

# Valorization of apple by-products by the extraction and purification of polyphenols: impact of the ultrasound Lu Wang

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## Par Lu WANG

Valorization of apple by-products by the extraction and purification of polyphenols : impact of the ultrasound

Thèse présentée pour l'obtention du grade de Docteur de l'UTC



Soutenue le 18 octobre 2019 **Spécialité :** Génie des Procédés : Transformations intégrées de la matière renouvelable (EA-4297)

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# Thèse présentée pour l'obtention du grade de Docteur de L'Université de Technologie de Compiègne, Sorbonne Université

Par

Lu WANG

# Valorization of apple by-products by the extraction and purification of polyphenols: impact of the ultrasound

Spécialité : Génie des Procédés

Date de soutenance le : 18/10/2019

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## Abstract

This thesis focuses on the intensification of polyphenols extraction from apple products (flesh, peel, and pomace) by ultrasound (US) and the purification of apple peel extracts by adsorption/desorption and membrane technology.

The selective extraction of phenolic contents from apple products has been analyzed. The obtained data evidenced the possibility of fine regulation of selective extraction of soluble matter, catechin and total polyphenolic compounds using different temperatures, ultrasound-assisted extraction (UAE) protocols, ethanol/aqueous mixtures. The selectivity of catechin extraction was also depended on the type of the tissue (flesh, peel or pomace) and apple variety (green or red).

The cavitation phenomenon generated by ultrasound could increase extraction of valuable components from fruit peels by damaging cell membranes of samples and accelerating heat and mass transfer by disrupted cell walls of samples. Meanwhile, the gas water solvents could enhance the extraction efficiency of polyphenols and antioxidant activity from apple peels by enhancing cavitation phenomenon generated by ultrasound.

The efficiency of polyphenols purification from apple peel extracts with adsorption/desorption process by ultrasound treatment with the polyaromatic amberlite adsorbent XAD-16 and with membrane electro-filtration were studied. The obtained data demonstrated that the sonication significantly facilitated adsorption kinetics and increased activation energy of polyphenols adsorption. In addition, the desorption ratio was positively affected by the sonication during the adsorption step. On the other hand, the results demonstrated that the membrane electro-filtration allowed the purification of polyphenols in the anode (+) space and obtaining larger volume of filtrates.

Key words: apple peel, polyphenols, ultrasound, selective extraction, cavitation phenomenon, cell damage, adsorption/desorption, membrane electro-filtration

# Résumé

Cette thèse porte sur l'intensification de l'extraction de polyphénols à partir de produits à base de pomme (chair, peau et marc) par ultrasons (US) et sur la purification d'extraits de peau de pomme par adsorption/désorption et technologie membranaire.

L'extraction sélective des polyphénols issus des produits de la pomme a été analysée. Les données obtenues ont démontré la possibilité d'une régulation fine de l'extraction sélective de la matière soluble, de la catéchine et des composés polyphénoliques totaux en utilisant différentes températures, protocoles UAE, mélanges éthanol/aqueux. La sélectivité de l'extraction de la catéchine dépendait également du type de tissu (chair, peau ou marc) et de la variété de pomme (verte ou rouge).

Le phénomène de cavitation généré par les ultrasons pourrait augmenter l'extraction de composants précieux des pelures de fruits en endommageant les membranes cellulaires des échantillons et en accélérant ainsi le transfert de chaleur et de masse. D'autre part, les solvants eau-gaz pourraient améliorer l'efficacité d'extraction des polyphénols des peaux de pomme en renforçant le phénomène de cavitation généré par les ultrasons.

L'efficacité de la purification de polyphénols d'extraits de pelure de pomme par adsorption/désorption assisté par ultrasons (adsorbant polyaromatique Amberlite XAD-16) et par électrofiltration sur membrane a été mise en évidence. Les données obtenues ont démontré que la sonication facilitait significativement la cinétique d'adsorption, la capacité d'adsorption accrue et augmentation de l'énergie d'activation de l'adsorption des polyphénols. En outre, le taux de désorption a été positivement affecté par la sonication au cours de l'étape d'adsorption. Par ailleurs, les résultats ont montré que l'électrofiltration sur membrane permettait de purifier les polyphénols dans l'espace anodique (+) et d'obtenir un volume plus important de filtrats.

Mots-clés : peau de pomme, polyphénols, ultrasons, extraction sélective, endommagement cellulaires, adsorption / désorption, membrane filtration

# **Publications list**

### **I.Publications**

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# **Nomenclature and Abbreviations**

## Nomenclature

А	Effective membrane area	$m^2$
Abs	Absorbance	-
°Brix	Concentration of total soluble matter content	g /100 g
С	Concentrations of bio-molecules	mg/mL
C <sub>cs</sub>	Weight fraction of cake-forming (colloidal and insoluble) solids	Kg/kg
	in the feed	
Cp	Specific heat capacity of solution	kJ/(kg•K)
$C_s$	Catechin concentration in solvent	mg/mL
d	Damage degree	-
D	The desorption ratio	-
DM	Dry matter	g
Е	Electric field strength	V/cm
E <sub>c</sub>	Critical electric field strength	V/cm
F	Load force	Ν
FW	Fresh weight	g
Ι	Colour intensity of filtrate	-
$I_f$	Intensity of the fluorescence emission	au
În	Inhibition of the polyphenols against the free radical DPPH	%
J	Filtrate flux	m/s
$K_r$	Rejection coefficients of different substances	-
m	Mass	g
MWCO	Molecular weight cut-off	kDa
n	Number of ultrasonic pulses	-
N <sub>d</sub>	Number of damaged cells	-
$N_t$	Total number of cells	-
Р	US intensity	W
$\Delta P$	Filtration pressure	Pa
Q	Adsorption capacity	mg/g
R	Sieving ratio	-
$R_e$	Adsorption/desorption efficiency (recovery) for bio-molecules	-
R <sub>m</sub>	Membrane resistance	$m^{-1}$
RM	Raw matter	g
t	Time	S
$\Delta t_u$	Duration of each ultrasound pulse	S
$\Delta t_w$	Pause duration	S
$\Delta T$	Temperature elevation	°C
TMP	Transmembrane pressure	bar
V	Volume of the liquid medium	mL
$V_{\rm m}$	Trans-membrane potential	mV
W	Specific ultrasound input energy	kW•h/kg
Y	Yield	-
Ζ	Extraction indexes	-
$Z_m$	Cell wall disintegration index	-
α	Specific filtration resistance of the filter-cake	m/kg
ρ	Density of filtrate	kg/m <sup>3</sup>

μ	Viscosity of filtrate Pa·s	,
σ	Electrical conductivity S/m	
δ	Thickness of fruit peels mm	
λ	Emission wavelengths nm	
$\lambda_{ m e}$	Excitation wavelengths nm	
<u>Subscript</u>		
a	US-assisted adsorption	
В	BSA	
С	Catechin	
d	Desorption experiments	
е	Equilibrium	
et	Ethanol	
f	Final filtrate after filtration	
G	Gallic acid	
h	$H_2O_2$	
i	Ionic	
pr	Proteins	
q	Quercetin	
r	Resin	
t	Trolox	
tf	Total polyphenols	
ts	Total solutes	
us	US treatment	
0	Initial	
<b>Abbreviation</b>	<u>ns</u>	
BSA	Bovine Serum Albumine	
BW	Bi-level images	
CA	Cellulose acetate	
CE	Conventional extraction	
CLSM	Confocal laser scanning microscopy	
DAD	Photodiode array detector	
FT	Frozen-thawed	
FTIR	Fourier transform infrared spectroscopy	
GAE	Gallic acid equivalent	
HPLC	High performance liquid chromatography	
HVED	High voltage electrical discharges	
MF	Microfiltration	
MW	Molecular weight	
NF	Nanofiltration	
PAC	Proanthocyanidins	
PAN	Polyacrylonitrile	
PARAFAC	Parallel factor analysis	
PCA	Principal component analysis	
PEF	Pulsed electric fields	
PES	Polyethersulphone	
PS DVDE	Polysulphone	
PVDF	Poly (vinylidene difluoride)	
KO	Reverse osmosis	
RP-HPLC	Reversed-phase high-performance liquid chromatography	

SEM	Scanning electron microscopy images
TFC	Total flavonoid content
TPC	Total polyphenols content
U	Untreated
UAE	Ultrasound-assisted extraction
UF	Ultrafiltration
UHPH	Ultra-high pressure homogenization
US	Ultrasound
UV/Vis	Ultraviolet-visible

# **General Introduction**

Apples contain various nutrients beneficial to human health with strong anti-inflammatory effects and high ability to prevent chronic diseases, especially polyphenols (flavanols, flavonols, phloridzin, procyanidin, chlorogenic acid, anthocyanin). Content of phenolics in apple pomace (peels, mesh, seeds, cores and stems) is much higher than in the juice or flesh of apples. Nowadays the extraction and purification of phenolic compounds from apple pomace, especially apple peels, attracts a great attention.

Many studies have turned to improve the polyphenols extraction from apple products. Among many extraction techniques, solvent extraction and thermal extraction are the most conventional ones. However, typically, such extraction methods are not selective. They can result in decrease of purity of extracts and require supplementary separation procedures. It is therefore necessary to find a compromise between extraction yield, extract quality, separation efficiency, and consumption energy.

Recently, different non-conventional extractions techniques (supercritical fluid, high pressure homogenization (HPH), ultrasound (US), microwave, high-voltage electrical discharges (HVED), and pulsed electric fields (PEP), enzymatic and other treatments) have been proposed to intensify extraction of polyphenols. Nowadays, US treatment has been widely used for polyphenols extraction from apple products. The ultrasound-assisted extraction (UAE) is widely recognized as a green technique with reduced energy consumption, and decreased processing time. Particularly, UAE also allows reduction of wastes and elimination of generation of hazardous substances.

US treatment generates air bubbles that propagate in the liquid medium with expansion and compression cycles. UAE is mainly based on acoustic cavitation and its hydrodynamic shear forces. The generated cavitation phenomenon from bubble collision can accelerate heat and mass transfer by plant cell walls disruption, leading to improved release of the target compounds from samples. In addition, the generated oxidative energy of radicals created during sonolysis of the solvent can result in a high extractive power of ultrasounds. Therefore, US treatment is beneficial to intensify extraction of polyphenols from material samples.

The conventional techniques for polyphenols purification of extracts are adsorption, chromatography-based techniques, nanofiltration (NF), and electrodialysis (ED). These purification techniques could be applied not only in laboratory but also in industry. Among these techniques, adsorption/desorption on the poly-aromatic amberlite adsorbent and

membrane filtration can offer more effective and cleaner technologies for the separation and clarification of extracts due to their flexibility and operating conditions respectful of the environment. The major problem for adsorption/desorption with macroporous resin is low recovery rate and long purification time. For membrane filtration technique, it is the rapid decrease in permeate flow following clogging of the membrane. Hence the adsorption/desorption procedure assisted by sonication and membrane electro-filtration will be used for the selective purification of polyphenols from apple peels.

This thesis aims to intensify the extraction of polyphenols from apple products by ultrasound treatment. Previous works were mainly focused on the impact of US on the improvement of aqueous extraction of solutes in general. Here, the focus will be done on the selective extraction of polyphenols from apple products and the effect of cavitation phenomenon produced by ultrasound on polyphenol extraction. On the other hand, purification polyphenols from apple peel extracts will also be studied. The application of adsorption/desorption procedure assisted by sonication and membrane electro-filtration for selective purification of polyphenols compared with proteins in extracts will be discussed.

This thesis consists of five chapters.

The Chapter I presents the research status on the apple polyphenols, their different extraction processes, and new techniques for improving extraction that can be applied. Then the bibliographic study also focuses on describing current knowledge concerning the purification and separation of polyphenols from apple extracts.

The Chapter II describes materials and methods used in this study, by presenting the raw materials, the experimental devices, the different applied processes as well as physico-chemical analyses and data calculation methods.

The Chapter III represents the results on the intensification of solute and polyphenols extraction from apple products (flesh, peel and pomace) with US pre-treatment, ethanol/water mixtures, and combined US and conventional heating. On the other hand the effects of CE and UAE protocols, and ethanol concentration on selectivity of polyphenols extraction from apple products will be presented.

The Chapter IV first focuses on the cavitation phenomenon produced by ultrasound leading to polyphenol extraction enhancement. On the other hand, the effect of cell damage and  $CO_2$  concentration in gas water solvents on UAE of valuable components from apple peels will be analyzed.

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The Chapter V discusses the application of adsorption/desorption procedure assisted by sonication for purification of polyphenols from apple peel extracts, and studies the selective purification of polyphenols from apple peels with membrane electro-filtration.

Finally, the general conclusions of this thesis will be summarized and some suggestions and perspectives for further work will be presented.

## **Chapter I Bibliography**

This chapter 1 will introduce the apple products (flesh, peel and pomace), the polyphenols content, the conventional extraction technologies and the new extraction techniques. In addition, an analysis of the literature on existing polyphenols purification processes will be presented.

#### I.1 By-products of the apple industry

#### I.1.1 Apple pomace and apple peels

Typical by-products of the apple industry are illustrated in Fig. I.1 (Perussello et al., 2017; Rabetafika et al., 2014). After the harvested apples are picked, they are washed and transported to the processing facilities. The apples are then pressed and juiced right away. Depending on the companies and end products, the apples will be processed in different ways before pressing. The fresh apple pomace and apple peels are produced at the same time with the raw apple juice.



Figure I.1. Conventional apple processing by products

During the processing of apple fruits for juice, cider, syrup or vinegar preparation, large amounts of solid residues (peel, core, seed, calyx, stem, and soft tissue) are generated (Kennedy et al., 1999), among them apple pomace accounts for approximately 25-30% of the weight of the fresh apple (Gullón et al., 2007). Apple pomace mainly contains about 54% flesh, 34% peel, 7% seeds, 4% seed core and 2% stem (Kolodziejczyk et al., 2007). Therefore, apple peel is the most main component in the apple pomace except underused apple flesh.

In addition, apple peels account for 13% of the weight of the fresh apple fruit (Tarazona-Díaz and Aguayo, 2013). Their by-products are obtained from apple sauce, canned apple manufacture, dried apple manufacture and fresh-cut apple (Md et al., 2017; Tian et al., 2010). It is estimated that approximately 9000 metrics tons of apple peels are generated annually in Chile, as a result of apple processing (Henríquez et al., 2010).

### I.1.2 Apple peels valorisations

At present, apple peels are typically used for non-valuable purposes with direct use including livestock feed, fertilizer, compost, press aid in the production of juice or vinegar and pressed into a cake. In addition, apple peels have began to be used as a source of pectin and fiber (Wolfe and Liu, 2003). The most common applications for apple peels are summarized in Table I.1 (Miceli-Garcia, 2014).

Application	Examples	References
Direct use	Animal feed, compost, source of dietary fiber in bakery products, press aid in fruit juice preparation.	(Gassara et al., 2011; Masoodi et al., 2002; Roberts et al., 2004; Singh and Narang, 1992)
Extraction of bioactive compounds	Dietary fibers, antioxidants, pectin, oil.	(Figuerola et al., 2005; Schieber et al., 2003; Tian et al., 2010; Wijngaard and Brunton, 2009)
Products obtained via fermentation	Organic acids, ethanol, aroma compounds, pigments, protein enriched animal feed, enzymes, heteropolysaccharides, baker's yeast.	(Attri and Joshi, 2005; Berovič and Ostroveršnik, 1997; Bhushan and Joshi, 2006; Hang and Woodams, 1984; Joshi and Sandhu, 1996; Longo and Sanromán, 2006; Stredansky and Conti, 1999; Villas-Bôas et al., 2003)
Fuel production	Biogas, ethanol	(Hang, 1987; JEWELL and CUMMINGS, 1984)
Substrate for edible mushrooms	Shiitake and oyster mushroom	(Worrall and Yang, 1992)

Table I.1. The utilizations of apple peels

Previous studies have demonstrated that apple peels contain a number of nutrient contents, such as phenolics, ascorbate, dietary fibers, and minerals (Łata and Tomala, 2007; Md et al., 2017). For example, apple peels have higher antioxidant activities (such as antioxidant concentration and antioxidant capacity) and higher health-promoting properties (such as greater inhibitory effects on cancer cells) than the apple flesh or whole apple (Drogoudi et al., 2008; Khanizadeh et al., 2008; Wolfe et al., 2003). In addition, several studies have reported that the dietary fibers and the minerals content are much higher in apple peels as compared to other edible parts of apples (Gorinstein et al., 2001; Henríquez et al., 2010; Leontowicz et al., 2003). Moreover, it is known that the concentration of total polyphenol contents is much

greater in the peel than in the flesh of apples (Burda et al., 1990; Ju et al., 1996). Previous studies had shown that the content of phenolic compounds in apple peels is higher than in apple flesh. The apple peels contain caffeic acid, catechins, chlorogenic acid, phloridzin, phloretin glycosides, and procyanidins as apple flesh. In addition, apple peel contains additional flavonoids not found in apple flesh, such as quercetin and cyanidin glycosides (Escarpa and Gonzalez, 1998; van der Sluis et al., 2005). Recycling of apple peels have been used as a source of phenolic compounds and dietary fibre in previous studies depending on different valuable compounds and different peel waste-derived materials (Table I.2). Since it is worth recovering these nutrients and developing value added products. Apple peels can be used in various food products to add phytochemicals and promote good health (Md et al., 2017).

Peel materials	Preservation conditions	Extraction solvent	Applications	Target compounds	References
Bulk peel powders	Drum-drying	70% Acetone (v/v)	Fibre formulation Functional foods	Dietary fibre; phenolic compounds	(Henríquez et al., 2010)
	1) Water blanching 2) Oven- drying (60°C, with air circulation)	Methanol	Fibre formulation Functional foods	Dietary fibre; phenolic compounds	(Rupasinghe et al., 2008)
	1) Water blanching; ascorbic acid dip	80% Acetone; 80% ethanol (v/v)		Phenolic compounds	
	2) Freeze- drying; air-drying; oven-drying (40/60/80°C, no air circulation)		Nutraceuticals		(Wolfe and Liu, 2003)
Antioxidant peel extracts	Freeze- drying	Methanol	Functional foods	Phenolic compounds Phenolic	(Huber and Rupasinghe, 2009) (Wegrzyn et al.,
		Ethanol ; methanol	Nutraceuticals	compounds Phenolic compounds	2008) (Tanabe et al., 1999)
	Freeze- drying	80% Acetone (v/v)	Nutraceuticals	Phenolic compounds	(Wolfe et al., 2003)

Table I.2.	The y	valorisations	of apple	peels (	Massini	et al., 2	2013)
1 4010 1.2.	Inc	aloributions	or uppic		1 abbilli	ot u1., 4	2013)

#### I.2 Polyphenols in apple peels

#### I.2.1 Introduction

Polyphenol is one of high value products of apple pomace, especially apple peels (Kennedy et al., 1999). Many studies were focused on the distribution of polyphenolic compounds in apple peels from different varieties and in comparison with different apple ingredients (flesh, whole apple or juice), the identification of polyphenolic components, and in specific cases the resulting concentration (Table I.3). The contents and composition of polyphenols can be rather different in dependence of the plant variety, environmental and post-harvest factors and the plant part (flesh, peels, seeds and pomace) (Heimler et al., 2017; Oszmiański et al., 2018). The differences between polyphenols of peel and flesh from distinct varieties of apples were compared (Kalinowska et al., 2014; McRae et al., 1990; Pérez-Ilzarbe et al., 1991). The authors confirmed the presence of flavonols and their glycosides in the peel of apples, while this compound was trace amounts in the flesh. The polyphenolic profiles and contents in peels and flesh from eight apple varieties were detected and compared (Tsao et al., 2003). Lamperi et al. (2008) compared the polyphenols content from peels and flesh respectively of three red apple cultivars from Italy (Annurca, Red Chief and Staynam Neepling). In addition, the total polyphenol content (TPC) from peels and flesh for different varieties apples were compared. The TPC for Fuji apples and organic Golden Delicious apples were corresponding to  $577.9 \pm 8.99$  mg/100g FW for peels and  $140.9 \pm 198$  mg/100g FW for flesh, and  $1204 \pm 76.2 \text{ mg/kg}^{-1}$  FW for peels and  $241 \pm 30.2 \text{ mg/kg}^{-1}$  FW for flesh. respectively. Moreover, catechins, procyanidins, hydroxycinnamic acids, flavonol glycosides, dihydrochalcone glycosides and cyanidin-3-O-galactoside could be identified both in the apple peels and flesh. Especially, procyanidins, catechins and flavonols were the main constituents of apple peels (Giomaro et al., 2014). Similar, previous researches have demonstrated that the total content of anthocyanins in apple peels was much higher than in apple flesh for different varieties of apples (Khanizadeh et al., 2008; Tsao et al., 2003).

Polyphanolia components	Apple veriety	Contant	Poforoncos
Foryprienone components	Apple vallety	Content	References
			(Awad et al., 2000; Awad and
			de Jager, 2000; Lata et al.,
Chlorogenic acid	2-18	0.03-24 mg/g DM	2009)
			(Duda-Chodak et al., 2011;
			Escarpa and Gonzalez, 1998;
			Khanizadeh et al., 2008;
	1-4	0.6-44 mg/kg FW	Veberic et al., 2005)
			(Guyot et al., 2002;
p-Coumaroylquinic acid	2	0.003-66.9 mg/g FW	Khanizadeh et al., 2008)

Table I.3. The content of polyphenolic components in apple peels

	1	0.05-0.19 mg/g DM	(Renard et al., 2007) (Awad at al., 2000; Fata at al.
(+) Catechin	1-18	0.99-4.42 mg/g DM	(Awad et al., 2000, Lata et al., 2009) (Escarpa and Gonzalez, 1998;
(-) Epicatechin	2-4 18	0.008-50.5 mg/g FW 1.24-5.75 mg/g DM	Guyot et al., 2002; Khanizadeh et al., 2008) (Łata et al., 2009) (Choi et al., 2012; Escarpa and Gonzalez, 1998; Guyot et
	1-4	0.036-2.589 mg/g FW	al., 2002; Khanizadeh et al., 2008; Veberic et al., 2005) (Escarpa and Gonzalez, 1998; Guyot et al., 2002; Khanizadah et al. 2008;
Procyanidin B2	2-7	0.069-2.772 mg/g FW	Khanizaden et al., 2008, Kolodziejczyk et al., 2007) (Escarpa and Gonzalez, 1998; Khanizadeh et al. 2008:
Procvanidin B1	2-7	0.014-1.551 mg/g FW	Kolodziejczyk et al., 2007)
Procyanidin C1	7	97-213 mg/kg FW	(Kolodziejczyk et al., 2007) (Awad et al., 2001, 2000; Awad and de Jager, 2000:
Phloridzin	2-13	0.46-18 mg/g DM	Lata et al., 2009) (Choi et al., 2012; Escarpa and Gonzalez, 1998; Guyot et al., 2002; Khanizadeh et al.,
	1-4	0.013-15.9 mg/g FW	2008; Veberic et al., 2005)
Quercetin-3-galactoside	1	213.4 mg/kg FW	(Khanizadeh et al., 2008)
Quercetin-3-glucoside	1	161.9 mg/kg FW	(Khanizadeh et al., 2008) (Awad et al., 2000; Awad and
	2	0.53-8.71 mg/g DM	de Jager, 2000) (Khanizadeh et al., 2008;
	1-3	0.131-95.1mg/g FW	Veberic et al., 2005) (Awad et al., 2000: Choi et
Quercetin-3-rhamnoside	1	0.58-2.66 mg/g DM	al., 2012) (Huber and Rupasinghe
	1-4	0.172-3.050 mg /g DM	2009; Vieira et al., 2011) (Duda-Chodak et al., 2011; Guyot et al., 2002:
Total phenolic compounds	1-2	1.407-215.4 mg/g FW	Khanizadeh et al., 2008) (Guyot et al. 2002:
Total Procyanidins	1-2	1.282-61.95 mg/g FW	(Awad et al., 2001; Awad and de Jager, 2000; Vieira et al.
Total Flavonoids	1-2	0.568-14.66 mg/g DM	2011)

DM: dry matter, FW: fresh weight.

