greater amount in the residue compared to the extracts. Flavonols were not well extracted, which could be solved by a pre-treatment of apple peels. Phloridzine was not well recovered, probably due to its polarity, even though dihydrochalcones were better extracted by ultrasounds than by the conventional technique. Yields on ultrasound assisted extraction are greater for catechin, epicatechin and flavonols, which implies a partial destructuring of the apple epidermis, suggesting a not full destructuring of apple epidermis by ultrasounds in this naturally resistant fraction. As for flavonols, yields are variable, possibly due to their solubility in the buffer. Ultrasounds increase extract yield in 6–8% for dihydrochalcones and phenolic acids, although results suggest the bonds between polyphenols and polysaccharides were not broken, since polyphenols present a strong interaction with apple cell walls (Le Bourvellec et al., 2004; Le Bourvellec and Renard, 2005; Le Bourvellec et al., 2009). This amount of retention of polyphenols has already been observed in the literature (Kołodziejczyk et al., 2009).

Both extractions (conventional and optimized) follow first-order kinetics, with a fast period from 0 to 10 min and a slow period from 10 to 40 min of extraction as represented in the Fig. 5 together with their respective coefficients. Indeed, the coefficients at the fast period are of 0.162 min⁻¹ for the ultrasound and 0.158 min⁻¹ for the conventional extraction; while for the slow period, the coefficients are of 0.088 min⁻¹ for the ultrasound and of 0.085 min⁻¹ for the conventional extraction. Since the difference between the coefficients for both equations were not significant, it is possible to conclude that the ultrasound treatment did not change the kinetics of the extraction, even though the extract yield for the ultrasound treatment is more important, which can be explained by the cavitation phenomena.

Some studies on the effects of ultrasound-assisted extraction using electronic microscopy (Veillet et al., 2010) showed that the cavitation phenomena is responsible for modifications on the plant

Table 3
Yields and polyphenol composition of apple pomace and its water extracts obtained by conventional and ultrasound-assisted optimized extraction. Yields are in % dry matter, and polyphenol composition in mg/kg of dry weight. The values in italics are the yields recorded for individual components.

	Yields (g/g DW)	Flavans-3-ols				Dihydrochalcones		Phenolic acids		Flavonols						TPC		
		Monomers		PCA	XPL	PLZ	CQA	pCA	Rut	Hyp	Iso	Rey	Gua	Avi	Qc		SUM	
		CAT	EC	PCA														DP
Initial Pomace	1.00	52	244	3408	3.6	142	1008	960	94	10	122	42	54	161	24	40	453	6360
Conventional extract	0.27	114	383	1249	3.1	180	1014	1321	133	22	150	52	56	169	24	38	511	4905
Conventional residue	0.70	37	193	4132	4.4	103	928	545	50	10	147	47	66	201	32	45	549	6537
Optimized extract	0.28	153	477	1335	4.0	199	1093	1399	141	27	211	71	80	242	35	53	721	5517
Optimized residue	0.69	40	197	4304	4.7	108	1050	602	52	10	157	49	69	205	33	47	570	6923
pSTD		30	88	283	0.6	14	91	99	10	4	30	10	11	31	4	6	97	360
Initial Pomace	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Conventional extract	0.59	0.42	0.10	0.34	0.27	0.37	0.38	0.63	0.33	0.34	0.28	0.28	0.26	0.25	0.30	0.21		
Conventional residue	0.50	0.55	0.85	0.51	0.64	0.40	0.38	0.74	0.85	0.78	0.85	0.87	0.93	0.79	0.85	0.72		
Optimized extract	0.83	0.55	0.11	0.39	0.30	0.41	0.42	0.80	0.49	0.48	0.41	0.42	0.40	0.37	0.45	0.24		
Optimized residue	0.53	0.56	0.87	0.53	0.72	0.43	0.39	0.76	0.89	0.81	0.88	0.88	0.92	0.80	0.87	0.75		

CAT: (+)-catechin; EC: (-)-epicatechin; PCA: procyanidins; DP: number average degree of polymerisation; XPL: phloretinxyloglucoside; PLZ: phloridzin; CQA: 5'caffeoyl-quinic acid (chlorogenic acid); pCA: paracoumaroylquinic acid; Rut: rutin (quercetin-3-O-rutinoside); Hyp: Hyperoside (quercetin 3-O-galactoside); Iso: Isoquercitrin (quercetin 3-O-glucoside); Rey: reynoutrine (quercetin 3-O-xyloside); Gua: guajaverin (quercetin 3-O-arabinopyranoside); Avi: avicularin (quercetin 3-O-arabinoside); Qc: quercitrin (quercetin 3-O-rhamnoside); pSTD: pooled standard deviations.

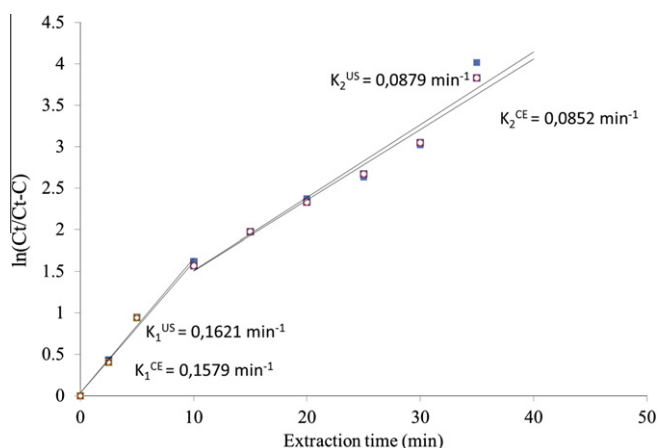


Fig. 5. Kinetics and respective constants for conventional (CE-□) and ultrasound-assisted extraction (US-■).

material inducing disruption of the cells, due to the burst of the cavitation bubble on the surface of the matrix (Vinatoru, 2001). Studies can be done directly in the cell wall to verify the state before and after extraction, nevertheless due to the heterogeneity and complexity of our matrix (apple pomace), cytological or histological studies of these samples would not provide reliable statistical results.

3.4. Antioxidant activity

The antioxidant activity was evaluated for both conventional and ultrasound-assisted extraction carried out in the optimized conditions. The experiments were monitored by UV/VIS spectroscopy by recording the accumulation of the lipid hydroperoxides ($\lambda_{max} = 234 \text{ nm}$) in the absence of antioxidant (constant peroxidation rate R_p^0) and in the presence of the antioxidant (initial peroxidation rate R_p). The IC_{50} parameters (antioxidant concentration corresponding to 50% inhibition, i.e. $R_p/R_p^0 = 0.5$) were calculated for both samples. Sonicated extracts present a lower IC_{50} ($4.90 \mu\text{M}$), representing a better antioxidant activity for those samples when compared to the activity of extracts obtained from maceration ($7.05 \mu\text{M}$). Quercetin presented an IC_{50} value of $0.58 \mu\text{M}$.

3.5. Ultrasound effects on extracted molecules

In order to verify the innocuousness of ultrasound, three isolated compounds of apple pomace (namely (-)-epicatechin, phloridzin and chlorogenic acid) were submitted to the optimized ultrasound extraction conditions. The degradation of these isolated products was evaluated comparing the initial mass to quantified final mass after treatment using HPLC-DAD (Fig. 6) against standards. These compounds were chosen due to their high concentration in the apple and/or importance of application, like phloridzin, which is mainly present in the apple fruit. We observed no specific reaction products after ultrasound treatment. For chlorogenic acid 97.6% of the initial mass was quantified after US treatment, against 94.7% for epicatechin and 99.2% for phloridzin. This loss of 5% in weight can be due to experimental error.

3.6. Large scale ultrasound extraction

While conventional procedures such as maceration are often time and/or energy consuming, ultrasound-assisted extraction provides numerous advantages from an industrial perspective. Ultrasound as a food processing technology has shown large commercial large scale application, with high returns on capital investment (with the break-even point about 4 months). Improvements in product efficiency, process enhancement and low maintenance cost are achievable on a commercial scale. Also, depending on the application, the required energy is comparable to other operation units currently utilized in the industry (Patist and Bates, 2008; Paniwnyk et al., 2009). Only 40 min in water (a green environmental solvent) are needed to recover polyphenols from apple pomace with higher yields compared to conventional extraction procedures. The recycling of an industrial byproduct such as apple pomace using a rapid technique consuming less energy is advantageous from an environmental point of view. For this purpose, a pilot study was performed in a 30 L extraction tank (Fig. 7) consisting of a quadruple output of ultrasound at 25 kHz and 4×200 Watts in the optimum conditions obtained from the previous experiments. Polyphenol yields in the ultrasound extraction were comparable to the lab scale experiments and 15% higher when compared to the conventional procedure using maceration (560 mg catechin equivalent per 100 g of dry weight for sonicated samples against