#### I.2.2 Polyphenols varieties

Five major groups of polyphenolic compounds are found in apple: anthocyanins, dihydrochalcones, flavan-3-ols/procyanidins (flavanols), flavonols and hydroxycinnamic acids (Mazza and Velioglu, 1992; Pérez-Ilzarbe et al., 1991; Schieber et al., 2003). Among these polyphenolic compounds, many of them are often associated with sugar moieties

(Merken and Beecher, 2000; Tsao et al., 2003). Catechin, epicatechin, chlorogenic acid, cyanidin 3- glycosides, phloridzin, phloretin and quercetin 3-glycosides are the major individual polyphenolics in apples (Awad et al., 2001, 2000). These polyphenolic compounds, located in the plastids/vacuoles of the cell, are related to the physical characteristics of apples such as the color of the peel (green, red and yellow), the flavor of the flesh and therefore to the differences between different varieties of apples (Petkovska et al., 2016).

The flesh and the peel of the apples showed a different type and distribution of phenolics compounds. The dihydroxycinnamic acids (caffeic acid, chlorogenic acid and neochlorogenic acid), hydroxybenzoic acids (gallic acid), monomeric flavanols (catechins and epicatechins), oligomeric flavanols (procyanidins) and dihydrochalcones (phloridizin and phloretin glycosides) were found in both flesh and peel, whereas flavanol (quercetin glycosides) and anthocyanins (cyanidin 3-galacoside) were exclusively found in the peel (Giomaro et al., 2014; Kalinowska et al., 2014) (Fig.I.2.). In apple peels, the predominant group of polyphenolics was the procyanidins, followed by quercetin glycosides (Fig.I.3), which was similar with previous studies (Table.I.3). Especially, flavonoids (hydroxycinnamic acids) composed 90% of the total polyphenolics in the peel (Amiot et al., 1992). Content of total polyphenol compounds is much larger in the peel than in the flesh of the apples (Burda et al., 1990; Escarpa and Gonzalez, 1998; Ju et al., 1996), especially procyanidins, flavonols and dihydrochalcone (Francini and Sebastiani, 2013; Le Bourvellec et al., 2007).



Figure I.2. Selected phenolic compounds in apples (Kalinowska et al., 2014).



Figure I.3. Main polyphenols constituents of apple peels (Giomaro et al., 2014).

#### **I.2.3 Polyphenols properties**

#### I.2.3.1 Interaction with proteins, alkaloids and polysaccharides

Properties of polyphenols are greatly affected by their interactions with other constituents of the food. The astringency of polyphenols could be altered by the presence of various molecules, including polysaccharides and proteins (Cheynier, 2005; El Gharras, 2009). The most important chemical characteristic of apple polyphenols is the binding reaction between polyphenols and proteins (Cheynier, 2005). Interactions of polyphenols with proteins is

responsible for astringency perception (resulting from interactions of tannins with salivary proteins), for the formation of haze and precipitates in beverages, and for inhibition of enzymes and reduced digestibility of dietary proteins (Fig. I.4). At present, the/gloves-hand zero reaction model proposed by Haslam et al. in the 1980s is generally accepted. It is based on the reaction theory of plant polyphenols combining proteins with hydrophobic bonds and multi-point hydrogen bonds (de Freitas and Mateus, 2012; Haslam, 1974). The main reaction mechanism is that plant polyphenols first approach the surface of protein molecules through hydrophobic bonds, then polyphenol molecules enter hydrophobic bag, and subsequently multi-point hydrogen bond occurs. The complex molecular reactions of plant polyphenols with alkaloids, polysaccharides and even with biological macromolecules such as nucleic acids and cell membranes are similar to that reaction of polyphenols combing proteins (de Freitas and Mateus, 2012; El Gharras, 2009).



Figure I.4. Schema of the interaction between condensed tannins and proteins (Santos-Buelga and De Freitas, 2009).

#### I.2.3.2 Complexation with metal ions

Many ortho-phenolic hydroxyl groups in plant polyphenols can be used as a kind of polyradical ligand to complexate with metal ions by forming stable five-membered ring chelates (Dumur et al., 2011; Rahim et al., 2014). Due to the multiple coordination groups, strong complexation ability and stability of polyphenols complex, most metal ions form precipitation after complexing with polyphenols (Slabbert, 1992) (presented in Fig. I.5). Under alkaline conditions, polyphenols and metal ions can easily form multi-donor ligand (Cherrak et al., 2016). For polyphenols interacting with some high-valent metal ions, such as  $Cr^{6+}$  and  $Fe^{3+}$ , the metal ions are complexed and reduced from high-valence state to low-valence state at the same time (Zhu et al., 2015). The pervious researches demonstrated that

the complexation of polyphenols with metal ions was the chemical basis of many applications (Castaneda-Ovando et al., 2009; McDonald et al., 1996).



Figure I.5. Schema of the complexation between quercetin and metal ions

(Kasprzak et al., 2015).

#### I.2.3.3 Antioxidant and free radical scavenging ability

The O-phenolic hydroxyl group of plant polyphenols (catechol or pyrogallol) is easily oxidized into quinone structure, with consuming oxygen in the environment, and has a strong ability to capture free radicals such as reactive oxygen species, which makes polyphenols have strong antioxidant and free radical scavenging ability as following reaction (Rice-Evans, 2001):

PH (polyphenol) + ROO• (lipid free radical) $\rightarrow$ P•+ROOH (I.1)

In apples, the contribution of anthocyanins to antioxidant activity was higher than the contribution of flavonols, flavan-3-ols and phenolic acids (Jakobek et al., 2009). Authors concluded that the anthocyanins can be regarded as major phenolic antioxidants of apples. The antioxidant activity was influenced by polyphenols content, and by the presence of stronger phenolic antioxidants (catechins, ellagic acid, and cyaniding-3-glucoside). In addition, plant polyphenols have strong ultraviolet (UV) absorption characteristics between 200 and 350 nm, especially in the high UV region with high energy and destructive power (Chen et al., 2002; Pfeilschifter et al., 2003). Therefore, plant polyphenols can be used as effective ingredients of anti-ageing agents and sunscreen.

#### I.2.4 Polyphenols analysis

A variety of methods have been used to determine the polyphenols contents of foods. Several spectrophotometric methods and some useful instrumental techniques involving separation include high-speed counter-current chromatography, supercritical fluid chromatography, gas chromatography, and capillary electrophoresis are used to quantify the total phenolic contents and individual classes of phenolic compounds (Craft et al., 2012; Ignat et al., 2011). Among these approaches, the Folin-Ciocalteu analysis is widely used to determine the total phenolics (Ignat et al., 2011). High performance liquid chromatography (HPLC) coupled with detection by absorbance, mass spectrometry or fluorimetry is one of the most frequently used method to quantify individual phenolics (Corradini et al., 2011; Pyrzynska and Sentkowska, 2015). In recent years, the fluorescence spectrum has been used to determine minor components in complex food samples, especially for polyphenols.

#### I.2.4.1 Folin-Ciocalteu analysis

The spectrophotometric method is widely used to determine total polyphenols content. Folin-Ciocalteu method became the most frequently prescribed reagent and the most suitable reagent for spectrophotometric estimation of total polyphenols (González-San José and Diez, 1992; Kanner et al., 1994; Swain, 1963). The total polyphenols content is measured by the Folin–Ciocalteu method based on a colorimetric oxidation/reduction reaction of phenols (Singleton et al., 1999). 0.2 mL of diluted extract, 1 mL of Folin–Ciocalteu reagent (diluted 1:10), and 0.8 mL of Na<sub>2</sub>CO<sub>3</sub> (75 g/L) were mixed. The sample is incubated for 10 min at 50 °C and then cooled at room temperature. For the control sample, 0.2 mL of extraction solvent is taken. The absorbance is measured at 750 nm by the ultraviolet-visible (UV/Vis) spectrophotometer. Gallic acid or catechin is used for making the calibration curve. Results are expressed as g gallic acid equivalent (GAE)/100 g dry matter (DM) or as g catechin equivalent (CE) /100 g DM (Boussetta et al., 2011).

Pingret et al. (2012) used Folin-Ciocalteu method to detect polyphenols content from apple pomace (257-460 mg CE/100 g DM), and demonstrated that ultrasound treatment could enhance aqueous extraction of polyphenols from apple pomace (555 mg GAE/100 g DM and 420 mg CE/100 g DM) in both lab and pilot-scale extraction. Suárez-Jacobo et al. (2011) evaluated the effect of ultra-high pressure homogenisation (UHPH) treatments on extraction polyphenol content from apple juice with Folin-Ciocalteu method (49.3 $\pm$ 0.9-52.4 $\pm$ 0.8 mg GAE/L), in comparison from raw (11.7 $\pm$ 1.4 mg GAE/L) and pasteurised apple juice (14.5 $\pm$ 1.3 mg GAE/L). In addition, Matthes et al. (2009) used Folin-Ciocalteu colorimetric method to detect total polyphenol content of whole apple and proved that polyphenol content and antioxidant capacity of apple were affected by cultivar and storage conditions of apples (Matthes et al., 2009). Optimal parameters (extraction time, extraction temperature and pomace to water ratio) for aqueous polyphenols extraction of apple pomace were determined by Candrawinata et al. (2015). The total polyphenol content ranged from 832.5 to 1257.6 µg GAE/g detecting with Folin-Ciocalteu colorimetric method. The comparative evaluation of bio-solvents for the efficient extraction of polyphenolic phytochemicals of apple waste peels

was studied by Blidi et al. (2015). In this study, Folin-Ciocalteu colorimetric method was used to determinate the total polyphenol content (18.34-18.96 mg GAE/g DM). No matter in the past or at present, almost all the researchers use Folin-Ciocalteu colorimetric method as a standard method to determinate polyphenol content in the samples.

#### 1.2.4.2 High Pressure Liquid Chromatography analysis

High pressure liquid chromatography (HPLC) is almost the most useful analytical technique for characterization of polyphenolic compounds, which is preferred for the separation and quantification of polyphenols in samples (Ignat et al., 2011). The chromatographic conditions of HPLC methods include the use of a reversed-phase C18 column, UV–Vis diode array detector, and a binary solvent system containing acidified water (solvent A) and a polar organic solvent (solvent B). The reversed phase columns could considerably enhance the HPLC separation of phenolic compounds. In addition, various supports and mobile phases are available for the analysis of different varieties of polyphenols such as flavanones, flavonols, flavones, flavan-3-ols, anthocyanins, procyanidins, procyanidins, and phenolic acids (Naczk and Shahidi, 2006).

HPLC coupled to fluorimetric, diode array or mass detectors was the most employed technique to characterize and quantify phenolic compounds in food (Albani, 2012; Fontana and Bottini, 2014; Gonçalves et al., 2012). HPLC method was used to investigate the profiles of polyphenolic compounds (various hydroxycinnamics, dihydrochalcones, procyanidins, flavonols and dihydrochalcones) in eight different apple varieties and different parts (peel and flesh) of apples (Tsao et al., 2003). In addition, a solid-liquid extraction procedure followed by reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with a photodiode array detector (DAD) for the determination of polyphenols (catechin, epicatechin, 5-caffeoylquinic acid, p-coumaric acid, phloridzin, hyperoside, isoquercitrin, rutin, quercitrin and ideain) from freeze-dried apple peel and flesh was reported (Alonso-Salces et al., 2005). The HPLC technique was also used for the analyze of the major polyphenols (chlorogenic acid, caffeic acid, syrigin, procyanidin B2, (-)-epicatechin, cinnamic acid, coumaric acid, phlorizin and quercetin) from apple pomace and for the study of the optimal extraction conditions with microwave treatment (Bai et al., 2010). The phenolic composition from the unripe apple extracted by ultrasound (US) was determined by HPLC in Figure I.6 (Yue et al., 2012). The effect of processing conditions on polyphenols composition ((-)-epicatechin, procyanidin B2, chlorogenic acid, and procyanidin B1) from young apple was studied by HPLC. HPLC analysis method has been widely used for polyphenols analysis from apple solids and apple juice. Qualities of commercial apple juice/stored juice were also evaluated on the basis of the polyphenols content with HPLC analysis (Gliszczynska-Swiglo and Tyrakowska, 2003). Results presented that storage of juices resulted in a decrease of phenolic acids from 5% to 21%, and flavonoids from 8% to 19%. Apple juice produced by different apple cultivars was studied. 17 kinds of polyphenols were analyzed by HPLC analytical techniques, and the content of every polyphenol depends on the apple cultivars and processing method (Kahle et al., 2005). In addition, individual phenol (hydroxycinnamic acids 64.9%, flavonols 21.6% and dihydrochalcones 13.6%) of apple juice was quantified by HPLC-DAD analytical technique to reflect that UHPH treatment was useful to prevent degradation of polyphenols compounds (Suárez-Jacobo et al., 2011).



Figure I.6. HPLC chromatogram at 280 nm of unripe apple polyphenols extract (1, procyanidin B1; 2, (+)-catechin; 3, chlorogenic acid; 4, procyanidin B2; 5, caffeic acid; 6, (-)-epicatechin; 7, quercetin-3-D-galactoside; 8, quercetin-3-glucoside; 9, phlorizin; 10, quercetin (Yue et al., 2012).

#### I.2.4.3 Fluorescence spectroscopy

Compared to other spectroscopic techniques, fluorescence spectroscopy is more sensitive and more selective. It significantly reduces spectral complexity and suppresses light-scattering interferences (Andrade-Eiroa et al., 2010). Due to its unique advantages, fluorescence spectroscopy is especially useful for studies of minor components in complex food samples with the presence of interferences (Christensen et al., 2006). Normally, fluorescence intensity is measured as a function of the simultaneously scanned emission and excitation wavelengths.

During the last decade, fluorescence spectroscopy has been widely used for polyphenols analysis of food materials (Andersen et al., 2008; SádeCká and Jana, 2007). Especially, Merzlyak et al. (2008) recorded apple samples' chlorophyll fluorescence excitation and diffuse reflection spectra in the visible and near UV regions to examine whole apple fruit differing in pigment content and composition. The authors identified the spectral bands sensitive to the pigment concentration and built linear models for non-destructive assessment of anthocyanins, carotenoids, and flavonols by using fluorescence spectroscopy. Nowadays, fluorescence spectroscopy has been more widely used to study apple juice than for apple tissue. The research demonstrated that the intensity of fluorescence spectra was correlated with the soluble solid contents of apple juice (Seiden et al., 1996). Apple juice produced from different apple varieties could be classified with different techniques based on fluorescence spectroscopy (Poryvkina et al., 2014). In addition, the profile of fluorescence spectroscopy was correlated with formed substances during the thermal processing of apple juices. In addition, the analysis of fluorescence spectroscopy could be used for monitoring the nonenzymatic browning of juice (Zhu et al., 2009). Recently, fluorescence spectra combined with parallel factor analysis (PARAFAC) was used to analyze varieties of polyphenols component from commercial apple juices with excitation/emission wavelengths at 270/315 nm, (310,370)/455 nm, 430/(550,680) nm (Włodarska et al., 2016). Front-face synchronous fluorescence spectra combined with principal component analysis (PCA) method was beneficial for the prediction of the total phenolic content (TPC=552  $\pm$  403 mg GAE/L), flavonoid content (TFC=134  $\pm$  69 mg CE/L), and the antioxidant activity (TAC=4.6  $\pm$  3.8 mmol (mM)) of apple juices (Fig. I.7) (Włodarska et al., 2017).



Figure I.7. Total synchronous fluorescence (TSyF) spectra of the apple juices: a) clear reconstituted from concentrate (TPC = 198.4 mg)GAE/L. TFC = 52.7 mg CE/L, TAC = 1.06 mM), b) cloudy produced from concentrate with added apple pulp (TPC = 386.5 mg GAE/L, TFC = 153.4 mg CE/L, TAC = 4.43 mM), c) pasteurized naturally cloudy produced not from concentrate (TPC = 174.2 mg GAE/L, TFC = 54.7 mg CE/L, (TPC = 391.2 mg)TAC = 1.36 mM), and d) freshly squeezed juices GAE/L, TFC = 42.2 mg CE/L, TAC = 3.05 mM) (Włodarska et al., 2017).

From large amounts of previous literatures analysis, the Folin-Ciocalteu method and HPLC method were the most widely used methods for quantitative and qualitative analysis of polyphenols of apple samples, respectively. The Folin-Ciocalteu method is simple, whereas it can only give an estimation of the total phenolic content. This is because the color reaction will occur with any oxidizable phenolic hydroxy group (Gómez-Caravaca et al., 2006). The HPLC method is useful for the separation and quantification of polyphenolics in samples. Nevertheless, HPLC methods present limitations in detection of complex samples due to the low sensitivity (Ignat et al., 2011). Recently, fluorescence spectroscopy was a new determination method for specific polyphenols of apple samples because of good sensitivity and specificity (Porgalí and Büyüktuncel, 2012; Rodríguez-Delgado et al., 2001).

#### **I.2.5 Polyphenols applications**

#### I.2.5.1 Food applications

#### I.2.5.1.1 Functional foods

Apple polyphenols can prevent hypertension and dental caries, resist tumor, senility, mutation, radiation and allergy, and enhance muscle strength with improvement of the activity of enzyme free radical scavenger (superoxidase dismutase, catalase and glutathione-peroxidase) in human body (Padmini et al., 2008). Nowadays, apple polyphenols have be used in the development and production of functional foods (Landete, 2012; Lea, 2010; Terao, 2009; Wang and Bohn, 2012; Watson and Preedy, 2010).

#### I.2.5.1.2 Meat products

Apple polyphenols are beneficial to prevent oxidation and deterioration of meat products. The antioxidant property of apple polyphenols could improve the freshness of meat products and extend shelf life (Beriain et al., 2018; Velasco and Williams, 2011; Yu et al., 2015). In addition, apple polyphenols could reduce the fishy smell with inhibiting the production of volatile trimethylamine (Lakshmanan, 2000). Previous studies presented that apple polyphenols can significantly inhibit the oxidation of fat in meat and improve the stability of the red color of meat (Luciano et al., 2009; Rojas and Brewer, 2008; Xue-ping and others, 2007).

#### I.2.5.1.3 Wine and beverage

Apple polyphenols have been used as a clarifying agent for wine and beverage (Chung et al., 2016; Shahidi and Ambigaipalan, 2015). The connection of the phenolic hydroxyl group and amide group in protein with hydrogen bond can precipitate the component forming with

gelatin and tannin, and can remove other suspended solids (Marchal and Jeandet, 2009; Siebert, 2009, 2006). Adding apple polyphenols to the vitamin C solution can effectively inhibit the degradation of vitamin C and the fading of pigments, especially  $\beta$ -carotene (Huvaere and Skibsted, 2015). Previous studies have demonstrated that apple polyphenols (1%) can keep the color of beverage during the shelf life, especially for beverages containing  $\beta$ -carotene (Chung et al., 2016; Shahidi and Ambigaipalan, 2015; Sun-Waterhouse, 2011). In addition, phloridzin and xylose-phloridzin have been widely used as the characteristic indexes of apple and its products to detect the mutual admixture of apple and pear products. At present, this detection method has played an important role in identifying product quality in international trade (Górnaś et al., 2015; Thavarajah and Low, 2006; Versari et al., 1997).

#### I.2.5.1.4 Vegetables and fruits

Apple polyphenols had antibacterial effects, such as bacillus, escherichia coli, pseudomonas and other tested bacteria. Apple polyphenol extracts have good thermal stability in the bacteriostatic activity, especially under the condition of pH=5-6 and low concentration of inorganic salt (<0.3mol/L) (Cai-wen et al., 2008; Vivek et al., 2012). Spraying low-concentration apple polyphenol solution on fresh products is beneficial for the preservation and corrosion of fruits and vegetables by inhibiting bacterial reproduction and keeping the original color (Akazome, 2004; Marshall et al., 2000; Zhang et al., 2015).

#### I.2.5.1.5 Bubble gum

Previous studies demonstrated that polyphenols, especially tea polyphenol, could effectively reduce the amount of methyl mercaptan produced and remove bacteria during chewing bubble gum (Porciani and Grandini, 2012; Tao et al., 2013). Especially, apple polyphenols can be added in bubble gum because of its good inhibition of halitosis.

In addition, apple polyphenols have been used as new additives in bread, cake, pastry and oil production (Sudha et al., 2007; Yilmaz, 2006).

#### I.2.5.2 Cosmetics applications

Apple polyphenol is a kind of natural product with unique physiological and chemical activities, which has good convergence and adhesion (Sugiyama et al., 2007; Zillich et al., 2015). Apple polyphenols can maintain the synthesis of collagen, inhibit elastase, protect collagen and improve skin elasticity to avoid or reduce the generation of wrinkles and keep the appearance of delicate skin (Gaudout et al., 2008, 2007, 2004). Apple polyphenol is often added as an active ingredient in cosmetics, playing an important role in anti-oxidation, anti-

aging, anti-ultraviolet, anti-radiation, sunscreen, whitening and moisturizing (Baldisserotto et al., 2012; Gaudout et al., 2006). Especially, condensed tannin extracted from apples can play an anti-allergic role with effectively inhibiting the dissociation of histamine and hyaluronidase activity (Magrone and Jirillo, 2012). In addition, apple polyphenols are used to prepare vitamin C or vitamin E and polyphenols complex whitening agents because of phenolics can inhibit the activity of tyrosinase and catalase and remove reactive oxygen species (Shoji et al., 2005).

#### I.2.5.3 Other applications

Apple polyphenols have been used to formulate antibacterial edible films (Alberto et al., 2006; Du et al., 2011). In addition, grafting apple polyphenols onto the polymer chain can not only reduce the environmental damage caused by phenolic waste discharged in the processing process, but also can be used as a product to treat wastewater containing heavy metal ions (Celik and Demirbaş, 2005; Chand et al., 2015).

Based on above applications, apple polyphenols are nowadays becoming more and more important in both food industrial area and other non-food domains. Therefore, the intensification of phenolic compounds extraction from apple products (peels, seeds, flesh and pomace) begins to attract a great attention of many researchers (Ćetković et al., 2008).

#### **I.3** Polyphenols extraction technologies

#### I.3.1 Conventional extraction

The conventional method of polyphenols recovery is based on a solid-liquid solvent extraction. Water, alcohols (methanol, ethanol, and alcohol–water mixtures), acetone, and ethyl acetate are commonly used for the extraction of polyphenols from apples (Wijngaard and Brunton, 2009). Depending on the type of solvent used, the cell membranes could be more vulnerable which facilitates the release of polyphenols. Solid–liquid extraction is defined as a mass transport phenomenon in which solids contained in a solid matrix migrate into a solvent brought into contact with the matrix (Ignat et al., 2011). Mass transport phenomena can be enhanced by changes in concentration gradients, diffusion coefficients or boundary layer (Corrales et al., 2009). The extraction efficiency is known to be a function of process conditions. The concentration of the polyphenol components in extracts is affected by some factors, including temperature, time, solid-liquid ratio, and flow rate (Hayouni et al., 2007; Pinelo et al., 2004; Rubilar et al., 2003). Conventional extraction as heating, boiling, or refluxing can be used to extract natural phenolic compounds. But these conventional methods

are time and energy consuming, and some methods even will take a few hours. Moreover, they involve extensive subsequent solid–liquid separation and purification steps.

#### I.3.2 Intensification of extraction process

Recently there has been an increasing demand for new extraction techniques that are environmentally friendly, faster, and more efficient than conventional extraction methods. Among these techniques, pressurized fluid, supercritical fluid, microwave, high hydrostatic pressure, ultrasound (US), pulsed electric fields (PEF) and high voltage electrical discharges (HVED) have shown their efficiencies for the extraction of polyphenols from apple products (Table I.4).

Technologies	Advantages	Disadvantages	References
Solvent	Cell membranes more	Time-consuming, low yields and	(Bai et al., 2010;
diffusion	vulnerable, easy operation	submit polyphenols to thermal	Guo et al., 2007)
		degradation or oxidation	
Enzyme	High selectivity	Cost of enzymes, difficult to	(Oszmiański et
		scale-up	al., 2011; Pinelo
D 1	T I (	<b>TT 1 1 1 7 7 7</b>	et al., 2008)
Pressurized	Lower solvent	High implementations costs	(Grigoras et al.,
IIulu	costs with chemicals and		2015)
	effluent treatment smaller		
	ecological impacts		
Supercritical	Higher process efficiency	Difficult to scale-up, not	(Adil et al., 2007;
fluid	and lesser detrimental	efficient in the extraction of	Massias et al.,
	ecological effects, lower	polar polyphenols	2015)
	operation temperatures,		
	preserving the material		
	composition; reducing		
T TI44000000 d	degradation reactions	I and time and high tamp mature	(Complex et al
Ultrasound	energy and permit the	Long time and mgn temperature	(Corrales et al., 2008: Pingret et
	reduction of solvents		al 2012)
Microwave	Reduce both extraction	Long time and a distinct	(Hemwimon et
	time and solvent	temperature increase	al., 2007; Spigno
	consumption		and De Faveri,
	-		2009)
Pulsed electric	Preserve the structure of	Less effective from dried raw	(Boussetta et al.,
fields	the product, low	materials	2015; Grimi et al.,
<b>TT 1</b>	temperature increase		2010)
High voltage	Low temperature	More difficult to a subsequent	(Boussetta et al.,
discharges	increase, short times of	solid to liquid separation,	2012; Gros et al., $2003$ )
uischarges	consumption	membranes difficult to scale-up	2003)
	consumption	memoranes, unneur to scale-up	

Table I.4. Polyphenols extraction methods.

I.3.2.1 Microwave

Microwave-assisted extraction is based on the direct effect on molecules by ionic conduction and dipole rotation (Pérez et al., 2014). Microwave energy can heat the solvents in

contact with solid samples and extract interest compounds from solid samples into solvents (Garcia and de Castro, 2002), by increasing the internal pressure inside the cells that subsequently facilitates the rupture of cellular walls and the release of active compounds to solvent (B. Zhang et al., 2008a). The electromagnetic waves caused by microwave can produce a synergistic combination of two transport phenomena: heat and mass gradients working in the same direction (Chemat et al., 2009), which is different in conventional extractions (Fig. I.8). Microwave-assisted extraction has some advantages, such as short-extraction time, low-energy requirement, high extraction efficiency and minimum degradation of target compounds (Barba et al., 2015b; Camel, 2000).



Figure I.8. Basic heat and mass transfer mechanisms in microwave and conventional extraction of natural products (Périno-Issartier et al., 2011).

Microwave treatment has been widely used to extract polyphenols from industrial apple pomace. Microwave-assisted extraction at optimal conditions (microwave power of 650.4 W, extraction time of 53.7 s, ethanol concentration of 62.1% and the ratio of solvent to raw material of 22.9:1) could get a higher extraction yield of TPC ( $62.68\pm0.35$  mg GAE/100 g) with lower solvent consumption compared to the conventional extraction (Bai et al., 2010). Similar results have shown that higher yields of polyphenolic compounds from apple pomace could be extracted by the combination of microwave treatment and application of ethanol-water solvents (Rezaei et al., 2013). In addition, the effects of US and microwave treatment on polyphenols extraction from apple pomace were compared (Ajila et al., 2011). Nowadays, the effects of solvents, temperature, time and detergents during the extraction of polyphenolics by US and microwave-assisted extraction methods have been studied by researchers for the development of better extraction and recovery method for polyphenolics. *1.3.2.2 Pulsed electric fields* 

Pulsed electric fields (PEF) treatment is a non-thermal technique providing electrical pulses of a few microseconds. PEF action is mainly localized on a microscopic scale. Pores are formed in cell membranes thus accelerating the release of intracellular compounds into

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solvents. The pore could be formed on the cell membrane (electroporation) by PEF treatment (Fig. I.9). The mechanism of PEF technique can be explained by the capacitor model. The cell membrane which is composed of two lipidic layers can be represented by a capacitor. The cell cytoplasm acts as an electrical conductor. The conductivity of the extracellular media is represented by a resistor in parallel and two resistances in series. When an electric field is applied, there is charges accumulation on both sides of the membrane. The cell membrane is polarized and a trans-membrane potential ( $V_m$ ) appears. Pores are formed on the membrane with  $V_m$  values higher than the dielectric characteristic of the membrane. This phenomenon would be similar to the breakdown of an electrical capacitor. The advantage of PEF assisted-extraction is to preserve the structure of the products and promote the purification of the subsequent extracts due to PEF results in electroporation of cell membranes but does not fragment the products.



Figure I.9. Schematic representation of electroporation mechanism in biological cell membrane exposed to an electric field. E is the electric field intensity and  $E_c$  is the critical electric field intensity (Kumari et al., 2018).

PEF treatment allowed selective extraction of different bio-molecules from apples (e.g. carbohydrates, proteins, pectin, pigments and flavors) (Vorobiev and Lebovka, 2010). Moreover, obtained data demonstrated that PEF pretreatment can significantly enhance extraction of polyphenols from apple flesh, peel, pomace and juice (Lohani and Muthukumarappan, 2016; Turk et al., 2012; Vorobiev and Lebovka, 2009; Wiktor et al., 2016). PEF combined with pressing could become a good alternative to traditional process for production of higher quality apple juices (Grimi et al., 2011). In addition, the effects of PEF treatment (E = 450 V/cm; t = 10 ms) and apple mash size on juice yield, polyphenolic compounds, sugars, and malic acid were studied (Turk et al., 2010). Study results demonstrated that the yield (Y) of apple juice increased significantly after PEF treatment of large mash (Y = 71.4%) and of small mash (45.6%) combined with conventional press. For apple pomace, PEF treatment could successfully release the bound phenolics and enhance the total polyphenols content and antioxidant activity (Lohani and Muthukumarappan, 2016). In

addition, PEF pretreatment has been beneficial for preserving important properties (purity, color, texture, flavor and nutrients) of apples and these properties would be destroyed by traditional mechanical, thermal or chemical pretreatments (Vorobiev and Lebovka, 2010). Moreover, previous research found that PEF application to the whole apple in an aqueous medium can be sensitive to changes in electric field induced inside tissue and depends on the conductivity of the aqueous medium (Grimi et al., 2010).

### I.3.2.3 High voltage electrical discharges

High voltage electrical discharges (HVED) introduce energy directly into an aqueous solution through a plasma channel formed by a high-current/high-voltage electrical discharge between two submerged electrodes. HVED includes a large range of current  $(10^3-10^4 \text{ A})$ , voltage  $(10^3-10^4 \text{ V})$  and frequency  $(10^{-2}-10^{-3} \text{ Hz})$  (Chang et al., 2000), while requires short treatment time (a few ms), low energy consumptions (10-50 kJ/kg) and low temperature elevations (<5 °C) (Gros et al., 2003). HVED results in several phenomena (such as thermal, photonic, acoustic and mechanical phenomena) that can appear separately or in combination. The phenomena generated by HVED strongly depend on the environment in which they are applied: aqueous or gaseous medium (Vorobiev and Lebovka, 2010). It is widely believed that HVED produces two types of discharges in liquids: slow discharge (subsonic) and rapid discharge (supersonic). The shock waves and the cavitation bubbles produced by HVED can alter and disrupt the cells thus resulting in the fragmentation of the cells (Fig. I.10). Since HVED could damage both cell walls and cell membranes of samples. Meanwhile, HVED can produce free radicals that make very reactive environments (Grémy-Gros et al., 2009).





Nowadays, few researches have studied the application of HVED-assisted extraction from apple products. However, HVED has been widely applied to extract polyphenols from other food samples or wine products. Many researchers studied the optimal conditions for HVED-assisted extraction of polyphenols from food materials. At the optimal conditions, the

intensification of the extraction of total polyphenols was different for various raw materials at both laboratory and pilot scales (Table I.5).

Materials	HVED	Polyphenols content	References
Orange peels	222 kJ/kg	0.7 g/100 g DM	(El Kantar et al., 2018)
Olive kernels	66 kJ/kg	626.6 mg/L	(Roselló-Soto et al., 2015a)
Grape stems	188 kJ/kg	$6.6 \pm 0.2 \text{ g}/100 \text{ g RM}$	(Brianceau et al., 2016)
Blackberries	9.6 kJ/kg	$932.69 \pm 33.45 \ mg/100 \ g \ DM$	(Barba et al., 2015a)
Vine shoots	10 kJ/kg	34.5 mg/DM	(Rajha et al., 2014)
Sesame cake	83 kJ/kg	$20.3 \pm 0.9 \text{ g/100 DM}$	(Sarkis et al., 2015)
Grape peels	120 kJ/kg	$21.4 \pm 0.8 \ \mu mol/g \ DM$	(Boussetta et al., 2009)
Grape pomace	80 kJ/kg	$2.8\pm0.4~g/100g~DM$	(Boussetta et al., 2011)
Grape seeds	26.4 kJ/kg	9 g/100 g DM	(Boussetta et al., 2012)
Papaya seeds	22.1 kJ/kg	95 mg/L	(Parniakov et al., 2015)
Grape seeds	24 kJ/kg	8.3 g/100 DM	(Liu et al., 2011)

Table I.5. The HVED-assisted extraction of polyphenols from various food materials.

DM: dry matter, RM: raw matter

It was demonstrated that HVED was more efficient than PEF for extraction of polyphenols from grape peels (Boussetta et al., 2009). HVED was applied for intensification of the extracted polyphenols from grape pomace (Boussetta et al., 2011). The results demonstrated that when the electric energy input increased to 80 kJ/kg, both total polyphenols content and antioxidant activity significantly increased. In addition, the grape seeds treated by HVED were clearly fragmented at the optimal treatment conditions (total specific energy input of 160 kJ/kg, pressure shock wave higher than 100 bar) for both laboratory and pilot scales (Boussetta et al., 2012). HVED-assisted extraction allowed an increase by 35% of TPC in comparison with a conventional extraction in the same conditions (pH = 2.5 and ethanol)concentration of 50% in water) from grape stems (Brianceau et al., 2016). Polyphenols from flaxseed cake were also extracted by HVED (Boussetta et al., 2013). The researchers studied the effects of HVED, mild temperature (20-60 °C) and ethanol addition in water (0-25%) on the extraction of polyphenols from the whole and crushed flaxseed cakes. The experiment results showed that HVED noticeably enhanced the aqueous and hydro-ethanolic extractions of polyphenols from both crushed and non crushed flaxseed cakes. In addition, the application of HVED increased significantly the aqueous and hydro-ethanolic extractions of total polyphenols, and proteins of the recovered extracts from olive kernels than the application of PEF and US (Roselló-Soto et al., 2015a). HVED pretreatment was efficient to enhance the extraction yields of reducing sugars (19 g/100 g DM) and polyphenols (0.7 g/100 g DM) from orange peels (El Kantar et al., 2018). Nowadays, HVED has been

regarded as an effective technique for disrupting sample tissues and improving the release of polyphenols.

## I.3.2.4 Ultrasound

## I.3.2.4.1 Introduction

Ultrasound (US) is composed of sound waves with frequency beyond the limit of human hearing. US technique is relatively low cost, simple and energy saving, and thus has been widely utilized in many food industrial fields, such as improving food quality during processing and storage, intensifying extraction, freezing and drying process (Awad et al., 2012). Especially, US treatment is beneficial for the extraction of different kind of compounds from various fruits and vegetables (Chemat et al., 2017b, 2011), such as polyphenols (Dimitrov et al., 2019; Wang et al., 2018), polysaccharides (Chen et al., 2015; Hromadkova and Ebringerová, 2003) and esters (Rodrigues et al., 2009; Wu et al., 2001).

## I.3.2.4.2 Mechanisms of action

US treatment generates air bubbles that propagate in the liquid medium with expansion and compression cycles. A local pressure inside of air bubbles can attain 50 MPa and a local temperature up to 5000°C (Mason, 1997). The cavitation phenomenon resulting from bubble collision is capable of damaging proximate cell membranes of samples (Chemat et al., 2004). The generated cavitational effects can accelerate heat and mass transfer by plant cell walls disruption, leading to improved release of the target compounds from several natural sources (Roselló-Soto et al., 2015b). In addition, there is also the oxidative energy of radicals created during sonolysis of the solvent (hydroxyl and hydrogen peroxide for water), which results in a high extractive power of ultrasounds (Chemat et al., 2011; Rawson et al., 2011). Ultrasoundassisted extraction (UAE) is mainly based on acoustic cavitation and its hydrodynamic shear forces (principle presented in Figure I.11). US treatment has some advantages and benefits to extract valuable bio-compounds from samples. In particular, US offers greater penetration of the solvent into cellular material, shorter processing and residence time, higher product yields and reproducibility, lower solvent and emulsifiers consumption, higher processing throughput, significant savings in maintenance, less energy needed for processing, and finally, due to the mentioned facts, greener and cumulative cheaper processing (Barba et al., 2015b; Deng et al., 2015; Roselló-Soto et al., 2015b).



Figure I.11. The principle of ultrasound-assisted extraction (Leong et al., 2011). I.3.2.4.3 Treatment equipment

The two main types of devises for ultrasound treatment in the laboratory are the ultrasonic bath and the ultrasound probe (Fig. I.12). Ultrasonic bath is widely used for disinfection and material cleaning whereas ultrasonic probe is generally used for extractions. The source of ultrasound power for both systems is based on a transducer, such as the piezoelectric transducer, which is one of the most common type used in the majority of ultrasonic reactors (Chemat et al., 2017b). The piezoelectric transducer has ability to deliver high powers which vary with the variation of the amplitude. The waves produced by transducer propagate mainly in axial directions and dissipate strongly in radial directions.



Figure I.12. Commonly used ultrasonic systems (a: Ultrasound bath, b: Ultrasound probe) (Chemat et al., 2017b).

## I.3.2.4.4 Influencing parameters of ultrasound assisted extraction

## Ultrasound power and frequency

Ultrasound input power is the actual heat dissipated in the solvent, which is commonly calculated by calorimetry as Eq. (I.2) (Contamine et al., 1995; Toma et al., 2011).

$$P = C_p m \frac{dT}{dt} \tag{I.2}$$

where  $C_p$  is the heat capacity of the solvent at constant pressure (J/(g•°C)), m is the mass of solvent (g), and dT/dt is the temperature gradient.

In general, the increase of ultrasound power can reduce extraction time and enhance extraction yield (Chemat et al., 2017b; Wei et al., 2010). Yue et al. (2012) showed that the TPC extraction yield from apple increased with the increase of ultrasound power. US frequency can also impact the extraction process due to effect on the bubble resonance size (Chemat et al., 2017b). The most commonly applied frequencies for UAE processes are in the diapason from 20 kHz to100 kHz. Chukwumah et al. (2009) reported a selective extraction of phenolics from peanuts with ultrasound frequency of 25 kHz. González-Centeno et al. (2014) demonstrated that 40 kHz was the most effective for the extraction of phenolics from grape pomace by comparing three frequencies (40 kHz, 80 kHz and 120 kHz).

#### Ultrasound intensity

Ultrasound intensity presents the transmitted energy every second and every square meter of emitting surface. It is directly related to the amplitude of the transducer and calculated as the following Eq. I.3 (Tiwari, 2015).

$$UI = \frac{P}{S}$$
(I.3)

Where *UI* is the ultrasound intensity (W/cm<sup>2</sup>), *P* is the ultrasound power (W), as calculated by the Eq. I.2, and **S** is the emitting surface of the transducer (cm<sup>2</sup>).

Previous studies showed that 20.9 W/cm<sup>2</sup> was the optimal ultrasound intensity for soybean oil extraction due to the yield decreasing at higher *UI* (Li et al., 2004). A similar tendency was observed by W. Wang et al. (2015) for ultrasound pectin extraction at 20 kHz indicating that *UI* (from 10.18-14.26 W/cm<sup>2</sup>) should be subjected to optimization, since the highest value of *UI* did not lead to the highest yields. Therefore, the optimal *UI* needs to be found for different products, extraction conditions and extracted components.

## Characteristics of ultrasonic probe

The shape and diameter of ultrasonic probe have influences on the extraction behavior. Generally, the stepped probe shape can give the highest amplitude magnification and the exponential probe shape can offer the smallest diameters (Chemat et al., 2017b). In addition, the material of ultrasonic probe has influence on the extraction effectiveness due to the erosion of probe, which can release metal particles into extraction medium (Cravotto et al., 2008). At present, the most material used for ultrasound probe emitters is titanium alloy, since this material is thermo-resistant and behaves well under corrosive conditions (Chemat et al., 2017b).

#### Solvent

The molecular affinity between the solvent and the solute, and the solubility of the extracted components are the most important factors for the selection of a solvent. Cavitation phenomena produced by US are related to the physical properties of the solvent, including viscosity, surface tension and vapor pressure (Mason, 1967). They found that the intensity of cavitation decreased with the increase of viscosity, vapor pressure and surface tension. Li et al. (2004) proved that the higher oil yield was obtained using UAE with isopropanol than with hexane as a solvent. This was due to the fact that vapor pressure of hexane is in five times higher than that of isopropanol. The collapse of cavitation bubble is more intense in solvents with lower vapor pressure (Flannigan and Suslick, 2010).

#### Characteristics of samples

Effectiveness of UAE for different samples depends on the particle size, structure, texture and composition of the sample (Vilkhu et al., 2011). The characteristics of samples can affect their sensitivity to the ultrasonic pressure waves (Vinatoru, 2001). Cavitation bubbles can more easily interact with samples having porous surface (Li et al., 2004). In addition, the turgidity of samples and the mobility of certain particles (such as starch and protein) in the cell cytoplasm both could influence the energy dissipation of ultrasound (Zhang et al., 2005). *Temperature* 

The temperature is another factor to affect solvent's characteristics, such as viscosity, surface tension and vapor pressure. High temperature can reduce the sonochemical effects due to the decrease of collapse of cavitation bubbles (Sališová et al., 1997). Previous study showed that the increase of temperature could lead to higher extraction yield (BARROSO et al., 2013). The effect of temperature (from 20 °C to 70 °C) for UAE was studied and compared with non-sonicated extractions (Shahidi and Ambigaipalan, 2015). The study presented that the extraction yields increased with the increase of extract temperature. However, the effectiveness is decreased when the temperature is close to the solvent's boiling point. Some studies demonstrated that relatively low temperature was beneficial for UAE (Esclapez et al., 2011; Palma and Barroso, 2002). The efficiency of aqueous UAE of polyphenols from dried apple pomace at mild temperatures (40 °C) was confirmed (Pingret et al., 2012). Optimal temperature (30 °C) was permitted to obtain the highest yield of extracted oil from flaxseed (Z.-S. Zhang et al., 2008). However extraction temperature varies according to the type of samples and the target compound of extraction (H.-F. Zhang et al., 2009; Q.-A. Zhang et al., 2009).

## I.3.2.4.5 Ultrasound assisted extraction from apples

Nowadays, some studies have proved the potential of ultrasounds as an energetic assistance in polyphenols extraction from apples in terms of yields and duration of the process (Virot et al., 2010). UAE was beneficial to increase the antioxidant activity of apple pomace extracts (Pingret et al., 2012). TPC obtained by UAE were on 30% higher than those obtained by conventional extraction. US-assisted extraction of polyphenols from apple is a time-saving, value-added, and environment-friendly process for the preparation of natural strong antioxidants (Yue et al., 2012). Abid et al. (2014) proved that US (frequency 25 kHz, intensity of 2 W/cm<sup>2</sup>), applied at 20 °C for 30 min enhanced effectively the TPC extraction from apple juice. The UAE for recovery of polyphenols from the unripe apple at different concentrations of ethanol (40-90% v/v), temperature (30-80 °C), extraction time (10-30 min), and ultrasound power (280-560 W) has been tested (Yue et al., 2012). At optimum extraction conditions (ethanol concentration of 50%, temperature of 50 °C, extraction time of 30 min, ultrasonic power of  $\approx 520$  W), the maximum TPC of  $13.26 \pm 0.56$  mg GAE/g could be obtained. Application of UAE at optimal conditions (US of 25 kHz and 150 W, 50% v/v ethanol/aqueous solvent, solid/liquid ratio of 15% w/v and moderate temperatures 16–34 °C) increased the TPC more than 20% as compared with conventional extraction (Virot et al., 2010). Similar results were obtained for UAE using ethanol 56% at 80 °C for 30 min or acetone 65% at 25 °C for 60 min, respectively (Wijngaard and Brunton, 2010). In addition, application of aqueous UAE allowed increasing the TPC from dried apple pomace by 30% as compared with conventional extraction at mild temperature (40 °C) (Pingret et al., 2012). Note that different processing factors can affect the efficiency of UAE of polyphenols and their purity (Chemat et al., 2017b; Pingret et al., 2012).

Previous studies showed that the US-assisted extraction was widely recognized as green technique with reduced energy consumption and processing time, low quantity of wastes and easily used on industrial scale (Chemat et al., 2017a, 2017b). Particularly, US treatment is beneficial on selectivity of polyphenols extraction from apple products (Pingret et al., 2012; Virot et al., 2010; Wang et al., 2018; Yue et al., 2012). Therefore, the US treatment was selected as the intensified technology for extracting polyphenols in present thesis. In addition, purification of extracted polyphenols is required in order to obtain higher concentration of polyphenols.