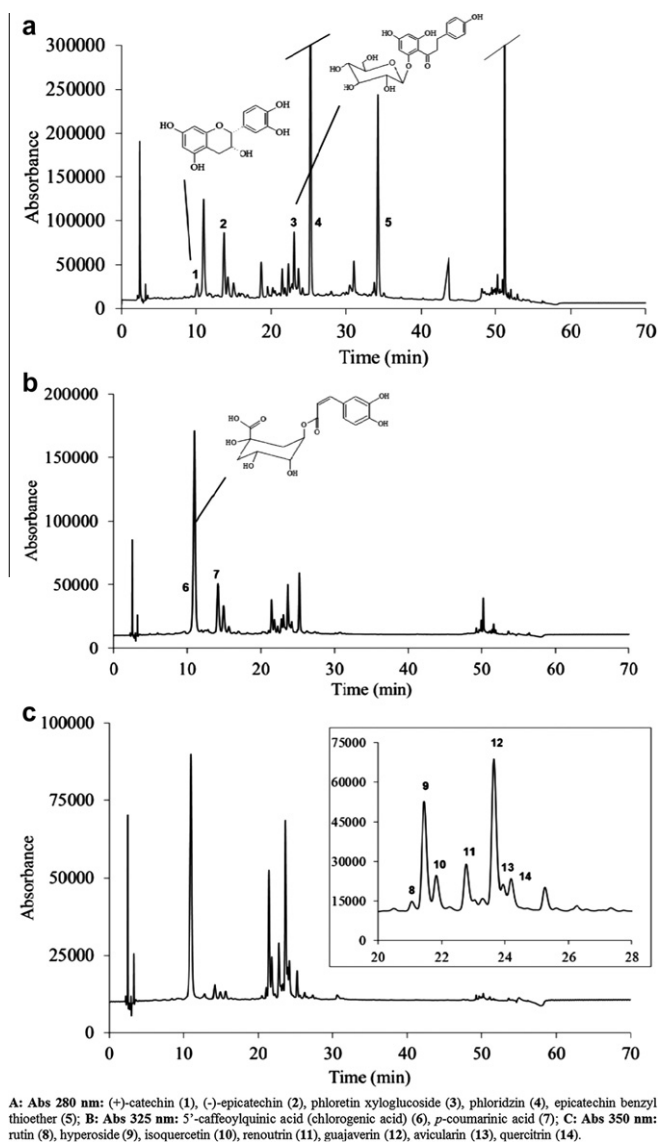
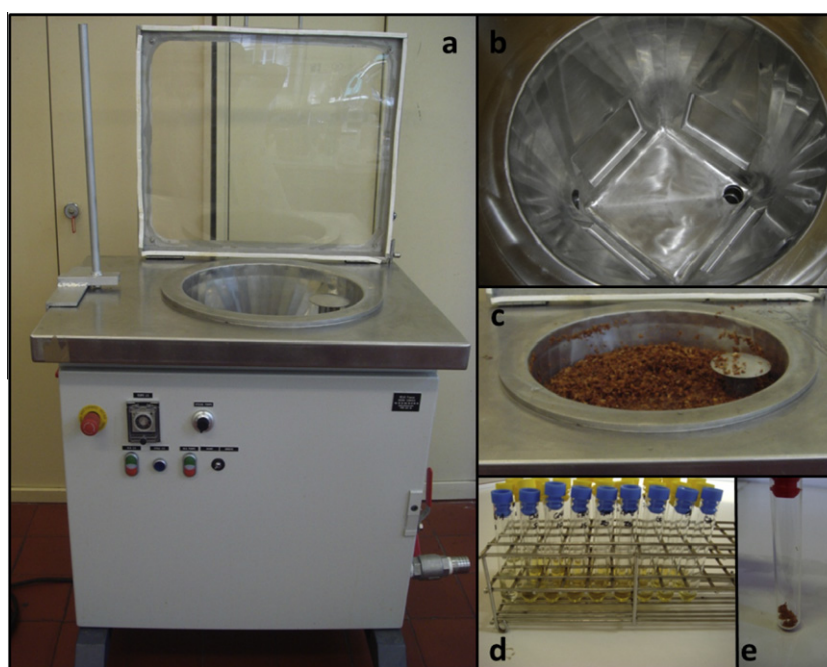


Fig. 6. C18 reverse phase HPLC-DAD chromatograms of ultrasound-assisted extracted apple pomace polyphenols at A: 280 nm; B: 320 nm; C: 350 nm.



Large scale ultrasound apparatus (a), inside view of apparatus (b), UAE of apple pomace (c), liquid extract (d), and lyophilized extract (e).

Fig. 7. Large scale ultrasound-assisted extraction.

487 mg catechin equivalent per 100 g of dry weight for conventional ones).

4. Conclusion

When compared to conventional maceration extraction, optimized ultrasonic treatment showed an increase of more than 30% in total phenolic content after 40 min, which was confirmed by large scale experiments, showing the potential applicability of the technique in industries. At the same time, the HPLC-DAD data clearly showed that there was no modification or degradation of the extract and its composition regarding the polyphenolic species.

Acknowledgements

This work was funded by Agence Nationale de la Recherche within Project ANR-07-PNRA-030TEMPANTIOX “New processes for production of fruit derived products with optimized organoleptic and nutritional qualities”.

References

- Akiyama, H., Sakushima, J.-ichi, Taniuchi, S., Kanda, T., Yanagida, A., Kojima, T., Teshima, R., Kobayashi, Y., Goda, Y., Toyoda, M., 2000. Antiallergic effect of apple polyphenols on the allergic model mouse. *Biological & Pharmaceutical Bulletin* 23, 1370–1373.
- Barbero, G., Liazid, A., Palma, M., Barroso, C., 2008. Ultrasound-assisted extraction of capsaicinoids from peppers. *Talanta* 75 (5), 1332–1337.
- Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escalera, L.A., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta* 76, 965–977.
- Caldeira, I., Pereira, R., Clímaco, M.C., Belchior, A.P., Bruno de Sousa, R., 2004. Improved method for extraction of aroma compounds in aged brandies and aqueous alcoholic wood extracts using ultrasound. *Analytica Chimica Acta* 513 (1), 125–134.
- Cao, X., Wang, C., Pei, H., Sun, B., 2009. Separation and identification of polyphenols in apple pomace by high-speed counter-current chromatography and high-performance liquid chromatography coupled with mass spectrometry. *Journal of Chromatography A* 1216 (19), 4268–4274.
- Cetkovic, G., Canadanovicbrunet, J., Djilas, S., Savatovic, S., Mandic, A., Tumbas, V., 2008. Assessment of polyphenolic content and in vitro antiradical characteristics of apple pomace. *Food Chemistry* 109 (2), 340–347.
- Chemat, F., Zill-e-Huma, Khan, M.K., 2011. Applications of ultrasound in food technology: processing, preservation and extraction. *Ultrasonics Sonochemistry* 18 (4), 813–835.
- Chen, F., Sun, Y., Zhao, G., Liao, X., Hu, X., Wu, J., Wang, Z., 2007. Optimization of ultrasound-assisted extraction of anthocyanins in red raspberries and identification of anthocyanins in extract using high-performance liquid chromatography-mass spectrometry. *Ultrasonics Sonochemistry* 14 (6), 767–778.
- Guyot, S., Marnet, N., Drilleau, J., 2001. Thiolytic-HPLC characterization of apple procyanidins covering a large range of polymerization states. *Journal of Agricultural and Food Chemistry* 49 (1), 14–20.
- Guyot, S., Marnet, N., Laraba, D., Sanoner, P., Drilleau, J., 1998. Reversed-Phase HPLC following thiolytic for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a French cider apple variety (*Malus domestica* Var. Kermerrien). *Journal of Agricultural and Food Chemistry* 46 (5), 1698–1705.
- Guyot, S., Marnet, N., Sanoner, P., Drilleau, J., 2003. Variability of the polyphenolic composition of cider apple (*Malus domestica*) fruits and juices. *Journal of Agricultural and Food Chemistry* 51 (21), 6240–6247.
- Herodez, S.S., Hadolin, M., Skerget, M., Knez, Z., 2003. Solvent extraction study of antioxidants from Balm (*Melissa officinalis* L.) leaves. *Food Chemistry* 80 (2), 275–282.
- Hilt, P., Schieber, A., Yildirim, C., Arnold, G., Kläiber, I., Conrad, J., Beifuss, U., Carle, R., 2003. Detection of phloridzin in strawberries (*Fragaria × ananassa* Duch.) by HPLC–PDA–MS/MS and NMR spectroscopy. *Journal of Agricultural and Food Chemistry* 51 (10), 2896–2899.
- Kanda, T., Akiyama, H., Yanagida, A., Tanabe, M., Goda, Y., Toyoda, M., Teshima, R., Saito, Y., 1998. Inhibitory effects of apple polyphenol on induced histamine release from RBL-2H3 cells and rat mast cells. *Bioscience, Biotechnology, and Biochemistry* 62 (7), 1284–1289.
- Khan, M.K., Abert-Vian, M., Fabiano-Tixier, A.S., Dangles, O., Chemat, F., 2010. Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel. *Food Chemistry*.
- Kołodziejczyk, K., Kosmala, M., Milala, J., Sójka, M., Uczciwek, M., Król, B., Markowski, J., Renard, C.M.G.C., 2009. Characterisation of the chemical composition of scab-resistant apple pomaces. *Journal of Horticultural Science and Biotechnology* 84, 89–95.
- Kyi, T.M., Daud, W.R.W., Mohammad, A.B., Wahid Samsudin, M., 2005. The kinetics of polyphenol degradation during the drying of Malaysian cocoa beans. *International Journal of Food Science and Technology* 40, 323–331.
- Le Bourvellec, C., Guyot, S., Renard, C.M.G.C., 2004. Non-covalent interaction between procyanidins and apple cell wall material Part 1: effect of some environmental parameters. *Biochim. Biophys. Acta* 1672, 192–202.
- Le Bourvellec, C., Renard, C.M.G.C., 2005. Non-covalent interactions between procyanidins and apple cell wall material. Part II: quantification and impact of cell wall drying. *Biochim. Biophys. Acta* 1675, 1–9.