### I.4 Polyphenols purification Technology

## I.4.1 Introduction

Commonly, purification of extracted polyphenols is required in order to get a higher concentration of polyphenols by removing the residual solid particles and undesirable molecules (such as proteins and sugars) (Boussetta et al., 2015). Solvent extraction is a conventional technique for the recovery of polyphenols, but its selectivity for polyphenols recovery is poor. Efficient, selective and environmentally friendly purification technologies are still required. In recent years, many polyphenol purification technologies were studied, including adsorption and desorption on macroporous resin, precipitation, crystallization, and membrane filtration (Conidi et al., 2015; Nawaz et al., 2006; Sun et al., 2013; Tsujita et al., 2011; Watson, 2018; Yang et al., 2000). Pilot scale resin adsorption was used to recover and fractionate apple polyphenols (D. R. Kammerer et al., 2010). The efficiency of eight macroporous resins for simultaneous separation and purification of total polyphenols, chlorogenic acid, and phlorizin from thinned young apples were compared. Results demonstrated that X-5 resin presented the best adsorption and desorption efficiency for total polyphenols (Sun et al., 2013). The purification process by ultrafiltration (UF) after extraction of polyphenols from grape seeds could provide high extraction rates and extraction selectivity. The obtained results showed that the maximum amount of polyphenols (11.4% of the weight of total seeds) from grape seeds could be recovered by UF process (Nawaz et al., 2006). The combination of an adsorption/desorption system (adsorbents resins) with a membrane filtration system (UF and nanofiltration, NF) produces more purified fraction of phenolic compounds from artichoke wastewaters compared to an individual process fully based on the filtration with the use of adsorbents resins or membrane (Conidi et al., 2015). In addition, the previous study presented that dialysis technology, using Diaion HP-20 and Sephadex LH-20 columns, was beneficial for the purification of extracted polyphenols from chestnut astringent peels (Tsujita et al., 2011). Among these purification technologies, the adsorption and desorption with resins and the membrane filtration are most widely used for the purification of polyphenols extracts.

## I.4.2 Adsorption and desorption

#### I.4.2.1 Introduction

Nowadays, many types of absorbents have been applied for adsorption and desorption processes, such as activated carbons, mineral adsorbents (siliceous materials, clay, natural zeolites), and resins (ion-exchange resins and macroporous resins). Especially, concentration

and purification of polyphenols have been widely studied using macroporous resins (J. Kammerer et al., 2010; Silva et al., 2007; Soto et al., 2011). Polyphenols are firstly adsorbed on the resin surface due to the strong noncovalent bonding and aromatic stacking. Following desorption of polyphenols from resins occurs into organic solvents. The previous studies demonstrated the effectiveness of macroporous resin adsorption/desorption for purification and concentration of bio-compounds obtained from the extracts of fruits and vegetables (T. Liu et al., 2010; Seeram et al., 2005; T. Wang et al., 2015; Barkakati et al., 2010; Saleh et al., 2008; Li and Chase, 2009; Silva et al., 2007).

### I.4.2.2 Operational parameters influencing adsorption and desorption

Adsorbent characteristics affect importantly adsorption (Brune et al., 1999; Cheng et al., 2006; Dargaville et al., 1996; Geng et al., 2009; Navarro et al., 2009; Pan et al., 2005). There are various absorbents used for polyphenols purification, such as activated carbon, reverse phase silica gels and resins. For instance, activated carbon is used to recover polyphenols from grape juice (Soto et al., 2008); reverse phase silica gels are beneficial for anthocyanin recover from aronia melanocarpa (Kraemer-Schafhalter et al., 1998). Among these absorbents, macroporous resins (Table I.6) have been widely used in the separation and purification of plant polyphenols due to their physicochemical stability, high adsorption selectivity and easy recycling. This concerns especially AB-8 macroporous resin (Wan et al., 2014). Macroporous resins are durable polar, non-polar or slightly hydrophilic polymers with high adsorption capacities for organic molecules through appropriate surface area and nuclear pore size, electrostatic force, hydrogen bonding interactions, hydrophobic interactions and complexation (Fu et al., 2006; Gao et al., 2007). Polyphenol molecules can form hydrogen bonds by interactions between surface functional groups (hydroxyl and carboxyl groups) of polyphenols and macroporous resins. Therefore macroporous resins are beneficial for polyphenols separation and purification (J. Kammerer et al., 2010). In addition, internal porous structure of absorbents and properties of target compounds of solution also have influence on the adsorption and desorption processes (Garcia-Araya et al., 2003).

Table I.6. Comparison of various macroporous resins and their physical properties

(Li and Chase, 2010)

Resin	Polarity	Matrix	Surface	Pore (Å)	Particle	References
			area(m <sup>2</sup> /g)	diameter	size (µm)	
AB-8	Weak	SDVB	480-520	130-140	300-1250	(Ma et al., 2009a,
						2009b; Yang et al.,
						2009)

ADS-5	Non	SDVB	520-600	250-300	300-1250	(Fu et al., 2007; W.
						Liu et al., 2010)
ADS-17	moderate	acrylic	250-300	90-150	300-1250	(Ma et al., 2009a)
AL-2	Polar	SDVB	550-600	150-250	300-1250	(Fu et al., 2006)
D101	Non	SDVB	480-550	90-150	300-1250	(Fu et al., 2005; Wan
						et al., 2008)
DM11	Non	SDVB	≥500	70-80	315-1250	(Lv et al., 2008)
DM-301	Weak	SDVB	≥330	140-170	300-1250	(Lv et al., 2008)
DS-401	Weak	SDVB	≥480	120-140	300-1250	(Wan et al., 2008)
HP-20	Non	SDVB	600	520	300-1250	(Yang et al., 2009)
HP-100	Non	SDVB	650-700	85-90	300-1250	(Jia and Lu, 2008; Lv
						et al., 2008; Yang and
						Tan, 2008)
HP-200	Non	SDVB	700-750	85-90	300-1250	(Ma et al., 2009a,
						2009b)
HP-500	Polar	SDVB	500-550	100-120	300-1250	(Lv et al., 2008)
HP-600	Polar	SDVB	500-550	100-120	300-1250	(Ma et al., 2009a)
HP-800	moderate	SDVB	700-750	90-110	300-1250	(Gao et al., 2007)
HP-850	Polar	SDVB	1100-1300	85-95	300-1250	(B. Zhang et al.,
						2008b)
LSA-10	moderate	methacrylic	500-540	85-90	300-1250	(Fu et al., 2005; Ma et
						al., 2009b)
LSA-20	Non	methacrylic	420-500	85-90	300-1250	(Fu et al., 2005)
NKA11	Polar	SDBV	160-200	145-155	300-1250	(Lv et al., 2008)
XAD-7	Weak	acrylic	450	90	250-840	(Li and Chase, 2010)
XAD-16	Non	SDVB	630	210	250-840	(Li and Chase, 2010)
XAD-	Non	SDVB	700	400	250-840	(Yang et al., 2009)
1180						
XAD-	Non	SDVB	800	150	250-840	(Yang et al., 2009)
1600						
XAD-1	Non	SDVB	800-1000	85-95	300-1250	(Fu et al., 2005; Jin et
						al., 2008)
XAD-6	Polar	SDVB	450-500	120-160	300-1250	(Ma et al., 2009b)

SDVB: styrene–divinylbenzene.

Apart from adsorbent characteristic, the values of pH, ionic strength, polarity and the temperature of extracts will also affect the effectiveness of adsorption and desorption

processes (Baycin et al., 2007; Ramos et al., 2004; Silva et al., 2007). The interactions of solutes and adsorbents presented different mechanisms at different pH (Caetano et al., 2009; Carmona et al., 2006). At acidic pH range, the adsorption of phenolics will be enhanced because the phenols are undissociated and the dispersion interactions predominate (Caqueret et al., 2008; Dabrowski et al., 2005; Mohanty et al., 2005; Scordino et al., 2004; Silva et al., 2007; Ugurlu et al., 2005). While the adsorption will be decreased at alkaline pH due to the dissociation of hydroxyl and carboxyl groups (Fu et al., 2006). Both positive and negative effects of temperature have been reported for polyphenols purification by macroporous resins (Gökmen and Çetinkaya, 2007; Ugurlu et al., 2005). High temperature can increase the rate of mass transfer through cell membrane and into the pores of absorbent due to the decrease of solution viscosity. However, high temperatures may promote irreversible interactions (Qiu et al., 2007). Optimal adsorption temperature depends on the purification component, applied absorbent and properties of extracts (Garcia-Araya et al., 2003; Ku and Lee, 2000). In addition, the presence of molecular oxygen in aqueous solutions can also influence the adsorption of polyphenolics onto resins (Saleh et al., 2008). The adsorption of aromatic compounds will be increased with the increase of molecular oxygen in aqueous solutions by dispersive/repulsive, donor/acceptor and hydrogen-bonding interactions (Dabrowski et al., 2005; Franz et al., 2000).

## I.4.2.3 Application of adsorption and desorption for polyphenols purification

In previous studies, adsorption and desorption technique has been applied for separation and purification of anthocyanins from mulberry (Chen et al., 2016) and blueberry (Buran et al., 2014), total polyphenols, chlorogenic acid and phlorizin from thinned young apples (Sun et al., 2013), individual phenolic compounds from apple juice (Kammerer et al., 2007) and phenolic compounds from grape marc (Zagklis and Paraskeva, 2015). For extracts of unripe apples, X-5 macroporous resin was confirmed to present the best adsorption capacity and desorption ratio for total polyphenols compared with different macroporous resins. After adsorption and desorption with X-5 resin, the polyphenols content was increased in 2.12-fold (from 35.17% to 74.64%) with a recovery yield of 89.35%. In addition, chlorogenic acid and phlorizin were selectively purified using X-5 resins, with the purities of 15.20% and 97.52%, and recovery yields of 89.16% and 64.95%, respectively (Sun et al., 2013). Quantification and selective purification of phenolic compounds presented in apple juice (Kammerer et al., 2007) and apple pomace (Carle et al., 2001; Kammerer et al., 2011; Saleh et al., 2008; Schieber et al., 2003) were done. Numerous studies confirmed that adsorption/desorption process was effective for the purification of valuable natural products from food and plant sources. However, the purity of the products may vary from 10 to 90% depending on the complexity of the composition of the crude extracts (Li and Chase, 2010). Therefore, more effective separation methods are required in order to improve the separation and purification efficiency.

## I.4.3 Membrane filtration

## I.4.3.1 Classification of membrane filtration

Efficiency of the membrane filtration is mainly dependent on the rejection of micro- and macromolecular species (sugars, bio-molecules, polymers and colloidal particles). A membrane, as a selective barrier between two phases, can separate the feed into permeate and retentate (Fig. I.13). The permeate is the part of the feed passing through the membrane, while the retentate is another part of the feed, which does not pass through the membrane. The goal component can be retained by the membrane in the retentate or pass through the membrane in the permeate. Nowadays, pressure driven membrane operations have been widely employed for various food products, such as dairy products, fruit juices, wine, vegetable oils, potable water, and agricultural wastewaters. The driving force of these processes is represented by a pressure gradient applied between the two sides of membrane. According to the transmembrane pressure (TMP) and the membrane pore size, membrane filtration is usually divided into four pressure-driven processes: microfiltration (MF, 0.1-5 µm, 1-10 bar), ultrafiltration (UF, 0.5–100 nm, 1–10 bar), nanofiltration (NF, 0.5–10 nm, 10–30 bar) and reverse osmosis (RO, <0.5 nm, 35-100 bar) (Cui et al., 2010). The application of different membrane separation and purification processes based on particle or molecular sizes is presented in Fig. I.14. RO process is widely used for desalination and water purification, while the UF and MF processes are widely used in food and bioprocessing. Especially, UF is most widely used for the purification of polyphenols by separating of undesirable components from liquids. UF membranes can retain molecules within the molecular weight (MW) more than 1000 MW (i.e. protein molecules) from passing through the membranes, thus low molecular weight components can be purified (García, 1999). Since the MW of various polyphenols are less than 1000 (Table I.7), polyphenol molecules are allowed to pass through UF membrane and thus to be purified from high molecular weight components (Nawaz et al., 2006).



Figure I.13. Schema of membrane separation process (Tsuru, 2008).



Figure I.14. The applicability ranges of different separation processes based on membrane pore sizes (Cui et al., 2010).

Polyphenol type	Molecular weight (MW)
Catechin	290.3
Epi-catechin	290.3
Epi-catechin gallate	442.4
Procyanidin dimer	578.5
Procyanidin trimer	870
Procyanidin tetramer	1160
Kaempferol	286.2
Gallic acid	170.1
Quercetin	448.4
Caffeic acid	180.2
Coumaric acid	164.2
Chlorogenic acid	354.3

Table I.7. Molecular weight of various polyphenols (García, 1999)

# I.4.3.2 Materials of filtration membranes

A membrane is a thin layer of semi-permeable material that permits the separation of certain species containing in a fluid. Membranes for filtration are usually divided into two types from materials (polymeric membranes and inorganic membranes). Especially,

polymeric membranes have had more applications for practical use in the areas of food, beverage, and biotechnology (Bhave, 2012). Among polymeric membranes, materials for MF and UF are mainly Polyacrylonitrile (PAN), Cellulose acetate (CA), Polysulphone (PS), Polyethersulphone (PES) and Poly (vinylidene difluoride) (PVDF) (Cassano et al., 2017). Each of these materials has different properties with respect to the surface charge, degree of hydrophobicity, pH, oxidant tolerance, strength and flexibility. At present, most common polymers used for producing polymeric membranes applied for polyphenols purification are PES and PVDF (Fig. I.15). PES is a less hydrophobic polymer because of containing more sulphur dioxide groups. While PVDF, contains an aliphatic chain with multiple carbon–fluorine bonds, is more hydrophobic due to less susceptible to hydrogen bonds.



polyethersulphone polyvinylidene fluoride Figure I.15. Chemical structural units of PES and PVDF (Cassano et al., 2017).

# I.4.3.3 Models of membrane filtration

Filtration models are generally classified according to the flow direction of the feed. The principle of main filtration models are presented in Fig.I.16.

# (1) Dead-end filtration

The principle of the dead-end filtration is to filter the feed perpendicularly to the surface of the membranes (Fig. I.16a). All particles in feed with the size larger than the pore sizes of the membrane are stopped at its surface to form the cake layer. The thickness of the cake layer is determined by the TMP and the flow rate, which are related to the specific resistance of the cake.

### (2) Cross-flow filtration

For cross-flow filtration, the feed flows tangentially to the surface of the membrane, thus establishing a trans-membrane pressure which limits the accumulation of cake (Fig. I.16b). Cross-flow filtration allows small particles to pass through the membrane and remains larger particles to flow along the membrane by cleaning the membrane surface. Finally, the equilibrium can be reached and the flow can stabilize in a stationary state. The cross-flow filtration can prevent larger particles from building up a cake layer on the membrane surface. (3) Dynamic filtration with rotating disk

For the dynamic filtration with rotating disk, the feed flows perpendicularly to the surface of the membranes as in dead-end filtration. However, dynamic filtration with rotating disk can produce a relative movement between the membrane and the cell, which generates a higher shear. The filter cell is equipped with a stirrer near the membrane surface, generating high shear rates on the surface of membrane to decrease membrane fouling. The intensity of shear mainly depends on the feed. The feed between stirrer and membrane is tangential to the membrane, therefore a part of the particles are kept in suspension and do not remain on the surface of the membrane (Fig. I.16c). In addition, dynamic filtration with rotating disk allows working at lower operating pressure and higher shear rates. Thus, dynamic filtration.



Figure I.16 Principle of different filtration models. a) Dead-end filtration (Ketola, 2016), b) Cross-flow filtration (Ketola, 2016), c) Dynamic filtration with rotating disk (Bouzerar et al., 2000).

### I.4.3.4 Application of membrane filtration

Membrane filtration performs purification, recovery, concentration and fractionation of solutes from by-products and biomass wastes (Castro-Muñoz et al., 2016). Nowadays,

membrane technologies (especially UF with PES and PVDF membranes) have been widely used for the subsequent step of polyphenol purification and concentration from different raw materials (Borneman et al., 2001; Liu et al., 2013; Youn et al., 2004; Zhu et al., 2014). For instance, UF had been used to purify polyphenols of flaxseed hull extracts by separating proteins (Loginov et al., 2013). A cross-flow UF pilot was effective to separate and purify polyphenols from pomegranate juice (Conidi et al., 2017). In addition, the effect of PES membranes with a molecular weight cut-off (MWCO) of 50 kDa during UF process for the purification of polyphenols in retentates from vine-shoot extracts was compared and analyzed (Rajha et al., 2015). For apple products, many previous studies analyzed the effect of membrane pore size on the permeate flux and the purification of polyphenols from apple juice (Vladisavljević et al., 2003; Zárate-Rodríguez et al., 2001) and apple cider (Zhao et al., 2015). Different kinds of membranes were compared to purify polyphenols from apple juice in the dead-end mode (Borneman et al., 2001) and from apple cider in the cross-flow mode (Zhao et al., 2015).

## I.4.3.5 Membrane electro-filtration

Nowadays, the biggest problem during membrane filtration is membrane fouling. The fouling reduces permeate flux and decreases the polyphenols purification efficiency by modifying membrane permeability and molecular selectivity (Merkel et al., 2002). Membrane fouling is due to pore constriction, pore blocking and cake layer formation (polyphenols may interact together and with other compounds to form large particles) on the membrane surface (Guo et al., 2012). In order to reduce the membrane fouling during polyphenols purification process, many technologies have been studied (Cai et al., 2009; Duclos-Orsello et al., 2006; Jaffrin, 2008; Rai et al., 2006; Wakeman and Williams, 2002), especially the "force field-assisted methods" (including electrical, magnetic and sonic forces) (Chen et al., 2007; Wakeman and Williams, 2002).

Membrane electro-filtration is a process in which charged solutes (such as proteins, organic colloids, and bacteria) are taken away from the surface of the membrane by employing direct current (DC) electrical field (Li et al., 2018). Membrane electro-filtration can reduce concentration polarization and membrane fouling (Lee et al., 2008), consequently the particles mass transfer and permeate flux may be enhanced (Song et al., 2010; Tarleton and Wakeman, 1988). The effects of membrane electro-filtration for various solutions have been studied (Liu et al., 1999; Zhao et al., 1998). For instance, membrane electro-filtration could improve liquid-solid separation, permeate flux, quality of filtrate, as well as fouling removal of drinking water and waste water (Mostafazadeh et al., 2016). Dead-end membrane

electro-filtration could improve biopolymer recovery and permeate flux (Hofmann and Posten, 2003). Cross-flow membrane electro-ultrafiltration was beneficial to enhance permeate flux of synthetic juice (mixture of sucrose and pectin) (Sarkar et al., 2008) and rapidly purify dilute protein solutions (Song et al., 2010). Therefore membrane electro-filtration was regarded as an effective filtration method to decrease membrane fouling and improve purification efficiency of valuable components from product extracts.

#### I.5 Conclusions and research objectives

Phenolic composition of apple peels is very rich and consists mainly of flavanols and dihydrochalcones. Content of phenolics in apple peels is higher than in the juice or flesh of apples, and it can vary among different varieties of apples. Nowadays the extraction and purification of phenolic compounds from apple products attract great attention. The conventional method is based on a solid-liquid extraction by various solvents with damaging cell membranes to release polyphenols. The conventional extraction needs relatively long duration, high temperature, high energy consumption and the addition of organic solvents. Therefore, many alternative technologies have been emerged, such as high pressure, ultrasound, and electric technologies, to improve the polyphenols extraction process. Nowadays, the ultrasound-assisted extraction is most widely used alternative technology for polyphenols extraction from apple products. However, the impact of ultrasound-assisted extraction on the selectivity of valuable compounds recovery from apple products was not yet elucidated enough. The objective of the first part of this thesis was to selectively extract valuable compounds (especially polyphenols) from apple products with ultrasound treatment.

The ultrasound treatment induces cavitation, shockwaves and mixing phenomena leading to increasing solid–liquid extraction yield. Especially, cavitation phenomenon was most important for the extraction efficiency due to the formation, growth and collapse of bubbles inside solvents. The bubbles collapse could cause the rupture of cell walls to enhance solvent contact with the available extractable cell material and accelerate diffusion. The mechanisms of cavitation phenomena produced by ultrasound need to be further understood.

In the third step, purification process was implemented for obtaining higher concentration of polyphenols extracts. The adsorption/desorption and membrane filtration have emerged. These techniques have the advantage of being selective, high productivity, short duration and low energy and operation consumption. Adsorption/desorption with macroporous resins and membrane techniques have shown their interests but sometimes they are confronted with some obstacles. Ultrasound-assisted adsorption/desorption and membrane electro-filtration were studied to increase purification efficiency and reduce filtration time.

In this context, the issues addressed by this work are for the extraction and purification of polyphenols from apple peels. The main objectives of this thesis are:

(1) Study the effects of ultrasound treatment on extraction selectivity of valuable compounds from apple products.

(2) Study the mechanisms of cavitation phenomenon induced by ultrasound.

(3) Analysis of correlations between disintegration degree of peel cells induced by US and efficiency of bio-compounds extraction from fruit peels.

(4) Compare and increase the performance of the two purification technologies (adsorption/desorption filtration and membrane filtration) of apple peel extracts.

#### **Chapter II Materials and Methods**

#### **II.1 Raw materials**

Peels, flesh and pomace of apples and some other fruit peels (bananas, persimmons and plums) were used as raw materials (Fig. II.1). The introduction of each of these products will be detailed in the following.

### II.1.1 Apple peels

Commercial green apples (Granny Smith) and red apples (Red delicious, Gala) were selected as the raw material for investigation (Fig. II.1a). The apples with good and uniform quality and near-spherical shape were purchased at the local supermarket (Compiegne, France). The initial moisture content of apple peel on wet basis (83.07 g/100 g for green apples, 85.39 g/100 g for red apple (Red delicious) and 84.34 g/100 g for red apple (Gala)) was determined using MA 160 infrared moisture analyzer (Sartorius, Göttingen, Germany).

#### II.1.2 Apple flesh

Commercial green apples (Granny Smith) and red apples (Red delicious) were purchased at the local supermarket (Compiegne, France) (Fig. II.1b). The apples were with good and uniform quality and near-spherical shape. The initial moisture content of apple flesh on wet basis (83.74 g/100 g for green apples, and 87.44 g/100 g for red apple) was determined using MA 160 infrared moisture analyzer (Sartorius, Göttingen, Germany).

#### II.1.3 Apple pomace

Fresh apple pomace was provided by Institut Francais des Productions Cidricoles (Rheu, France) (Fig. II.1c). The pomace was packed in plastic bags and stored at 4 °C before extraction experiments. The storage time never exceeded 1 week. The initial moisture content of apple pomace on wet basis (81.13 g/100 g) was determined using MA 160 infrared moisture analyzer (Sartorius, Göttingen, Germany).



Figure II.1 Photos of the three raw materials of apples: (a) peel; (b) flesh; (c) pomace

#### II.1.4 Other fruit peels

Commercial bananas (Cavendish), persimmons (Ribera del Xùquer), plums (Angeleno), and bottled gas water (Perrier) were purchased at a local supermarket (Compiegne, France).

The samples of good and uniform quality (with nearly spherical shape for persimmons and plums, and near the same size for bananas) were stored at 4 °C. Perrier water was stored at room temperature until needed. All experimental data were collected within 10 days of the purchase of the samples. The initial moisture content of peels on wet basis (89% for bananas, and 76% for persimmons) was determined using MA 160 infrared moisture analyzer (Sartorius, Göttingen, Germany).

#### II.1.5 Adsorbents

The hydrophobic poly-aromatic adsorbent Amberlite XAD-16 (Sigma Aldrich, France) was used in adsorption experiments. The resin XAD-16 has a surface area of 900 m<sup>2</sup>/g, mean pore radius of 5.0 nm, pore volume of 1.82 mL/g and dry density of 1.08 g/mL.

### II.1.6 Filtration membranes

The main properties of used membranes are presented in Table II.1. Four types of hydrophilic polyethersulphone (PES) filtration membranes were all purchased from Microdyn-Nadir (GmbH, Germany).

Table II.1 Properties of used membranes				
Name of membrane	e Molecular weight cut-off (MWCO)	Pore size, d <sub>m</sub>		
MP005	45660 kDa	50 nm		
UP150	150 kDa	10 nm		
UH030	30 kDa	4.35 nm		
UP010	10 kDa	3.02 nm		

For every filtration test, new membrane was used.

### **II.2 Extraction and purification experiments**

#### **II.2.1** Sample preparation

The flesh tissue (the disks with diameter of 20mm and thickness of 10 mm) was taken from the central part of the apple.

The peel tissue (thin slices with length of 20 mm, width of 10 mm) was removed from apples and persimmons with a razor blade (thickness of  $\approx 0.1$  mm) and from bananas by hand. For preparation of the freeze-thawed products, the peel tissues (20 mm x 10 mm) were frozen at -20 °C for 12 h and then thawed at room temperature ( $\approx 23$  °C) for 12 h.

The apple peel powders were prepared with peel tissues (20 mm x 10 mm). The fresh apple peel tissues were dried in a convection oven (UL50, MEMMERT, Germany) at 105 °C for

24h and then were ground into powders by a grinder (MCG2013B-16, 150 W, 50 Hz, Mandine, France) for 30s.

#### **II.2.2** Polyphenols extraction

#### II.2.2.1 Conventional extraction

20 g of samples were put in a glass beaker filled with 200 mL of distilled water/ethanol aqueous solution ( $C_{et}$ =0-50%), so the solid/liquid ratio was 1/10. During extraction experiments, the glass beaker was covered with aluminum foil to reduce solvents evaporation. Three different extraction temperatures were used T = 20, 50, 60, and 75 °C for conventional extraction (CE). Room temperature was always fixed at 20 °C. The detection temperature for TPC using the Folin–Ciocalteu method was 50 °C (Singleton et al., 1999). Many previous researches showed that the polyphenol yield was increased with increasing temperature, but also that beyond a certain value (80 °C) most phenolic compounds would be denatured (Gironi and Piemonte, 2011; Pinelo et al., 2008; Spigno et al., 2007). Therefore, T in range of 20-75 °C was selected for CE in this thesis. The distilled water for extraction was preheated to specified temperature (50-75 °C). The extraction experiment with ethanol aqueous solution was all at room temperature (20 °C). The temperature during extraction was stabilized using a water thermal bath Polystat 36 (Fisher Scientific, France). The total extraction time was up to 3 h.

#### II.2.2.2 Ultrasound- assisted extraction

20 g of samples were put in a glass beaker filled with 200 mL of ethanol aqueous solution ( $C_{et}$ =0-50%), so the solid/liquid ratio was 1/10. Ultrasonic treatment of solution was done directly in the glass beaker using an ultrasonic processor UP 400S (400 W, 24 kHz, Hielscher GmbH, Stuttgart, Germany) (Fig. II.2a). The titanium ultrasonic probe (H14, Hielscher GmbH, Stuttgart, Germany) with a tip diameter of 14 mm, and the length of 100 mm was used. The ultrasonic treatment was applied in pulsed mode. The different ultrasonic powers (P=28-150 W) were selected for ultrasound-assisted extraction process. The extraction T by ultrasound with distilled water was in the range of 20-75 °C, and with ethanol aqueous solution was at room temperature (20 °C).

The scheme of applied pulsed sonication treatment is illustrated in Fig. II.2b. The ultrasound treatment was done in pulsed mode with sequential application of n=1-10 ultrasonic pulses. A pause with duration of  $\Delta t_w=50-1000$  s was applied after each ultrasound pulse with duration of  $\Delta t_u=10-180$  s. In order to avoid increasing temperature by ultrasound

treatment, the glass beaker was put in an ice/cold water box to keep the temperature at specified temperature. The total time of UAE was  $t_e=n(\Delta t_u+\Delta t_w)=0-5050$  s.



Figure II.2 (a) The experimental ultrasound generator. (b) Scheme of applied pulsed sonication treatment.

The specific input energy, W, produced by ultrasound treatment was estimated from the temperature elevation  $\Delta T$  in sample using following equation:

$$W = mC_p \Delta T / \Delta t_u$$
 II.1

where m and C<sub>p</sub> ( $\approx$ 4.18 kJ per kg K) are the mass and specific heat capacity of solution, respectively,  $\Delta t_u$  is the duration of sonication. In this study, W was in the range of 6.4-327.3 kW/kg.

For ultrasound-assisted extraction experiments, the effects of operated parameters ( $C_{et}$ , T, n,  $\Delta t_w$ ,  $\Delta t_u$ ,  $t_e$ , P, W) on polyphenol yields were analyzed. The extracts from apple flesh and apple peels were stored at 4 °C for further analysis. The extracts of apple pomace were firstly centrifuged for 10 min at 4000 rpm (Laborzentrifugen 3-10, SIGMA, Osterode am Harz, Germany) to separate supernatant and solid sediment before stored the supernatant at 4 °C for further analysis. For the extracts of apple peel powders, the supernatant obtained after centrifuging extracts for 10 min at 4000 rpm was concentrated to remove the ethanol in a vacuum rotary evaporator (LABOROTA 4001, Heidolph Instrument, Schwabach, Germany) at 40 °C. Then the concentrated aqueous solution was filtrated with filter papers (No.474, VWR, Geldenaaksebaan, Leuven). Finally, the filtrate was stored at 4 °C for further analysis.

#### **II.2.3** Polyphenols purification

#### II.2.3.1 Adsorption/desorption procedure

#### II.2.3.1.1 Adsorbents preparation

The adsorbent Amberlite XAD-16 (10g) was firstly soaked in 95% ethanol (100 mL) for 6h and rinsed with distilled water (until eluate was clear) in order to remove the impurities,

porogenic agents and monomers trapped in the pores the resin. Then the absorbent was soaked in 5% hydrochloric acid, HCl (100 mL) for 6h, rinsed with distilled water (until neutral effluent), soaked in 5% sodium hydroxide, NaOH (100 mL) for 6h, and rinsed with distilled water (until neutral effluent). Finally, it was dried in a convection oven UL50 (Memmert, Germany) at 70 °C for 24h. The final moisture content of resin was 80.39%.

II.2.3.1.2 Adsorption procedure

The adsorption experiments were performed at 25-40 °C in a glass beakers by mixing the adsorbent amberlite XAD-16 (2 g) with polyphenol extracts (100 mL) at different concentrations of total polyphenols,  $C_0$  (0.01-6 mg/mL). The beakers were kept in shaking water bath SW22 (Julabo GmbH, Seelbach, Germany) at a rate of 150 rpm. The total time of adsorption experiments was up to 1440 min.

In the adsorption step the probes of liquid extract were periodically taken, centrifuged using Galaxy Mini Centrifuge C1413-230EU (VWR, Paris, France) for 1 min at 6000 rpm, and analyzed for the total polyphenol content, *TPC*.

Adsorption capacity (Q, mg/g dry resin) at equilibrium was calculated as:

 $Q = (C_o - C_e) V / M_r$  II.2

where  $C_o$  and  $C_e$  are initial and equilibrium (final) concentrations of bio-molecules (e.g., polyphenols) (mg/mL) in extract solutions,  $M_r$  is the mass of the resin (g) and V is the volume of the liquid medium (mL) in adsorption experiments.

In experiments with US-assisted adsorption, the preliminary pulsed sonication was applied at different US intensity, P (0-400W), in the same manner as described in II.2.2.2. The total number of pulses was n=20, US treatment time,  $t_{us}=60$  min, and the total time of US-assisted adsorption was  $t_a = 100$  min. Then the adsorption in shaking bath was continued.

II.2.3.1.3 Desorption procedure

Desorption experiments were done using the absorbents previously saturated with biomolecules in the adsorption step. The adsorbent (2g) was put into the shaking glass beaker filled with aqueous ethanol solution (100 ml,  $C_{et}$ =0-95%). Desorption experiments were performed at 25°C for 180 min. In the course of desorption, the probes of liquid extract were periodically taken, centrifuged and analyzed for the soluble matter content, <sup>o</sup>Brix, and total polyphenol content, *TPC*.

The desorption ratio, *D*, was calculated as:

$$D = C_{ff} V_d / [(C_o - C_e) V_a]$$
 II.3

where  $C_{tf}$  is the concentration of polyphenols (mg/mL) in the aqueous-ethanol solution, and  $V_d$  is the volume of the liquid medium (mL) in desorption experiments.

The adsorption/desorption efficiency (recovery),  $R_e$ , for phenolics, proteins and soluble matter content was calculated using the following equation:

$$R_e = C_f / C_o$$
 II.4

where  $C_o$  and  $C_f$  are the initial (before adsorption) and final (after desorption) concentration of bio-molecules (mg/mL), respectively.

II.2.3.2 Membrane filtration

## II.2.3.2.1 Dead-end electrofiltration

Filtration setup is presented in Figure II.3. Dead-end electrofiltration was performed with an effective membrane area of  $1.809*10^{-3}$  m<sup>2</sup>. For each experiment, 90 mL of feed solution was used. 4 types of hydrophilic polyethersulfone filtration membranes (Microdyn-Nadir GmbH, Germany) with MWCO of 10, 30 and 150 kDa (specific description of membranes is presented in II.1.5) were used to filtrate apple peel extracts. The new membrane was used for each set of experiments. Filtration pressure of 1, 4, 6 and 10 bar (1-10  $*10^5$  Pa) was supplied and continuously monitored by a pressure gauge. Electrofiltration voltage of 5, 10 and 15 V was provided by FLUKE 45 and Consort EV261. The filtrate was collected in beakers placed on an electronic balance and the mass of filtrate was recorded by computer software (Service électronique UTC, Compiègne, France).



Figure II.3 Scheme presentation of dead-end electrofiltration. II.2.3.2.2 Calculated parameters

The sieving ratio R was defined as ratio of different substances presented in solution after and before filtration (concentration of total polyphenols  $C_{tf}$ , concentration of proteins  $C_{pr}$ , concentration of total solutes  $C_{ts}$ , and colour intensity I). The rejection coefficients of different substances are calculated as  $K_r=1-R$ .

The rejection coefficient  $K_{rtf}$  and sieving ratio  $R_{tf}$  of total polyphenols are defined as:

$$K_{rtf} = 1 - R_{tf} = 1 - C_{tf'} C_{tf0}$$
 II.5

where  $C_{tf}$  and  $C_{tf0}$  are the concentration of total polyphenols in filtrate and initial feed (mg/mL), respectively.

The rejection coefficient  $K_{rpr}$  and sieving ratio  $R_{pr}$  of proteins are defined as:

$$K_{rpr} = 1 - R_{pr} = 1 - C_{pr} / C_{pr0}$$
 II.6

where  $C_{pr0}$  and  $C_{pr0}$  are the concentration of proteins in filtrate and initial feed (mg/mL), respectively.

The rejection coefficient  $K_{rts}$  and sieving ratio  $R_{ts}$  of total solutes are defined as:

$$K_{rts} = 1 - R_{ts} = 1 - C_{ts}/C_{ts0}$$
 II.7

where  $C_{ts}$  and  $C_{ts0}$  are the concentration of total solutes in filtrate and initial feed, respectively.

The rejection coefficient  $K_{rI}$  and sieving ratio  $R_I$  of colour intensity are defined as:

$$K_{rI} = 1 - R_I = 1 - I/I_0$$
 II.8

where I and  $I_0$  are the colour intensity of filtrate and initial feed, respectively.

Volume ratio  $(V_r)$  is calculated as:

$$V_r = V_f / V_0$$
 II.9

where  $V_f$  and  $V_0$  are the volume of final filtrate after filtration and initial feed (m<sup>3</sup>), respectively.

The filtrate flux (J) is calculated as:

$$J = d_V / A d_t$$

where V is the filtrate volume at time t ( $m^3$ ), A is the effective membrane area ( $m^2$ ), and t is the filtration time (s).

II.10

The Ruth–Carman's equation which was applied for estimation of filter cake resistance in the dead-end filtration is calculated as (Liu et al., 2011; Loginov et al., 2011).

$$\frac{t}{V} = \frac{\alpha C_{cs} \rho \mu}{2A^2 \Delta P} V + R_m \frac{\mu}{A \Delta P}$$
 II.11

where t and V are the filtration time (s) and the filtrate volume (m<sup>3</sup>), respectively,  $\alpha$  is the specific filtration resistance of the filter-cake (m/kg), C<sub>cs</sub> is the weight fraction of cake-forming (colloidal and insoluble) solids in the feed (kg/kg),  $\rho$  is the density of filtrate (kg/m<sup>3</sup>),  $\mu$  is the viscosity of filtrate (Pa·s), A is the effective membrane area (m<sup>2</sup>),  $\Delta$ P is the filtration pressure (Pa), R<sub>m</sub> is the membrane resistance (m<sup>-1</sup>).

#### **II.3** Physico-chemical analysis

### II.3.1 Dry matter

The dry matter (DM) content in sample was determined after drying the apple flesh in MA 160 infrared moisture analyzer (Sartorius, Göttingen, Germany).

## II.3.2 Analysis of extracts

#### II.3.2.1 Soluble matter content

The concentration of total soluble matter, °Brix (g of DM/100 g solution), was measured using the refractometer (Atago, USA) at room temperature.

## II.3.2.2 pH

The pH of solution was detected by a multifunction pH meter (PCE-PHD 1, PCE Instruments France EURL, France).

#### II.3.2.3 Electrical conductivity

The electrical conductivity,  $\sigma$  (ms/cm), of solution was detected by a multifunction pH meter (PCE-PHD 1, PCE Instruments France EURL, France).

## II.3.2.4 Water holding capacity

A modified method was conducted to measure water holding capacity of apple peels (Huang and Ma, 2016). The surface moisture of apple peels after extraction experiment was wiped with filter paper. Then the apple peel samples were dried using MA 160 infrared moisture analyzer. Then ten milliliters of distilled water was added to 1 g of the dried apple peel samples. The suspension was homogenized in a vortex for 1 min and left at room temperature for 24 h. After centrifugation at 1000g for 5 min (Z200, Hermle Labortechnik GmbH, Wehingen), the supernatant was removed and the residue weighed. The water holding capacity was expressed as grams of water held by 1 g of fresh sample.

### II.3.2.5 Particle size distribution

The particle size distributions in liquid extracts were analyzed using light scattering method with Malvern Mastersizer X (Malvern Instruments S.A., France). The measurements were done using the 45 mm lens and the optical pathway of 14.3 mm.

#### II.3.2.6 Hardness

The hardness tests for fruit peels were performed in a Texture Analyser (model TA-XT plus, Rhéo, Champlan, France). The samples were placed on the platform of the texture analyzer to carry out force deformation tests. The load force, F, was detected at deformation of 0.5 mm for all samples.

#### II.3.2.7 Thickness

The thickness of fruit peels,  $\delta$ , was estimated using the scanning electron microscopy images (SEM) (Quanta FEG 250, Thermo Fisher Scientific, MA USA) at magnification of 50. The high voltage and mode of SEM was 20 kV and SE, respectively. The length and width of the extraction region for all samples was 5 mm and 3.5 mm, respectively. The testing peel was

fixed between two iron plates. For thinner samples, the edges of SEM images were visually brighter because of the iron light reflection.

## II.3.2.8 Color

The colour of extracts (diluted 10 times) was measured by adsorbance at 420 nm (A<sub>420</sub>) with UV/Vis instrument (Thermo Spectronic Genesys 20, Thermo Electron Corporation, MA, USA) (Liu et al., 2011).

## II.3.2.9 Turbidity

The turbidity of extracts was measured with a XR turbidimeter (HACH Company, Loveland, USA). For the turbidity measurement, the adsorption experiments with initial concentration of polyphenols  $C_o=1$  mg/mL were performed for 1500 min in presence of sonication during the first 100 min at different ultrasonic powers, P=0-400 W. Then solutions were clarified by sedimentation in the Earth's gravity for 15 min (g-samples) or by centrifugation for 1 min at 6000 rpm (c-samples).

### II.3.2.10 Scanning electron microscopy

The scanning electron microscopy (SEM) images with magnification of 100 were registered using SEM instrument Quanta FEG 250 (FEI, Holland) applied in an accelerating voltage of 20 kV. The microscope was coupled to a CCD camera (Sony, Japan) connected to a computer.

### II.3.2.11 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) analysis of solutions was performed using Nicolet<sup>™</sup> iS5 (iD3 ATR, Thermo Scientific, TM, USA) instrument. The FTIR transmittance (%) was recorded between 600 and 4000 cm<sup>-1</sup>.

### II.3.2.12 Optical images

The optical images of gas water solvents with different  $CO_2$  concentration (0, 1.76, 3.53, 5.28, 7.05 mmol/L) before and after ultrasound treatment (W=0.242 kW•h/kg) were directly observed using a reflection optical microscope (Leitz Orthoplan, Germany) at magnification  $\times$  4. The microscope was coupled to a CCD camera (Sony, Japan) connected to a computer. The gas water solvent was carefully got with a dropper, placed on a glass plate and observed with a cold lamp. The software 'Archimed' was used to register images and analyse the bubble size. In each experiment, 3 images from three different samples of one solution were analysed.

#### II.3.2.13 Total polyphenol content

The total polyphenols content (TPC) was determined using the Folin–Ciocalteu method based on a colorimetric oxidation/reduction reaction of phenols (Singleton et al., 1999). 0.2

mL of diluted extract and 1 mL of Folin–Ciocalteu reagent (Merck, Darmstadt, Germany) (diluted 1:10 with water) were mixed. 0.8 mL of Na<sub>2</sub>CO<sub>3</sub> (75 g/L) (Prolabo, Fontenay-sous-Bois, France) was then added. The sample was incubated for 10 min at 50 °C and then cooled for 10 min at room temperature. For the control sample, 0.2 mL of distilled water was taken. The absorbance was measured at 750 nm using UV/Vis instrument (Thermo Spectronic Genesys 20, Thermo Electron Corporation, MA, USA). Gallic acid (Sigma-Aldrich, St-Quentin Fallavier, France) was used for the calibration curve (Fig. II.4). The results were expressed as mg GAE/g DM.



Figure II.4 Standard line of gallic acid concentratin for total polyphenol content analysis.

The relationship between absorbance *Abs* and the concentration of gallic acid,  $C_G$  (µg/mL) was near linear, as following:

# $Abs=0.0204C_G$ II.12

### II.3.2.14 Catechin content

The concentration of catechin,  $C_c$ , was estimated by fluorescence technique using the instrument Cary Eclipse Fluorescence Spectrofluorometer and 10mm fused-silica cuvette (Agilent Technologies, USA). The fluorescence spectra was obtained using the excitation at  $\lambda_e$ =280 nm and registration of emission  $\lambda$ =312 nm (Arts et al., 2000). Figure II.4 presents the calibration curve for the fluorescence of catechin in water. For calibration, the standard of catechin hydrate ( $\geq 0.98\%$ , Sigma–Aldrich) was used. Figure II.4 presents the calibration curve and examples of emission spectra (inset to Fig. II.5).



Figure II.5 Intensity of the fluorescence emission,  $I_f$ , versus the concentration of catechin in water,  $C_c$ . Inset shows examples of emission spectra at different concentrations of catechin.

The relationship between fluorescence intensity  $I_f$  (au) and catechin concentration  $C_c$  (mg/mL) was near linear, as following:

$$I_f = 7.14 * 10^4 C_c$$
 II.13

Figure II.6 presents examples of calibration curves for the fluorescence of catechin for concentration of ethanol ( $C_{et}=0$  and 10 %). For relatively small concentrations of ethanol ( $C_{et}\leq10\%$ ) the relationship between catechin concentration in solvent,  $C_s$  (mg/mL) and fluorescence intensity  $I_f$  (au) was practically linear (Fig. II.6).

$$C_s \approx k I_f$$
 II.14

Here,  $k=1.39\times10^{-5}$  mg/mL and  $k=0.72\times10^{-5}$  mg/mL are slopes for  $C_{et}=0$  and  $C_{et}=10\%$ , respectively.

For higher concentrations of ethanol ( $C_{et}$ >10%) the extracts were diluted to the concentration  $C_{et}$ =10 % and then analyzed. The used fluorescence technique allowed determination of  $C_c$  in extracts using relatively small quantities of tested solutions.



Figure II.6 Concentration catechin,  $C_c$ , in solvents versus the fluorescence intensity  $I_f$  (au) at ethanol concentrations  $C_{et}=0\%$  and  $C_{et}=10\%$ .

# II.3.2.15 Total flavonoid content

Total flavonoid content (TFC) was estimated according to the method previously described (Liu et al., 2009). 250  $\mu$ L of extract was mixed with 1250  $\mu$ L of distilled water and 75  $\mu$ L of 5% sodium nitrite (NaNO<sub>2</sub>). After 6 min, 150  $\mu$ L of 10% aluminium chloride (AlCl<sub>3</sub>) was added. Finally, 500  $\mu$ L of 1 M sodium hydroxide (NaOH) was added and the total volume was made up to 2500  $\mu$ L with distilled water. Absorbance was measured at 510 nm. Quercetin (Sigma-Aldrich, St-Quentin Fallavier, France) was used for calculating the calibration curve (Fig. II.7). Results were expressed as mg of quercetin equivalent per 100 gram of dry matter (mg/100g DM).



Figure II.7 Standard line of quercetin concentratin for total flavonoid content analysis.

The relationship between absorbance *Abs* and quercetin concentration  $C_q$  (µg/mL) was near linear, as following:

# $Abs=7.976*10^{-4}C_q$

### II.3.2.16 Proanthocyanidins content

Proanthocyanidins (PAC) was determined using the vanillin assay previously described (Sun et al., 1998). 0.5 mL extract was mixed with 3 mL of 4% vanillin–methanol solution and 1.5 mL HCl (37%). Absorbance was measured after 15 min of reaction in dark at 500 nm. Catechin (Sigma-Aldrich, St-Quentin Fallavier, France) was used for the calibration curve (Fig. II.8). Results were expressed as mg of catechin equivalent per 100 gram of dry matter (mg/100g DM).



Figure II.8 Standard line of catechin concentratin for total proanthocyanidins content analysis.

The relationship between absorbance *Abs* and catechin concentration  $C_c$  (µg/mL) was near linear, as following:

$$Abs=0.0152C_c$$
 II.16

## II.3.2.17 Antioxidant activity

The 2, 2-diphenyl-1-picrylhydracyl (DPPH) radical scavenging activity was used to evaluate the antioxidant capacity of polyphenols (Nixdorf and Hermossín-Gutiérrez, 2010; Yue et al., 2012). The DPPH radical scavenging activity assay was performed on the extractions following the procedure by previous described (Truong et al., 2007). 50  $\mu$ L extract was added into 1450  $\mu$ L of DPPH solution (0.06 mM in methanol) to initiate the reaction. After a reaction time of 30 min in dark at room temperature, the reaction had reached completion. The decrease in absorbance of DPPH free radicals was read at 515 nm against methanol as a blank using a UV/Vis instrument. Trolox (0, 25, 50, 100, 250, 500 and 1000 mM/L) was used as a standard antioxidant compound for the calibration curve (Fig. II.9). The antioxidant activity was reported in mmol of Trolox equivalents per litre (mM TE/L).



Figure II.9 Standard line of trolox concentratin for antioxidant activity analysis.