- Le Bourvellec, C., Le Quere, J.-M., Renard, C.M.G.C., 2007. Impact of noncovalent interactions between apple condensed tannins and cell walls on their transfer from fruit to juice. Studies in model suspensions and application. *Journal of Agricultural and Food Chemistry* 55 (19), 7896–7904.
- Lebourvellec, C., Guyot, S., Renard, C., 2009. Interactions between apple (*Malus domestica* Borkh.) polyphenols and cell walls modulate the extractability of polysaccharides. *Carbohydrate Polymers* 75 (2), 251–261.
- Lorimer, J.P., Mason, T.J., 1987. Sonochemistry. Part 1-The physical aspects. *Chemical Society Reviews* 16, 239.
- Lu, Y., 2000. Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chemistry* 68 (1), 81–85.
- Lu, Y., Foo, Y., 1997. Identification and quantification of major polyphenols in apple pomace. *Food Chemistry* 59 (2), 187–194.
- Ma, Y., Chen, J., Liu, D., Ye, X., 2009. Simultaneous extraction of phenolic compounds of citrus peel extracts: effect of ultrasound. *Ultrasonics Sonochemistry* 16 (1), 57–62.
- Mangas, J.J., Rodriguez, R., Suarez, B., Picinelli, A., Dapena, E., 1999. Study of the phenolic profile of cider apple cultivars at maturity by multivariate techniques. *Journal of Agricultural and Food Chemistry* 47 (10), 4046–4052.
- Mirhosseini, H., Tan, C.P., Hamid, N.S., Yusof, S., 2008. Effect of Arabic gum, xanthan gum and orange oil on flavor release from diluted orange beverage emulsion. *Food Chemistry* 107 (3), 1161–1172.
- Ozcan, E., 2006. Ultrasound assisted extraction of phenolics from grape pomace. Middle East Technical University, The graduate School of Natural and Applied Sciences, Ph.d., Chemical Engineering, Ankara.
- Paniwnyk, L., Cai, H., Albu, S., Mason, T., Cole, R., 2009. The enhancement and scale up of the extraction of anti-oxidants from *Rosmarinus officinalis* using ultrasound. *Ultrasonics Sonochemistry* 16 (2), 287–292.
- Patist, A., Bates, D., 2008. Ultrasonic innovations in the food industry: from the laboratory to commercial production. *Innovative Food Science & Emerging Technologies* 9 (2), 147–154.
- Price, K., 1999. A comparison of the flavonol content and composition in dessert, cooking and cider-making apples; distribution within the fruit and effect of juicing. *Food Chemistry* 66 (4), 489–494.
- Roche, M., Dufour, C., Mora, N., Dangles, O., 2005. Antioxidant activity of olive phenols: mechanistic investigation and characterization of oxidation products by mass spectrometry. *Organic & Biomolecular Chemistry* 3 (3), 423.
- Rodrigues, S., Pinto, G.A., Fernandes, F.A., 2008. Optimization of ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder by response surface methodology. *Ultrasonics Sonochemistry* 15 (1), 95–100.
- Sanoner, P., Guyot, S., Marnet, N., Molle, D., Drilleau, J.-F., 1999. Polyphenol profiles of French cider apple varieties (*Malus domestica* sp.). *Journal of Agricultural and Food Chemistry* 47 (12), 4847–4853.
- Shoji, T., Akazome, Y., Kanda, T., Ikeda, M., 2004. The toxicology and safety of apple polyphenol extract. *Food and Chemical Toxicology* 42 (6), 959–967.