The relationship between inhibition of the polyphenols against the free radical DPPH In (%) and trolox concentration  $C_t$  (mM/L) was near linear, as following:

 $In=0.099C_t$ 

II.17

### II.3.2.18 Hydrogen peroxide content

The measurement for the concentration of hydrogen peroxide,  $H_2O_2$ , was determined by the method previously proposed (Allen et al., 1952). The method was based on the oxidation of the iodide ion by hydrogen peroxide, which produced an adsorption peak at 350 nm proportional to the concentration of  $H_2O_2$ . Two reagents are used for detection, one is a solution of potassium acid phthalate of 20 g/L, the other one is a solution of 1% ammonium molybdate (0.2 g/L), sodium hydroxide (2 g/L) and potassium iodide (66 g/L). 0.3 mL of the sample was mixed with 0.75 mL of the each reagent. Absorbance was measured after 15 min in dark at 350 nm. Hydrogen peroxide,  $H_2O_2$  (VWR, France) was used for the calibration curve (Fig. II.10). Results were expressed as  $H_2O_2 10^{-4}$  mg/L.



Figure II.10 Standard line of H<sub>2</sub>O<sub>2</sub> for active species analysis.

The relationship between absorbance *Abs* and  $H_2O_2$  concentration  $C_h$  (10<sup>-4</sup> mg/L) was near linear, as following:

$$Abs=1.374*10^{3}C_{h}$$
 II.18

### II.3.2.19 The phenolic stability

The determination of phenolic compounds was performed using a Waters 717 HPLC (Waters, France), equipped with Millenium 32 software, a degasser, a binary gradient pump, a Waters 717 plus thermoautosampler, a column oven, and a Waters 996 diode array detector. The separation was carried out with an Aqua C18 column ( $150 \times 4.6$  mm; 0.5 µm particle size) (Hypersil Gold, Torrance, CA, USA) at 30 °C. The elution gradient was performed according to the previous method with some modifications (Boussetta et al., 2011); the column was initially equilibrated with distilled water and acetic acid (98:2) as solvent A for x min. Polyphenols were eluted with a three-stage linear gradient: from 92 to 76% of A in 20 min, from 76 to 60% of A in 10 min, and from 60 to 0% of A in 15 min with a flow rate of 1 mL/min. Acetonitrile and distilled water (98:2) was used as solvent B. Absorbance of mixture was measured at 280 nm. HPLC peaks were identified on chromatograms according to their retention times and their UV-visible spectra by comparison with available standard polyphenol compounds. 60 mg gallic acid, 8mg quercetin-3-O-glucoside, and 30 mg catechin were dissolved in 20 mL 10% acetonitrile aqueous solution at different pH (4.26, 5.47, 5.79, 5.91 and 5.93), respectively. Quantification was performed by reporting the measured integration area in the calibration equation of the corresponding standard. Phenolics degradation was calculated with  $C/C_0$ , C was the phenolic content in different pH and  $C_0$  is the initial content of gallic acid, quercetin-3-O-glucoside and catechin in 10% acetonitrile aqueous solution at pH=5.25, 3.54 and 4.17, respectively (4 mg/mL, 0.4 mg/mL and 1.5 mg/mL, respectively).

#### II.3.2.20 Protein content

The concentration of proteins was determined by means of Bradford method (Bradford, 1976). The details of analysis were presented in Technical Bulletin for Bradford Reagent (B 6916, Sigma-Aldrich). Seven standard solutions with various BSA (Bovine Serum Albumin) concentrations (0-25  $\mu$ g/mL) were prepared for standard curve. 1 ml of distilled water, 0.8 mL of Bradford reagent and 0.2 mL of diluted sample (distilled water for blank) were introduced into the tube successively, the mixture was mixed with a Vortex, and the absorbance was measured at 595 nm, then the concentration of protein was calculated using the standard curve (Fig. II.11). The results were also expressed as mg/mL.



Figure II.11 Standard line of BSA for protein concentration analysis.

The relationship between absorbance *Abs* and BSA concentration  $C_B$  (µg/mL) was near linear, as following:

$$Abs=0.041C_B$$
 II.19

#### **II.4 Indexes analyses**

#### II.4.1 Extraction indexes

The electrical conductivity,  $\sigma$ , and total polyphenol content, TPC, of extracts were measured during extraction experiments. The value of  $\sigma$  was measured using a conductometer (PCE-PHD 1, PCE Instruments France EURL, France). The value of TPC was determined using the Folin–Ciocalteu method based on a colorimetric oxidation/reduction reaction of phenols (specific description of method in II.3.2.13)

The ionic,  $Z_i$ , and total polyphenol,  $Z_{tf}$ , extraction indexes respectively (Boussetta and Vorobiev, 2014; Vorobiev and Lebovka, 2009), are defined as

$$Z_{i} = (\sigma_{\text{US}} - \sigma_{\text{U}}) / (\sigma_{\text{FT}} - \sigma_{\text{U}})$$
 II.20

$$Z_{tf} = (P_{US} - P_U) / (P_{FT} - P_U)$$
 II.21

Here, lower case symbols correspond to the ultrasound-assisted (US) and control extraction experiments with untreated (U) and frozen-thawed (FT).

### II.4.2 Cell disintegration index

The cell disintegration was analyzed using the confocal laser scanning microscopy (CLSM) analysis performed with a ZEISS LSM 710 (ZEISS, Oberkochen, Germany). A single line excitation and multiple channel emission technique were used. For visualization of multispectral image data, the different channels were associated with a colors value on the display system. The RGB-color mode with three primary display colors (red, green and blue) was used. UV excitation was used with laser line at 405 and 488/543 nm, the fluorescence

channel was given by the band pass filter (BP) 410–495 nm (blue), BP 495–564 nm (green) and longpass filter (LP) 563–685 nm (red).

The laser-induced fluorescence images from fruit peel samples were taken with a cooled, integrating CCD camera (AT 200) mounted on a ZEISS LSM 710 microscope with an HBO 50 excitation lamp (Zeiss, Oberkochen, Germany). Samples were detected under identical conditions and with identical buffer solutions. Single x-y images as well as a series of x-z images were taken from all samples (image size x\*y\*z was 212.55 µm\*212.55 µm\*13.63 µm), and the magnification times of images were 40.

Figure II.12 presents examples of cell images for samples obtained after U (a) and US (b) extraction experiments for studied fruit peels. The data were presented for the extraction time of  $t_e$ =2700 s. It corresponded to the US energy input, W=0.299 kW·h/kg in the US experiments. Cells were practically undamaged in fruit peels obtained after U experiments (Fig. II.12a), whereas in peels obtained after US experiments the spaces between cells became larger (apple), and many cells became connected together (banana and persimmon) (Fig. II.12b). Preliminary US extraction experiments with different US energy inputs (n=0–9, W=0–0.299 kW·h/kg) showed that with a higher US energy input, the cell membranes and cell walls were damaged more severely, and more cells were connected to each other, and the spaces between cells become larger.



Figure II.12 Examples of images for samples obtained after U (a) and US (b) extraction experiments for studied fruit peels (apple, banana, persimmons).

Processing of the CLSM images and calculation of cell damage degree were done using the wholly automatic procedure supported with Matlab (Version 2011a, the Mathworks Inc., Mass, USA). Figure II.13 shows the scheme applied for the processing and calculation of cell damage degree.
The following 5 steps were applied:

1) Pre-processing. For better identification of the cell walls the RGB image was transformed into the blue image. To reduce possible noise, the median-filtering technique (a special case of filter called rank-statistic filter), which allowed the edges to be preserved while filtering out the peak noise, was used (DU and SUN, 2005).

2) Segmentation. To remove background, the blue image was transformed into the gray level image and then to the binary image (called bi-level images, BW). The Otsu algorithm for edge detection is used and the threshold value was determined with by Sobel filter (Otsu, 1979). This filter is used in image processing for creation image emphasizing edges. It is based on the gradient approximation and convolving the image with a small, separable, and integer-valued filter.

3) Inversion and holes filling. The BW image was inverted, white became black, and black became white. Now, the black and white parts present cell walls and cell bodies, respectively. Then hole filling using a flood filling operation was applied (Soille, 2013). The flood filling operation is performed recursively on all elements connected to the node of interest.

4) The edges of cell image were smoothed by eroding the image with opening and closing algorithms. Opening is erosion followed by dilation, closing is a dilation followed by erosion (Roushdy, 2006).

5) Corresponding pixels of each cells were found based on eight nearly label algorithm (Charbit, 2010). In this algorithm, the label of a pixel is influenced by the labels of its eight nearest neighboring pixels.

6) Calculation of cell numbers and determination of damage degree. To calculate the total number of cells,  $N_t$ , all the cells including the cells with incomplete edges (damaged cell walls) were labeled, except the cells on the image borders. The number of pixels in every labeled cell and the average number of pixels in all labeled cell were calculated. To calculate the number of damaged cells,  $N_d$ , the cells with number of pixels smaller than the average value were regarded as damaged cells. The damage degree is evaluated as:

$$d=N_d/N_t$$
 II.22  
Finally, the cell disintegration index,  $Z_m$ , is calculated as:

$$Z_m = (\mathbf{d}_{\mathrm{US}} - \mathbf{d}_{\mathrm{U}}) / (\mathbf{d}_{\mathrm{FT}} - \mathbf{d}_{\mathrm{U}})$$
 II.23

Here, lower case symbols correspond to US, U and FT experiments.



Figure II.13 Scheme applied for processing of the images.

Figure II.14 presents example of temporal changes of the cell damage degree, d, in US, U and FT extraction experiments with apple peels. The obtained results presented that the damage degree, d, increased with increasing of extraction time,  $t_e$ , in U and US experiments, whereas in FT experiments the damage degree was approximately 0.91.



Figure II.14 Examples of temporal changes of cell damage degree, d, in US, U and FT extraction experiments for apple peels.

# **II.5 Statistical analysis**

All experiments and measurements of characteristics were repeated at least in triplicate. The mean values and the standard deviations were calculated. All experiments and measurements were repeated at least in triplicate. Data were expressed as mean ± standard deviation. A probability value (p value) of less than 0.05 was considered statistically significant. The error bars in figures correspond to the standard deviations. TableCurve 2D® (Systat Software Inc.) was used for data fitting.

# **Chapter III Selectivity of polyphenols extraction**

# **III.1 Introduction**

Nowadays, the ultrasound-assisted extraction (UAE) is widely recognized as a green technique for recovery of polyphenols from apple products (Chemat et al., 2017a, 2017b; Jacotet-Navaro et al., 2016; Khadhraoui et al., 2018; Lohani and Muthukumarappan, 2016; Pingret et al., 2012; Virot et al., 2010). In this chapter of thesis, the extraction of polyphenols from apple flesh, peel and pomace was studied using conventional aqueous extraction (CE), ultrasound-assisted extraction (UAE) and ethanol/water extraction. The catechin content (C<sub>c</sub>), total polyphenols content (TPC) and relative content of C<sub>c</sub> in TPC ( $R=C_c/TPC$ ) were evaluated. The efficiency of UAE and the extract purity can be governed by the combined action of thermal and sonication effects, different processing factors (such as temperature, ethanol concentration, solid/liquid ratio, extraction time, ultrasound power and treatment time (Chemat et al., 2017b; Pingret et al., 2012; Virot et al., 2010; Yue et al., 2012). However, the effects of CE and UAE protocols, and ethanol concentration on selectivity of polyphenols extraction from apple products were not previously discussed. Therefore, the selectivity of extraction of phenolic contents from apple products is interesting to be carried out for the purpose of:

1) Comparing efficiency and selectivity of aqueous extraction of soluble matter and phenolic contents from apple flesh for CE and UAE;

Analyzing the effects of pulsed ultrasonic treatment on the extraction of soluble matter, catechin and total polyphenols from the different parts (flesh and peel) of green and red apples;
 Investigating the effects of ultrasound treatment time (0–30 min) and concentration of ethanol (0–50%) on the extraction of phenolics from apple pomace;

4) The precise determination of catechin in extracts with the fluorescence technique.

# III.2 Article I Comparison of conventional and ultrasound-assisted aqueous extraction of soluble matter and phenolic compounds from apple flesh Summary

In the first part of this chapter (details are presented in article I *Comparison of conventional and ultrasound-assisted aqueous extraction of soluble matter and phenolic compounds from apple flesh*), the selectivity of phenolics extraction from apple flesh with conventional thermal and ultrasound-assisted aqueous extraction was studied. The CE was done at different temperatures (50, 60, and 75 °C). The UAE was done at fixed temperature, T = 50 °C, and different specific energy inputs, W, and total duration, t<sub>u</sub>. The kinetics data for CE and UAE were studied. The obtained kinetics data for the CE at different temperature evidenced for different mechanisms of soluble matter and catechin extraction from apple flesh. The significant acceleration in extraction of C<sub>c</sub> from apple flesh for UAE was observed compared to the extraction contents for CE. Correlations between the maximum (saturation) values of C<sub>m</sub> and TPC<sub>m</sub>, for CE and UAE were developed. In correlations between C<sub>m</sub> and TPC<sub>m</sub>, the two distinctive branches were observed for CE and UAE. The obtained results evidence the possibility of fine regulation of selective extraction of soluble matter, catechin and total polyphenolic compounds using different temperatures and ultrasound-assisted protocols.

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**ORIGINAL PAPER** 



# Comparison of conventional and ultrasound-assisted aqueous extraction of soluble matter and phenolic compounds from apple flesh

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#### Abstract

The extraction of soluble matter (°Brix), catechin and total phenolic compounds (TPC) from apple flesh was studied. A conventional aqueous extraction (CE) was done at different temperatures (50, 60, and 75 °C). A pulsed ultrasound-assisted extraction (UAE) was done at fixed temperature, T=50 °C, and different specific energy input and total duration. The main attention was focused on correlations between °Brix, concentration of catechin, *C*, TPC, and selectivity of phenolics extraction. For UAE, the significant acceleration in extraction of catechin was observed for the short duration protocol as compared with long duration one. The maximum (saturation) levels for concentrations of catechin, *C*<sub>m</sub>, and total phenolic compounds, TPC<sub>m</sub>, were compared. In correlation between *C*<sub>m</sub> and TPC<sub>m</sub>, the two distinctive branches were observed for CE protocols and UAE protocols. Moreover, the relative contents of catechin in TPC were noticeably higher for UAE protocols as compared with CE protocols.

Keywords Aqueous extraction · Ultrasound-assisted extraction · Soluble matter · Catechin · Total phenolic compounds · Selectivity

#### Introduction

Plant polyphenols are beneficial to human health, as they have strong anti-inflammatory effects and can effectively reduce the risk of getting some diseases, including coronary heart disease, cancer, neurodegenerative, gastrointestinal disorders and others diseases [1–4]. The main components of polyphenols include flavanols (catechins, epicatechins, procyanidins), anthocyanids (cyanidines, cyanidin glycosides), flavonols (quercetin), and phenolic acids (hydroxybenzoic and hydroxycinnamic acids) [5, 6]. Apples contain a rather high level of phenolic compounds and especially chlorogenic acid [7]. The contents and composition of polyphenols can

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<sup>2</sup> Institute of Biocolloidal Chemistry named after F. D. Ovcharenko, NAS of Ukraine, 42, blvr. Vernadskogo, Kyiv 03142, Ukraine be rather different in dependence of sort of plant, variety, environmental and post-harvest factors as well as the plant part (flesh, peels, seeds and residues) [8, 9].

The efficiency of conventional solvent extraction (CE) of polyphenols from plant materials depends on processing factors, including temperature, time of extraction, type of solvent, pH, and liquid–solid ratio [10]. The extraction of polyphenolic compounds at high temperatures and/or with using of organic solvents (e.g., ethanol) improves the extraction efficiency. However, typically, such extraction is not selective, it can result in decrease of purity of extracts and it requires supplementary separation procedures [11].

Recently, different nonconventional extractions assisted by physical methods (ultrasound, ohmic heating, pulsed electric fields, etc) have been tested [12–15]. These methods demonstrated high efficiency and possibility of selective recovery of plant polyphenols. The ultrasound-assisted extraction (UAE) is widely recognized as a green technique [16–19]. Concept of the green extraction ensures a safe and high-quality extracts, assumes the process with reduced energy consumption and processing time and allows use of alternative solvents and renewable natural products [20]. The differences in extraction kinetics of apple pomace

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polyphenols were observed for conventional and UAE [12, 17]. The efficiency of aqueous UAE of polyphenols from dried apple pomace at mild temperatures (40 °C) has been also evaluated [12]. Application of UAE allowed increasing the TPC by 30% as compared with CE. Note that efficiency of UAE and the extract purity can be governed by the combined action of thermal and sonication effects. However, the effects of CE and UAE protocols on selectivity of polyphenols recovery from apples were not previously discussed.

The objective of this study was to compare efficiency and selectivity of aqueous extraction of soluble matter and phenolic contents for conventional and ultrasound-assisted extraction (UAE). The apple flesh was selected as a model plant material. Attention was focused on the effects of thermal and pulsed ultrasound treatments on extraction kinetics of total solutes and catechin. Impact of extraction conditions on the efficiency of catechin recovery and its content in total phenolic compounds was also discussed.

#### **Materials and methods**

#### Material

Commercial green apples (Granny Smith) were selected as the raw material for investigation. Samples of good and uniform quality (with near-spherical shape) were purchased at the local supermarket (Compiegne, France). The initial moisture content ( $\approx 85\%$  wet basis) was determined after drying the apple flesh in MA 160 infrared moisture analyzer (Sartorius, Germany)

#### **Extraction techniques**

The disks (with diameter of d = 20 mm and thickness of h = 10 mm) were cut from the apple flesh. Then, disks (25 g) were put in a glass beaker filled with preheated distilled water (250 ml), so the solid/liquid ratio was 1/10. During extraction experiments, the glass beaker was covered with aluminium foil to reduce water evaporation. The different European Food Research and Technology (2018) 244:1683-1689

protocols of conventional and ultrasound-assisted aqueous extraction were tested. The total extraction time was up to 10,800 s (3 h).

#### **Conventional extraction**

For conventional extraction, three different temperatures were used T = 50, 60, and 75 °C for protocols  $S_1$ ,  $S_2$  and  $S_3$ , respectively (Table 1). The temperature was stabilized using a water thermal bath Polystat 36 (Fisher Scientific, France).

#### Ultrasonic-assisted extraction

Ultrasonic treatment of apple-water mixture was done directly in the glass beaker using an ultrasonic processor UP 400S (400 W, 24 kHz, Hielscher GmbH, Stuttgart, Germany). The titanium ultrasonic probe (H14, Hielscher GmbH, Stuttgart, Germany) with a tip diameter of 14 mm, and the length of 100 mm was used. The experiment with temperature elevation control of the apple-water mixture allowed estimation of ultrasonic power as ≈150 W. The ultrasonic treatment was applied in pulsed mode.

Figure 1 presents example of the typical protocol of ultrasound-assisted extraction. In this protocol  $(S_4)$ , apple samples were put initially to the distilled water at  $T = 50 \text{ }^{\circ}\text{C}$ and then ultrasonic pulse with duration of  $\Delta t_s = 10$  s was applied. It resulted in insignificant temperature elevation  $(\Delta T \approx 1.5 \text{ °C})$ . After application of n (n = 5 in Fig. 1) sequential ultrasonic pulses, the conventional aqueous extraction was continued for a long time up to 3 h.

The other tested protocols of UAE are presented in Table 1. These protocols were done at the fixed temperature, T = 50 °C. The protocols S<sub>4</sub> and S<sub>5</sub> use the same moderate specific energy inputs  $W \approx 27.3$  kJ/kg, but they have different total durations  $(t_t = n(\Delta t_s + \Delta t_w)), t_t = 5050s (S_4)$  and  $t_t$ =350 s (S<sub>5</sub>). The high-power protocol with  $W \approx 327.3$  kJ/ kg and  $t_t = 1800$ s was also tested.

Note that for different protocols presented in Table 1, the temperature elevation during the ultrasonic treatment never exceeded a few degrees ( $\approx 1.5-9$  °C). During the pause of

Table 1The tested protocolsof conventional extraction $(S_1, S_2, S_3)$ and ultrasound-assisted extraction combinedwith conventional extraction $(S_4, S_5, S_6)$	Protocol #	$T_{\rm w}(^{\rm o}{ m C})$	$\Delta t_{\rm s}$ (s)	$\Delta t_{\rm w}$ (s)	n	$t_{\rm t}({\rm s})$	W (kJ/kg)
	S <sub>1</sub>	50	Ξ.	÷	0	-	=
	$S_2$	60	-	-	0	-	-
	S <sub>3</sub>	75	-	-	0	-	-
	$S_4$	50	10	1000	5	5050	27.3
	S <sub>5</sub>	50	10	60	5	350	27.3
	S <sub>6</sub>	50	60	120	10	1800	327.3

Here,  $T_{\rm w}$  is the temperature of the water bath,  $\Delta t_{\rm s}$  and  $\Delta t_{\rm w}$  are the duration of pulsed sonication and cooling of the sample during one pulse, respectively, n is the number of pulses,  $t_t = n(\Delta t_s + \Delta t_w)$  is the total duration of pulsed sonication, W is specific energy of ultrasound treatment. The mean power of treatment was  $\approx$ 150 W. After pulsed ultrasound-assisted extraction, the conventional extraction continued up to 3 h

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Fig.1 Example of the pulsed ultrasound-assisted extraction protocols. Here, the temperature evolution during application of pulses is presented. The apple disks were put initially in the distilled water at T=50 °C and then a series of n=5 pulses with duration of  $\Delta t_s=10$  s was applied. During the pauses of  $\Delta t_w=60$  s, the temperature in the glass beaker was decreased by cooling in ice water. After the ultrasound-assisted extraction, the conventional extraction was continued up to  $\Delta t_w=3$  h

 $\Delta t_{wr}$ , the temperature in the glass beaker was decreased up to 50 °C by cooling in ice water. The used moderate temperatures allows avoiding thermal degradation of organic compounds and provide the efficient conditions for application of ultrasound [16, 21]. Note that no significant degradation of targeted compounds and specific reaction products after prolonged sonication (5–55 min) applied to the isolated phenolic compounds of apple pomace were previously observed [21].

## Analysis

The obtained extracts were analysed for soluble matter (<sup>o</sup>Brix), catechin and total polyphenol compounds (TPC).

#### Soluble matter content

The concentration of soluble matter was measured by a digital refractometer (Atago, USA) at room temperature. The results are expressed in °Brix (g of DM/100 g solution).

#### **Polyphenols content**

The catechin content in extract was estimated by the method of fluorescence spectroscopy using the instrument



Fig.2 Intensity of the fluorescence emission, *I*, at  $\lambda$ =312 nm versus the concentration of catechin in water, *C*. The excitation was done at  $\lambda_{a}$ =280 nm. Inset shows examples of emission spectra at different concentrations of catechin

Cary Eclipse Fluorescence Spectrofluorometer using a 10 mm fused-silica cuvette (Agilent Technologies, USA). The fluorescence spectra were obtained using the excitation at  $\lambda_e = 280$  nm and registration of emission at  $\lambda = 312$  nm. This excitation/emission wavelengths are close to that reported in [22, 23].

Figure 2 presents calibration curve for the fluorescence of catechin in water. For calibration, the standard of catechin hydrate ( $\geq 0.98\%$ , Sigma–Aldrich) was used. Figure 2 presents the calibration curve and examples of emission spectra (inset to Fig. 1). The relationship fluorescence intensity I(au) and catechin concentration C (mg/ml) was near linear.

$$7.14 \times 10^4 C$$

 $I \approx$ 

The total polyphenols content was determined spectrophotometrically using the Folin–Ciocalteu method based on a colorimetric oxidation/reduction reaction of phenols [24]. 0.2 mL of diluted extract and 1 mL of Folin–Ciocalteu reagent (Merck, Darmstadt, Germany) (diluted 1:10 with water) were mixed. 0.8 mL of Na<sub>2</sub>CO<sub>3</sub> (75 g/L) (Prolabo, Fontenay-sous-Bois, France) was then added. The sample was incubated for 10 min at 50 °C and then cooled for 10 min at room temperature. For the control sample, 0.2 mL of distilled water was taken. The absorbance was measured at 750 nm by the UV/Vis spectrophotometer (Thermo Spectronic Genesys 20, Thermo Electron Corporation, MA, USA). Gallic acid (Sigma–Aldrich, St-Quentin Fallavier, France) was used for the calibration curve. The results were expressed as mg of gallic acid equivalent

(GAE) per gram of dry matter (mg GAE/g DM).

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(1)

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#### Statistical analysis

All experiments and measurements of characteristics were repeated at least in triplicate. The mean values and the standard deviations were calculated and presented in figures. TableCurve 2D® (Systat Software Inc.) was used for data fitting.

## **Results and discussions**

Figure 3 shows kinetics of soluble matter content, °Brix,(a) and concentration of catechin, *C*, (b) for the extraction at different temperatures, protocols  $S_1(50 \text{ °C})$ ,  $S_2(60 \text{ °C})$ ,  $S_3(75 \text{ °C})$ . Both the °Brix and concentration of catechin increased with the time and the effects were more pronounced at higher temperatures. The non-zero values of °Brix and *C*, were observed at the initial moment of time (t=0) that can reflect fast releasing of extracted matter from the surface of cut disks. The detailed analysis has shown that the extraction curves can be well-fitted using the stretched exponential law:

$$Y = Y_o + (Y_m - Y_o) [1 - \exp(-(t/\tau)^{\beta})]$$
 (2)

where  $Y = {}^{\circ}\text{Brix}$  or *C*, and  $Y_{0}$  and  $Y_{m}$  are the initial and maximum (saturation) values at t = 0 and at long time of extraction, *t*. Here,  $\tau$  and  $\beta$  are the fitting parameters.



Fig.3 Kinetics of soluble matter content, "Brix (a) and concentration of catechin, C (b) for the extraction with different extraction protocols  $S_1$  (50 °C),  $S_2$ (60 °C) and  $S_3$ (75 °C) (Table 1). The symbols present the experimental data and solid lines were obtained using the stretched exponential function (Eq. 2)

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The solid lines were obtained by fitting experimental data (symbols in Fig. 3) using stretched exponential function with rather higher determination coefficients in the interval  $R^2 = 0.982-0.996$ . Recently, the stretched exponential equation was applied for fitting the pulsed electric energy-assisted extraction of bioactive molecules from exotic fruit residues (e.g., papaya seeds or mango peels) [25–27]. The stretched exponential law represents the generalisation of the simple first-order kinetics in presence of the distribution of extraction times. The stretching parameter  $\beta$  characterizes the broadness of the distribution of the extraction times. The case  $\beta = 1$  corresponds to the single extraction time and the larger deviation of  $\beta$  from 1 is, the broader the distribution of the extraction times. The called as [28]:

$$\langle \tau \rangle = \tau \beta^{-1} \Gamma(\beta^{-1})$$
 (3)

where  $\Gamma$  is the Euler gamma function.

Figure 4 presents the evaluated parameters °Brix<sub>m</sub> and  $C_m$  (a), and  $ractor and \beta$  (b) versus extraction temperature, *T*. The practically linear growth of both the °Brix<sub>m</sub> and  $C_m$  with increase of the temperature was observed (Fig. 4a). The mean time of extraction of soluble matter ractor noticeably decreased with increasing of *T*, whereas the inverse behaviour was observed in kinetics of  $C_m$  (Fig. 4b).

Note, that for extraction of soluble matter, the behaviour of  $\langle \tau \rangle$  naturally reflects the acceleration of diffusion processes with increase of temperature. For this case, the stretching parameter  $\beta$  was close to 1 ( $\beta \approx 0.9$ ) and the extraction kinetics can be described with the classical first-order kinetic equation with a single relaxation time (Fig. 4b). For extraction of catechin, the anomalous behaviour in  $\langle \tau \rangle$  was



Fig.4 The evaluated extraction parameters °Brix<sub>m</sub> and  $C_m$  (a), and < p and  $\beta$  (b) versus the temperature, *T*. Here, °Brix and *C* are the maximum (saturation) values at long extraction times, < r> is a mean extraction time and  $\beta$  is a stretching parameter

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observed. For this case, the stretching parameter  $\beta$  noticeably deviated from 1 ( $\beta \approx 0.55$ ) and this evidences the presence of broad distribution of extraction times. Such distribution can reflect the presence of different binding sites for catechin in the apple flesh. The differences in thermal activation of these sites can result in the observed anomalous behaviour of ( $\tau_{\gamma}$ .

Figure 5 presents the  $C_m$  versus "Brix<sub>m</sub> correlation dependencies. These correlations were rather distinct for the used protocol with three different temperatures. The noticeable increase of catechin content with elevation of temperature for the same level of "Brix was observed. It can also reflect the presence of different mechanisms of soluble matter extraction from apple flesh.

Figure 6 compares the extraction kinetics of °Brix and catechin for conventional aqueous extraction (protocol  $S_1$ ) and ultrasound-assisted extraction (protocols  $S_4$ ,  $S_5$ ,  $S_6$ ). All extractions were done at the same temperature, T=50 °C. The enhanced extraction of total solutes (°Brix) and catechin was observed for the ultrasound-assisted extraction (protocols  $S_4$ ,  $S_5$ ,  $S_6$ ) as compared to conventional extraction, protocol  $S_1$ . For the protocols  $S_4$  and  $S_5$ , using the same specific energy input ( $W \approx 27.3$  kJ/kg) and different total durations, 5050s ( $S_4$ ) and 350 s ( $S_5$ ), the remarkable distinctions in kinetics of °Brix, and catechin concentration were observed.

For the short duration protocol S<sub>5</sub> the more accelerated kinetics are expected, as it consumes a less time than the protocol S<sub>4</sub>. Such behaviour was really observed for kinetics of °Brix (Fig. 6a). However, such acceleration for extraction kinetics of catechin was even striking (Fig. 6b). For the high-power protocol S<sub>6</sub> ( $W \approx 327.3$  kJ/kg) with a long total duration (1800s), the most accelerated extraction of



Fig.5 Concentration of catechin, C, versus the soluble matter content, <sup>o</sup>Brix, at three different temperatures (protocols S<sub>1</sub>,S<sub>2</sub>,S<sub>1</sub>)



Fig.6 Soluble matter content, °Brix, and catechin concentration, C, versus the extraction time, t, for conventional aqueous extraction (protocol S<sub>1</sub>) and extraction assisted by ultrasound (protocols S<sub>4</sub>, S<sub>5</sub>, S<sub>6</sub>) (see the Table 1). The temperature was fixed at T=50 °C

total solutes (°Brix) was observed (Fig. 6a). However, this protocol still concedes to the short duration protocol S<sub>5</sub> in acceleration of catechin extraction (Fig. 6b). At long extraction time (t > 6000–7000s), the saturation levels of °Brix<sub>m</sub> were approximately the same for all protocols S<sub>4</sub>, S<sub>5</sub>, S<sub>6</sub>, but the saturation levels of catechin,  $C_m$ , increased in the order S<sub>4</sub> < S<sub>5</sub> < S<sub>6</sub>.

Figure 7 presents the  $C_m$  versus °Brix<sub>m</sub> correlations for conventional aqueous extraction (protocol S<sub>1</sub>) and extraction



Fig.7 Concentration of catechin, C, versus the soluble matter content, "Brix, for conventional aqueous extraction (protocol S<sub>4</sub>) and extraction assisted by ultrasound (protocols S<sub>4</sub>, S<sub>5</sub>, S<sub>6</sub>). The temperature was fixed at T=50 °C



Fig.8 Maximum (saturation) concentration of catechin,  $C_{\rm m}$ , versus the maximum concentration of total polyphenols content, TPC<sub>m</sub>, for different extraction protocols presented in the Table 1. The extraction time was 3 h (10,800 s). Inset shows relative contents of catechin in TPC (ratio  $C_{\rm m}/{\rm TPC_m}$ ) versus TPC<sub>m</sub>

assisted by ultrasound (protocols  $S_4$ ,  $S_5$ ,  $S_6$ ). The data demonstrates noticeable increase of catechin content for the extraction assisted by ultrasound. Moreover, the short duration protocol  $S_5$  allows the most efficient extraction of catechin even at moderate extraction of soluble matter.

This assumption is supported by the observed correlation between the maximum (saturation) concentration of catechin,  $C_{\rm m}$ , and maximum concentration of total polyphenols content,  $TPC_m$ , for different extraction protocols (Fig. 8). In general, the increase in  $TPC_m$  accompanies the increase in catechin concentration,  $C_{\rm m}$ . However, the two distinctive branches were observed for conventional extraction protocols S1, S2 and S3 and ultrasound-assisted protocols S4, S5 and S6. Moreover, for the protocols S4, S5 and S<sub>6</sub> using ultrasound the relative ratio of catechin in TPC was noticeably higher than for protocols S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub>. It is interesting to note that relative content of catechin in TPC (ratio  $C_{\rm m}$  /TPC<sub>m</sub>) decreases with increase of TPC<sub>m</sub>. Here, the two distinctive branches for the conventional extraction and ultrasound-assisted protocols were observed (see inset to Fig. 8). So, the obtained data evidenced the possibility of fine regulation of extraction selectivity of soluble matter and phenolic components with using the different protocols pulsed ultrasound-assisted extraction. The observed selectivity can reflect the distribution of phenolic compounds inside the apple flesh. The development of different processes in recovery of total polyphenols from apple pomace by ultrasound were previously identified [12]. In general, the mechanism of selectivity governed by ultrasound may include combination of the effects of fragmentation, local

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erosion, sonocapillarity, capillary effect, detexturation, and sonoporation [19].

#### Conclusions

The detailed studies of extraction of soluble matter (°Brix) and phenolic compounds from apple flesh were performed. The main attention was focused on correlations between soluble matter content, <sup>o</sup>Brix, concentration of catechin, C and total polyphenols content, TPC. The kinetics data for the conventional aqueous extraction evidenced for different mechanism of soluble matter and catechin extraction. For ultrasound-assisted extraction, the significant acceleration in extraction of catechin was observed. In correlations between maximum (saturation) values of  $C_{\rm m}$  and TPC<sub>m</sub>, the two distinctive branches were observed for the conventional aqueous extraction and ultrasound-assisted extraction. The obtained data evidence the possibility of fine regulation of selective extraction of soluble matter, catechin and total polyphenolic compounds using different temperatures and ultrasound-assisted protocols.

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#### **Compliance with ethical standards**

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights statement This article does not contain any studies with human or animal subjects.

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# III.3 Article II Selectivity of ultrasound-assisted aqueous extraction of valuable compounds from flesh and peel of apple tissues Summary

Apples contain a rather high level of phenolic compounds and especially chlorogenic acid (Podsędek et al., 2000). However, the contents and composition of polyphenols are rather different in dependence of sort of plant, variety, environmental and post-harvest factors as well as the plant part (flesh, peels, seeds and residues) (Heimler et al., 2017; Oszmiański et al., 2011). Meanwhile, the mechanism of the extraction selectivity of these polyphenols with UAE is still unclear. The mechanisms induced by UAE may include the combination of many effects related to cavitation bubble collapses, fragmentation, erosion, sonocapillarity, detexturation, and sonoporation (Chemat et al., 2017b; Pingret et al., 2012; Saleh et al., 2008). It can be speculated that manifestation of these effects is quite different in dependence on the structure of flesh and peel tissues, the ability of their swelling and rehydration, and distribution of phenolic compounds inside these tissues. Therefore, in the second part of this chapter (details are presented in article II *Selectivity of ultrasound-assisted aqueous extraction of phenolics from flesh and peel of apple tissues*), the selectivity of ultrasound-assisted aqueous extraction of phenolics from different part of apples (flesh and peel) and different varieties of apple was compared.

The effects of pulsed ultrasonic treatment during initial period of extraction (first 300–350 s) on the recovery of soluble matter, catechin and total polyphenols from different parts (flesh and peel) of green (Granny Smith) and red (Red delicious) apples were studied. The different impacts of W (0–26.8 kJ/kg) on the extraction of soluble matter and catechin were observed for flesh and peel tissues. For all UAE protocols and used variety of apple (green or red), the extraction levels of °Brix, C<sub>c</sub> and TPC for peel tissue were compared with flesh tissue. The significant acceleration in extraction of °Brix and C<sub>c</sub> of apple flesh and peel for UAE was observed to the extraction contents for CE. The extraction levels of C<sub>c</sub> and TPC were noticeably higher for peel tissue as compared to flesh one for all UAE protocols and used variety of apple (green or red). In addition, the different impacts of W on the extraction of soluble matter and catechin were observed for flesh and peel tissues. The obtained data evidenced more significant impact of the US pretreatment on extraction of catechin as compared to extraction of total soluble matter. The °Brix values increased continuously during the initial period of UAE and subsequently CE. Previous studies presented that ultrasound has an impact on the structural properties of vegetable tissue and may influence on the solute

diffusivity in the tissue matrix (Chemat et al., 2017b; Vinatoru, 2001). The observed differences in the catechin recovery from peels of green and red apples can reflect their different structure. Increasing of W resulted in increase of both the final values of  $C_m$  and TPC<sub>m</sub>. However, the different branches collecting the data for protocols were observed for flesh and peel tissues. It can be due to the different mechanisms of phenolic compounds extraction from flesh and peel tissues. The obtained data evidence that selectivity of catechin extraction (R=C<sub>m</sub>/TPC<sub>m</sub>) was depended on the type of the tissue (flesh or peel), apple variety (green or red) and applied ultrasound protocols.

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# Selectivity of ultrasound-assisted aqueous extraction of valuable compounds from flesh and peel of apple tissues



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ARTICLE INFO	A B S T R A C T
Keywords: Apple flesh and peel tissues Ultrasound-assisted extraction Soluble matter Catechin TPC	Ultrasound-assisted aqueous extraction (UAE) of soluble matter ("Brix), catechin and total phenolic contents (TPC) from flesh and peel of apple tissues were studied. The commercial green (Granny Smith) and red (Red delicious) apples were used in the investigation. All extractions were done at fixed temperature, $T = 50$ °C, and protocols with different specific energy inputs, $S_1$ ( $W = 0$ kJ/kg), $S_2(W = 6.5$ kJ/kg), $S_3(W = 10.6$ kJ/kg), $S_4(W \approx 21.3$ kJ/kg), and $S_5(W \approx 26.8$ kJ/kg) during the initial period of extraction (first 300–350 s). The total aqueous extraction time was up to 3 h. The kinetics of extractions, and correlations <sup>o</sup> Brix and C orcentration of catechin, <i>C</i> , were compared for flesh and peel tissues. The increase of energy resulted in increase of <sup>o</sup> Brix and <i>C</i> values. For both green and red apples, the values of <sup>*</sup> Brix and <i>C</i> were noticeably higher for peel as compared to flesh. The distinct correlations between saturation levels of $C_m$ and TPC <sub>m</sub> at the long extraction time ( $\approx 3$ h) were observed. However, the relative content of catechin in TPC (i.e., ratio $C_m/TPC_m$ ) evidenced the presence of selectivity in extraction of catechin. This selectivity depends from the type of tissue (flesh or skin), apple variety (green or red) and applied treatment protocols.

#### 1. Introduction

Apples contain various nutrients beneficial to human health with strong anti-inflammatory effects and high ability to prevent chronic diseases (González-Gallego, García-Mediavilla, Sánchez-Campos, & Tuñón, 2010). They include vitamin C, soluble fibre, and different dietary polyphenols (flavanols, flavonols, phloridzin, procyanidin, chlorogenic acid, anthocyanin) (Boyer & Liu, 2004; Weichselbaum, Wyness, & Stanner, 2010). The content of most abundant polyphenols in apple ranges between 19.6 and 55.8 mg for flavanols (e.g., (+)-catechin and (-)-epicatechin), 17.7-33.1 mg for flavonols (e.g., quercetin), and 10.6-80.3 mg for phenolic acids (e.g., chlorogenic acid) (McGhie, Hunt, & Barnett, 2005). Nowadays the extraction of phenolic compounds from apple pomace (peels, seeds, core, stem and calyx) attracts a great attention (Cetković et al., 2008). Apple pomace is the solid waste product resulting from industrial processing of apple juice or cider production and it can be considered as a potential source of food antioxidants (Lu & Foo, 2000).

The different methods of polyphenols extraction from plant products include conventional solvent extraction (CE), extraction assisted by enzymatic, ultrasound, microwave, pulsed electric fields, and other

treatments (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014; Ameer, Shahbaz, & Kwon, 2017; Barba et al., 2015; Caballero-Valdés, Olivares-Miralles, Soto-Maldonado, & Zúñiga-Hansen, 2016; Donsì, Ferrari, & Pataro, 2010).

Ultrasound has been previously used for polyphenols extraction from apples (Pingret, Fabiano-Tixier, Le Bourvellec, Renard, & Chemat, 2012; Virot, Tomao, Le Bourvellec, Renard, & Chemat, 2010; Yue, Shao, Yuan, Wang, & Qiang, 2012). Being environmentally friendly the ultrasound-assisted extraction (UAE) technique is widely recognized as "green and innovative", which typically involves reduced operating and maintenance costs, moderate energy consumption and small processing time, low quantity of water and solvents (Chemat et al., 2017a; Chemat et al., 2017b). Application of the UAE technique also allows reduction of wastes and elimination of generation of hazardous substances.

The UAE (25 kHz, 150 W) of polyphenols from apple pomace has been tested using the optimal concentration of ethanol/aqueous solution of 50% (v/v), solid/liquid ratio of 15% (w/v), and moderate temperatures  $16.0 \le T$  (°C)  $\le 34.0$  (Virot et al., 2010). Application of UAE for 45 min allowed increasing the total phenolics content (TPC) by more than 20% as compared with CE. The UAE for recovery of polyphenols from the unripe apple at different concentrations of ethanol

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(40–90% v/v), temperature (30–80 °C), time of extraction (10–30 min), and ultrasound power (280–560 W) has been tested. At optimum extraction conditions (ethanol concentration of 50%, temperature 50 °C, time of 30 min, ultrasonic power of  $\approx$ 520 W), the TPC value of 13.26  $\pm$  0.56 mg GAE/g was found (Yue et al., 2012). Different processing factors can affect the UAE efficiency of polyphenols recovery and their purity (Chemat et al., 2017b; Pingret et al., 2012). However, the impact of UAE protocols on the selectivity of valuable compounds recovery from apple products was not yet elucidated.

This work is focused on the effects of pulsed ultrasonic treatment on the initial period of extraction (300-350 s) of soluble matter (°Brix), catechin and TPC from the different parts (flesh and peel) of green (Granny Smith) and red (Red delicious) apples. The extraction experiments were done at fixed temperature (T = 50 °C). For UAE protocols with different power inputs the correlations between °Brix, TPC and catechin concentration, *C*, were evaluated.

#### 2. Materials and methods

#### 2.1. Material

Commercial green apples (Granny Smith) and red apples (Red delicious) were selected as the raw material for investigation. The apples with good and uniform quality and near-spherical shape were purchased at the local supermarket (Compiegne, France). In total, 60 apples were taken for experiment analyses. The initial moisture content on wet basis (83.74 g/100 g flesh and 83.07 g/100 g peel for green apples, and 87.44 g/100 g flesh and 85.39 g/100 g peel for red apple) was determined using MA 160 infrared moisture analyzer (Sartorius, Germany).

#### 2.2. Extraction experiments

The flesh tissue (the disks with diameter of 20 mm and thickness of 10 mm) was taken from the central part of the apple. The peel tissue (thin slices with length of 20 mm, width of 10 mm, and thickness of  $\approx 0.1$  mm) was removed from the apple with a razor blade.

In extraction experiments the flesh or peel tissue (20 g) were put into a glass beaker filled with preheated (50 °C) distilled water (200 mL), and solid liquid ratio was 1:10. The glass beaker was covered with aluminium foil in order to prevent water evaporation. The total extraction time was up to 3 h. UAE was done directly in the glass beaker using an ultrasonic processor UP 400S (400 W, 24 kHz, Hielscher GmbH, Stuttgart, Germany). The titanium ultrasonic probe (H14, Hielscher GmbH, Stuttgart, Germany) with a tip diameter of 14 mm, and the length of 100 mm was used.

The different extraction protocols presented in Table 1 were performed at fixed temperature (50 °C) of a thermal water bath Polystat 36 (Fisher Scientific, France). The protocol  $S_1$  corresponds to the aqueous CE and protocols  $S_2$ - $S_5$  correspond to the initial ultrasonic treatment with different pulsed modes. The moderate temperature of 50 °C was used to avoid destruction of organic compounds as well as provide an

#### Table 1

Parameters of applied extraction protocols,  $S_1$ - $S_5$ . Here,  $\Delta t_u$  and  $\Delta t_w$  are the duration of pulsed sonication and cooling of the sample during one pulse, respectively, n is the number of pulses,  $t_t = n(\Delta t_u + \Delta t_w)$  is the total duration of pulsed sonication, W is specific energy and P is the actual power of ultrasonic treatment. After UAE the CE continued up to 3 h.

Protocol #	$\Delta t_u$ ,s	$\Delta t_w$ ,s	п	t <sub>c</sub> , s	W, kJ/kg	<i>P</i> ,W
<b>S</b> <sub>1</sub>	-	-	0	-	-	-
S <sub>2</sub>	10	50	5	300	6.4	28
S <sub>3</sub>	10	50	5	300	10.7	47
S4	20	50	5	350	21.4	47
S <sub>5</sub>	10	50	5	300	26.8	118



**Fig. 1.** Scheme of pulsed UAE protocol  $S_2$  (**Table 1**). Here, the temperature evolution during application of pulses a series of n = 5 pulses with duration of  $\Delta t_u = 10 s$  is presented. During the pause ( $\Delta t_w = 50 s$ ), the temperature relaxes to the initial level of  $T_w = 50$  °C by cooling in cold water. After the initial UAE the CE was continued up to  $\Delta t_w^f = 3 h$ .

efficient application of ultrasound (Pingret, Fabiano-Tixier, & Chemat, 2013). Prolonged sonication can also cause degradation of targeted compounds. In our experiments the sonication time range chosen (from 50 to 100 s) was relatively short. Note that no specific reaction products after sonication (5–55 min) applied to the isolated phenolic compounds of apple pomace were observed (Pingret et al., 2013).

The actual ultrasonic power introduced to the system was estimated from the temperature elevation  $\Delta T$  in sample using following equation  $P = mCp\Delta T/\Delta t_u$  (1)

where *m* and  $C_p$  ( $\approx 4.18$  kJ/kg K) are the mass and specific heat capacity of sample, respectively,  $\Delta t_u$  is the duration of sonication.