- Shoji, T., Kobori, M., Shinmoto, H., Yanagida, A., Kanda, T., Tsushida, T., 2000. Inhibitory effects of apple polyphenols on differentiation of 3T3-L1 cells into adipocytes. *Food Science and Technology Research* 6 (2), 119–121.
- Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology Viticulture* 16 (3), 144–158.
- Spiro, M., Jago, D.S., 1982. Kinetics and equilibria of tea infusion. Part 3. -Rotating-disc experiments interpreted by a steady-state model. *Journal of the Chemical Society, Faraday Transactions* 1 78 (1), 295–305.
- Suárez, B., Álvarez, Á.L., García, Y.D., Barrio, G.D., Lobo, A.P., Parra, F., 2010. Phenolic profiles, antioxidant activity and in vitro antiviral properties of apple pomace. *Food Chemistry* 120 (1), 339–342.
- Svitelska, G., Gallios, G., et Zouboulis, A., 2004. Sonochemical decomposition of natural polyphenolic compound (condensed tannin). *Chemosphere* 56 (10), 981–987.
- Tiwari, B.K., Muthukumarappan, K., O'Donnell, C.P., Cullen, P.J., 2008. Effects of sonication on the kinetics of orange juice quality parameters. *Journal of Agricultural and Food Chemistry* 56 (7), 2423–2428.
- Toma, M., Fukutomi, S., Asakura, Y., Koda, S., 2011. A calorimetric study of energy conversion efficiency of a sonochemical reactor at 500 kHz for organic solvents. *Ultrasonics Sonochemistry* 18 (1), 197–208.
- Van der Sluis, A.A., Dekker, M., Skrede, G., Jongen, W.M.F., 2002. Activity and concentration of polyphenolic antioxidants in apple juice. 1. effect of existing production methods. *Journal of Agricultural and Food Chemistry* 50 (25), 7211–7219.
- Veillet, S., Tomao, V., Chemat, F., 2010. Ultrasound assisted maceration: An original procedure for direct aromatisation of olive oil with basil. *Food Chemistry* 123 (3), 905–911.
- Vinatoru, M., 2001. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrasonics Sonochemistry* 8 (3), 303–313.
- Virost, M., Tomao, V., Le Bourvellec, C., Renard, C.M., Chemat, F., 2010. Towards the industrial production of antioxidants from food processing by-products with ultrasound-assisted extraction. *Ultrasonics Sonochemistry* 17 (6), 1066–1074.
- Wang, J., Sun, B., Cao, Y., Tian, Y., Li, X., 2008. Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry* 106 (2), 804–810.
- Wijngaard, H., Brunton, N., 2009. The optimization of extraction of antioxidants from apple pomace by pressurized liquids. *Journal of Agricultural and Food Chemistry* 57 (22), 10625–10631.
- Wijngaard, H.H., Brunton, N., 2010. The optimisation of solid-liquid extraction of antioxidants from apple pomace by response surface methodology. *Journal of Food Engineering* 96 (1), 134–140.
- Xia, T., Shi, S., Wan, X., 2006. Impact of ultrasonic-assisted extraction on the chemical and sensory quality of tea infusion. *Journal of Food Engineering* 74 (4), 557–560.
- Yanagida, A., Kanda, T., Tanabe, M., Matsudaira, F., Oliveira Cordeiro, J.G., 2000. Inhibitory effects of apple polyphenols and related compounds on cariogenic factors of mutans *Streptococci*. *Journal of Agricultural Food Chemistry* 48 (11), 5666–5671.