The scheme of applied pulsed sonication treatment for protocol  $S_2$  is illustrated in Fig. 1. During the application of the ultrasonic pulse with duration of  $\Delta t_u = 10$  s the insignificant temperature elevation ( $\Delta T \approx 0.35$  °C) was observed. During the pause with duration of  $\Delta t_w = 50$  s, the temperature relaxes by cooling in cold water up to the initial value of 50 °C. After UAE with application of n = 5 sequential pulses, the CE was continued up to 3 h. The specific energy input for this protocol was  $W \approx 6.5$  kJ/kg. The total time of UAE was 300–350 s and the values of W were increased in the raw  $S_1$ - $S_4$ . The applied protocols allows testing the contribution of ultrasound power to the extraction yield during the first part of extraction (Chemat et al., 2012).

#### 2.3. Analysis

The obtained extracts were analyzed for <sup>o</sup>Brix, catechin concentration and TPC. The concentration of soluble matter <sup>o</sup>Brix (g of DM/100 g solution) was measured using the refractometer (Atago, USA) at room temperature.

The concentration of catechin, *C*, was estimated by fluorescence technique using the instrument Cary Eclipse Fluorescence Spectrofluorometer and 10 mm fused-silica cuvette (Agilent Technologies, USA). The fluorescence spectra were obtained using the excitation at  $\lambda_e = 280$  nm and registration of emission  $\lambda = 312$  nm (Arts, Van de Putte, & Hollman, 2000). Standard of catechin hydrate ( $\geq 0.98\%$ , Sigma-Aldrich) was used for the calibration curve.

The total polyphenols content was determined using the Folin-Ciocalteu method based on a colorimetric oxidation/reduction reaction of phenols (Singleton, Orthofer, & Lamuela-Raventós, 1999). 0.2 mL of diluted extract and 1 mL of Folin-Ciocalteu reagent (Merck,



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Darmstadt, Germany) (diluted 1:10 with water) were mixed. 0.8 mL of Na<sub>2</sub>CO<sub>3</sub> (75 g/L) (Prolabo, Fontenay-sous-Bois, France) was then added. The sample was incubated for 10 min at 50 °C and then cooled for 10 min at room temperature. For the control sample, 0.2 mL of distilled water was taken. The absorbance was measured at 750 nm using UV/ Vis instrument (Thermo Spectronic Genesys 20, Thermo Electron Corporation, MA, USA). Gallic acid (Sigma-Aldrich, St-Quentin Fallavier, France) was used for the calibration curve. The results were expressed as mg of Gallic acid equivalent (GAE) per gram of dry matter (mg GAE/ g DM).

#### 2.4. Statistical analysis

All experiments and measurements of characteristics were repeated at least in triplicate. The mean values and the standard deviations were calculated. The error bars in figures correspond to the standard deviations.

#### 3. Results and discussions

#### 3.1. Kinetics of extraction

Fig. 2 shows a soluble matter content, <sup>o</sup>Brix, (a, c) and concentration of catechin, C, (b, d) during solutes extraction from green apple flesh (a, b) and peel (c, d) (protocols  $S_1$ - $S_5$ ). Fig. 3 shows the similar data for the red apple. Both the Brix and catechin concentration were higher for UAE protocols S<sub>2</sub>-S<sub>5</sub> as compared with CE protocol S<sub>1</sub>. Both the <sup>o</sup>Brix and C values increased with the time and the effects were more pronounced for the treatments with higher ultrasound energy input W. The different effects of ultrasound power on the extraction of soluble matter and catechin were observed. The effects of ultrasound power on °Brix were insignificant, whereas the concentration of catechin continuously increased with increase of specific energy W (Table 1). Note that catechin contents from flesh and peel were in the range of 50–300 mg/ 100 g DM (Figs. 2 and 3). These results were in good correspondence with the previously reported values for different apple varieties 94 ± 4.8 mg/100 g DM (Leontowicz et al., 2003), 153 mg/100 g DM (García-Alonso, Rimbach, Rivas-Gonzalo, & De Pascual-Teresa, 2004), and 530 ± 50 mg/100 g DM (Heras-Ramírez et al., 2012).

3.2. Correlations between the concentration of catechin and soluble matter content

Fig. 4 presents examples of correlations between the concentration of catechin, C, and soluble matter content, "Brix, during extraction from flesh and peel tissues of green (a) and red (b) apples for the protocol  $S_5$  with the highest power,  $W \approx 26.8 \, {\rm kJ/kg}$ . It is remarkable that soluble matter content, "Brix, changed continuously during the preliminary UAE (300s) and CE (3 h) whereas the most accelerated extraction of catechin was only observed during the preliminary UAE. The quantity of extracted catechin was noticeably higher for skin tissue as compare to flesh one. The obtained data are in correspondence with the literature data. In apples the polyphenols are mainly located in peel and seeds (Francini & Sebastiani, 2013). For different varieties of apples, the total phenolic and flavonoid contents were highest in the peel (outermost tissue), followed by the flesh + peel and the flesh (Wolfe, Wu, & Liu, 2003).

For flesh tissue the kinetics of catechin extraction were comparable for both the green (Fig. 4a) and red (Fig. 4b) apples. However, the catechin extraction from the skin tissue of green apple was noticeably lower (Fig. 4a) as compared to extraction from red apple (Fig. 4b). It is known from literature, that the content and composition of polyphenols in apples are different in dependency of apple variety (Kalinowska, Bielawska, Lewandowska-Siwkiewicz, Priebe, & Lewandowski, 2014).

The correlations between final levels of  $C_m$  and <sup>o</sup>Brix<sub>m</sub> for the long extraction time (3 h) and different protocols  $S_1$ - $S_5$  (Table 1) are



**Fig. 2.** Soluble matter content, <sup>o</sup>Brix, (a,c) and concentration of catechin, *C*, (b,d) during solute extraction from green apple flesh (a,b) and peel (c,d) for different protocols  $S_1$ - $S_5$  (Table 1). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

presented in Fig. 5. Increasing of ultrasound specific energy input *W* (0–26.8 kJ/kg) resulted in insignificant increase of the °Brix value (by  $\leq$  1.25 times), and more noticeable increase of the final catechin content,  $C_{m_c}$  (by  $\geq$  1.58 times). The most significant increase of  $C_m$  (by  $\approx$  2.7 times) was observed for peel tissue of red apple, whereas ultrasound treatment practically had no influence on the final value of °Brix<sub>m</sub>. This study evidenced the more significant impact of the initial ultrasonic pretreatment on the catechin extraction comparatively to the total solutes extraction.

Increasing of ultrasound specific energy input *W* resulted in increase of both the final concentration of catechin  $C_m$  and the value of TPC<sub>m</sub> (Fig. 6). For flesh and peel tissues the different master curves (branches) collecting the data for protocols S<sub>1</sub>-S<sub>5</sub> were obtained. It can reflect the different extraction mechanisms of phenolic compounds from flesh and peel tissues. A clear improvement of the extraction of polyphenols from apple pomace (an increase in TPC yield by more than 1.32) was

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Fig. 3. Soluble matter content,  ${}^{\circ}Brix_{i}(a,c)$  and concentration of catechin, *C*, (b,d) during solute extraction from red apple flesh (a,b) and peel (c,d) for different protocols S<sub>1</sub>-S<sub>5</sub> (Table 1). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

attributed to ultrasonic cavitation (Pingret et al., 2012).

#### 3.3. Selectivity of catechin extraction

The relative content of catechin in total phenolic content,  $C_m/\text{TPC}_m$ , was in the interval of 15–30% (Fig. 7). The ratio  $C_m/\text{TPC}_m$  reflects the selectivity of extraction of catechin as compared to extraction of other phenolics.

The data evidenced that this ratio was nearly constant (within the data errors) for flesh of red apples and peel of green apples, whereas for flesh of green apples and peel of red apples it was dependent on the extraction protocols,  $S_1$ - $S_5$ . Surely it reflects the impact of extraction protocol n the selectivity of catechin extraction. For example, for peel tissue from red apple the CE gave high ratio of  $C_m/TPC_m$  ( $\approx 30\%$ ) at small value of TPC<sub>m</sub> ( $\approx 400$  mg GAE/100 g DM). Application of initial UAE with small energy (protocol  $S_2$ ) resulted in noticeable increase of TPC<sub>m</sub> ( $\approx 700$  mg GAE/100 g DM) and decrease of  $C_m/TPC_m$  ( $\approx 18\%$ ).

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Fig. 4. Examples of correlations between concentration of catechin, *C*, and soluble matter content, °Brix, during extraction from flesh and peel tissues of green (a) and red (b) apples for the protocol  $S_5$  with the highest power,  $W \approx 26.8 \text{ kJ/kg}$ . After the initial UAE for 300 s the CE was continued up to 3 h. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 5.** Correlations between final levels of soluble matter content <sup>o</sup>Brix<sub>m</sub> and concentration of catechin  $C_m$  for the long extraction time (3 h) and different protocols  $S_1$ - $S_5$  (Table 1). The data are presented for flesh and peel tissues of both green and red apples. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

However, further increase in energy (protocols  $S_2$ - $S_5$ ) resulted in increase of TPC<sub>m</sub> and stabilization of ratio  $C_m$ /TPC<sub>m</sub> at the level of  $\approx 25-26\%$ .

#### 4. Conclusions and final remarks

The effects of pulsed ultrasonic treatment during initial period of extraction (first 300–350 s) on the recovery of soluble matter (°Brix), catechin and TPC from different parts (flesh and peel) of green (Granny Smith) and red (Red delicious) apples were studied. The different ultrasound specific energy inputs *W* (0–26.8 kJ/kg) were compared using S<sub>1</sub> (CE) and S<sub>2</sub>-S<sub>5</sub> (UAE) protocols. All experiments were done at *T*  $\approx$ 



**Fig. 6.** Correlations between final levels of catechin concentration  $C_m$  and TPC<sub>m</sub> for the long extraction time (3 h) and different protocols  $S_1$ - $S_5$  (Table 1). The data are presented for flesh and peel tissues of both green (a) and red (b) apples. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



Fig. 7. Relative content of catechin in total phenolic content, Cm/TPCm, versus  $TPC_m$ . The data represent final levels for the long extraction time (3 h) and different protocols  $S_1$ - $S_5$  (Table 1) for flesh and peel tissues of both green and red apples. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

50 °C and UAE with short duration of pulses were used in order to avoid significant temperature elevation. The different impact of ultrasound power on the extraction of soluble matter and catechin was observed for flesh and peel tissues. For the given protocol and used variety of apple (green or red) the extraction levels of catechin or TPC were noticeably higher for skin tissue as compared to flesh one. The obtained data evidenced more significant impact of the initial ultrasonic pretreatment on extraction of catechin as compared to extraction of total soluble matter. The <sup>o</sup>Brix values increased continuously during the initial period of UAE and during CE (3 h). The most accelerated extraction of catechin was observed only for the initial UAE. Ultrasound has impact on the structural properties of vegetable tissue and may influence

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on the solute diffusivity in the tissue matrix (Chemat et al., 2017b; Vinatoru, 2001). The observed differences in the catechin recovery from skin of green and red apples can reflect their different structure. Increasing of ultrasound specific energy input W resulted in increase of both the final concentration of catechin C<sub>m</sub> and value of TPC<sub>m</sub>.

However, the different branches collecting the data for protocols S1-S5 were observed for flesh and peel tissues. It can be due to the different mechanisms of phenolic compounds extraction from flesh and peel tissues. The extraction mechanisms induced by ultrasound may include combination of many effects related with fragmentation, erosion, sonocapillarity, detexturation, and sonoporation (Chemat et al., 2017b). The relative content of catechin in total phenolic content,  $C_m/\text{TPC}_m$ , (i.e. selectivity of catechin extraction) was depended on the type of the tissue (flesh or skin), apple variety (green or red) and can be controlled by selecting the ultrasound protocol. The previous studies on large scale ultrasound extraction of polyphenols from apple pomace have demonstrated the potential towards the use of the UAE on a commercial scale (Pingret et al., 2012; Virot et al., 2010) and we believe that the obtained lab-scale results on extraction selectivity will help for the scale up of the UAE processes

However, the mechanism of the observed extraction selectivity is still unclear. The different UAE mechanisms based on cavitation bubble collapses were recently discussed(Saleh et al., 2016). The two extraction processes in recovery of total polyphenols from apple pomace by ultrasound were identified (Pingret et al., 2012). The enhancing of apple pomace polyphenols extraction by ultrasound was explained by better water absorption during the first 10 min of extraction. It was hypothesized that such absorption can favour solvent penetration inside of tissue and following polyphenols transfer out of the pomace. The extraction mechanism induced by ultrasound may include combination of many effects related with fragmentation, erosion, sonocapillarity, detexturation, and sonoporation (Chemat et al., 2017b). It can be speculated that manifestation of these effects is quite different in dependence on structure of flesh and peel tissues, ability of their swelling and rehydration, and distribution of phenolic compounds inside these tissues

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# III.4 Article III Effects of ultrasound treatment and concentration of ethanol on selectivity of phenolic extraction from apple pomace Summary

Content of phenolics in the apple pomace is much higher than in apple juice or apple flesh (Wolfe et al., 2003), and it can vary among different varieties of apples (Kalinowska et al., 2014). Apple pomace (peels, mesh, seeds, cores and stems) accounts for 25%–35% of the dry mass of apple (Gullón et al., 2007). This waste is considered as a potential source of food antioxidants, phenolics (Reis et al., 2014) and as food additives to obtain a fibre riched product (Lu et al., 2017). Extraction of phenolics from apple pomace have attracted great attention for researchers (Ćetković et al., 2008). The potential towards the use of the UAE on a commercial scale for extraction of polyphenols from apple pomace has been demonstrated in the previous studies (Jacotet-Navarro et al., 2016; Pingret et al., 2012; Virot et al., 2010). Therefore, in the last part of this chapter (details are presented in article III *Effects of ultrasound treatment and concentration of ethanol on selectivity of phenolic extraction from apple pomace*), the selectivity of phenolics extraction from apple pomace with ultrasound-assisted ethanol/aqueous extraction was studied.

The extraction kinetics and extraction yields of  $C_c$  and TPC were evaluated at different times of ultrasound treatment ( $t_u$ =0-30 min) and concentrations of ethanol ( $C_{et}$ =0-50%). The obtained results presented that the highest extraction efficiency of TPC from apple pomace was observed for  $t_u = 30$  min,  $C_{et} = 50\%$  and  $t_e = 60$  min. The relative content of catechin ( $R = C_c/TPC$ ) of applied extraction protocols was analyzed to demonstrate the selectivity of catechin extraction. The ratio ( $R = C_c/TPC$ ) was different in dependence of applied extraction protocols for apple pomace. The experimental dependence  $C_c$  (TPC) for different applied protocols was fitted by power equation  $C_c = a((TPC + 1)^b-1)$ , where  $a = 6.0\pm1.9$  mg per 100 g DM and  $b = 0.23\pm0.04$  are fitting parameters. The initial portions of extracts at a small level of TPC ( $\leq$ 50 mg per 100 DM) were richer in  $C_c$  ( $R\approx15\%$ -31%) as compared with last portions of extracts ( $R\approx2\%$ ) obtained using long duration UAE and extraction in concentrated ethanol/water mixtures at a high level of TPC(850 mg per 100 g). Experiment results presented simultaneous application of UAE and using of ethanol/water mixtures allow fine regulation of catechin extraction selectivity from apple pomace. Food Science +Technology

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## Original article

# Effects of ultrasound treatment and concentration of ethanol on selectivity of phenolic extraction from apple pomace

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**Summary** The extraction of polyphenols from apple pomace was studied using ultrasound, ethanol/water mixtures and moderate temperatures, 20–37 °C. The extraction kinetics and extraction yields of catechin and total phenolic content (TPC) were evaluated at different times of ultrasound treatment ( $t_u$ ) and concentrations of ethanol ( $C_e$ ). The highest extraction efficiency of TPC was observed at  $t_u = 30$  min,  $C_e = 50\%$  and total extraction time of  $t_e = 60$  min. The high selectivity of catechin extraction was demonstrated by analysing the relative content of catechin ( $R = C_e/\text{TPC}$ ) in dependence of applied extraction protocol. The initial portions of extracts at small levels of TPC ( $\leq 50$  mg per 100 DM) were richer in catechin content ( $R \approx 15\%$ -31%), whereas the last portions of extracts with high level of TPS were more exhausted in catechin ( $R \approx 2\%$ ).

Keywords Apple pomace, catechin, ethanol aqueous extraction, total phenolic content, ultrasound-assisted extraction.

#### Introduction

Apple pomace (peels, mesh, seeds, cores and stems) accounts for 25%-35% of the dry mass of apple (Gullón *et al.*, 2007). This waste is considered as a potential source of food antioxidants, phenolics (Reis *et al.*, 2014) and as food additive to obtain a fibreenriched products (Lu *et al.*, 2017). The main ingredients include procyanidins, flavonols, dihydrochalcones, hydroxycinnamic acids and catechins. Extraction of these compounds from apple pomace attracts great attention (Četković *et al.*, 2008). Content of phenolics in the pomace is higher than in the juice or flesh (Wolf *et al.*, 2003), and it can vary among different varieties of apples (Kalinowska *et al.*, 2014). The pomace is richer in procyanidins as compared to the flesh (Le Bourvellec *et al.*, 2007), and it contains high concentrations of flavonols and dihydrochalcones due to their dominating location in the peel and seeds (Francini & Sebastiani, 2013).

Efficiency of conventional solvent extraction (CE) of polyphenols from plant materials can depend on processing parameters, including the temperature, time, solvent, pH and liquid-solid ratio (Caballero-Valdés *et al.*, 2016). For efficient recovery of

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doi:10.1111/ijfs.13835 © 2018 Institute of Food Science and Technology polyphenols from apple pomace, methanol, acetone and their aqueous solutions are commonly used (Wijngaard & Brunton, 2009). Recently, different nonconventional methods (ultrasound, microwaves, pulsed electric fields, high-voltage electrical discharges,) have been proposed to intensify extraction of polyphenols (Bai *et al.*, 2010; Donsì *et al.*, 2010; Junjian *et al.*, 2013; Acosta-Estrada *et al.*, 2014; Ameer *et al.*, 2017).

Nowadays, the ultrasound-assisted extraction (UAE) is widely recognised as green technique with reduced energy consumption, processing time and low quantity of wastes (Jacotet-Navarro et al., 2016; Chemat et al., 2017a, 2017b; Khadhraoui et al., 2018). Particularly, in several works, an application of UAE for recovery of polyphenols from apple pomace has been reported (Virot *et al.*, 2010; Pingret *et al.*, 2012; Lohani & Muthukumarappan, 2016; Chemat *et al.*, 2017b). Obtained extracts were nondegraded and demonstrated high antioxidant activity. For example, application of UAE at optimal conditions (using 25 kHz, 150 W, 50% v/v ethanol/aqueous extractant, solid/liquid ratio of 15% w/v and moderate temperatures 16-34 °C) allowed increasing of the total phenolic content (TPC) by more than 20% as compared with CE (Virot et al., 2010). However, to our knowledge, the effects of UAE and ethanol concentration on the selectivity of

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compound extraction from apple pomace have never been discussed before.

Therefore, the main objective of this study was to investigate the effects of ultrasound treatment (24 kHz, 150 W, 0–30 min) and concentration of ethanol (0%–50%) on the extraction of phenolics from apple pomace. The fluorescence technique has been used for the precise determination of catechin in ethanol/water extracts. The extraction kinetics and correlations between catechin concentration and TPC were discussed. Obtained data were used for the demonstration of extraction selectivity.

#### Materials and methods

#### Apple pomace

Fresh apple pomace was provided by Institute Français des Productions Cidricoles (Rheu, France). The pomace was packed in plastic bags and stored at 4 °C before extraction experiments. The storage time never exceeded 1 week. The dry matter (DM) content in fresh apple pomace ( $\approx 0.81$  g/g) was determined after drying the apple flesh in MA 160 infrared moisture analyser (Sartorius, Germany).

#### Extraction experiments

The pomace (20 g) was randomly taken from the raw material, mixed with distilled water (20 °C, 200 mL) and after 1 min of intensive stirring at 500 r.p.m. (C-MAG HS7, IKA, Staufen, Germany) the mixture was centrifuged for 10 min at 4000 r.p.m. (Laborzentrifugen 3–10, SIGMA, Osterode am Harz, Germany). The supernatant and solid sediment were separated. The supernatant was used for determination of catechin concentrations,  $C_c^i$  (=4.74 ± 0.09 mg per 100 g DM) and TPC<sup>i</sup> (24.90 ± 0.25 mg per 100 g DM) in apple pomace.

95% ethanol (Sigma-Aldrich, St-Quentin Fallavier, France) and distilled water were used for preparation of ethanol/water mixtures with different concentrations of ethanol ( $C_e = 0\%-50\%$ ). The solid sediment was loaded into a glass beaker and the ethanol/water mixture was added (sediment/solvent ratio was 1/10). The beaker was covered with plug to reduce solvent evaporation. The sample was intensively stirred for 1 min at 500 r.p.m. (C-MAG HS7, IKA, Staufen, Germany).

After preliminary washing and mixing for 1 min, the UAE experiments were carried out and extraction time,  $t_e$ , was counted starting from this moment. The general schema of extraction experiments is presented in Figure S1. The ultrasound treatment was done directly in the beaker using an instrument UP 400S (24 kHz, 400 W, Hielscher GmbH, Stuttgart, Germany) and a titanium ultrasonic probe (H14, Hielscher

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GmbH, Stuttgart, Germany) with a tip diameter of 14 mm and the length of 100 mm.

The actual ultrasonic power introduced to the system was estimated from the temperature elevation  $\Delta T$  in sample using following equation

$$P = mC_p \Delta T / \Delta t_u \tag{1}$$

where *m* and  $C_p$  ( $\approx$ 4.18 kJ per kg °C) are the mass and specific heat capacity of sample, respectively,  $\Delta t_u$ is the duration of sonication. For the applied treatment, it was  $P \approx 118$  W.

The time of ultrasound treatment was  $t_u = 0-30$  min. The initial temperature before treatment was 20 °C. During the ultrasound treatment the beaker was put inside ice/water bath and the temperature elevation for the longest time of the treatment ( $t_u = 30$  min) never exceeded 37 °C. The moderate temperatures were used to avoid thermal degradation of organic compounds as well as provide an efficient application of ultrasound (Pingret *et al.*, 2013; Jacotet-Navarro *et al.*, 2016). In principle, the prolonged sonication can cause degradation of targeted compounds. Note that no specific reaction products after sonication (5-55 min) applied to the isolated phenolic compounds of apple pomace were previously observed (Pingret *et al.*, 2013).

Then conventional solvent extraction was continued for a time up to  $t_e = 60$  min at 20 °C. Finally, the samples were centrifuged for 10 min at 4000 r.p.m. and the phenolics in supernatant were analysed.

#### Phenolic content analysis

The concentration of catechin,  $C_c$ , in extract was estimated by fluorescence technique and the total polyphenols content (TPC) was determined spectrophotometrically using the Folin–Ciocalteu method (see Supplementary Information for the details). Figure S2 presents examples of calibration curves for the fluorescence of catechin.

#### Statistical analysis

All experiments and measurements of characteristics were repeated at least in triplicate. The mean values and standard deviations were calculated, and the error bars in figures correspond to the standard deviations. Statistical fitting of experimental data was done using the package Table Curve 2D, version 5.01 (© SYSTAT Software Inc., San Jose, CA, USA).

#### **Results and discussions**

Figure 1 shows concentration of catechin,  $C_c$ , and TPC obtained immediately after adding of ethanol/water mixture and intensive mixing for 1 min. The

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**Figure 1** The concentration of catechin,  $C_c$ , and total polyphenols content, TPC, obtained immediately after adding of ethanol/water mixture and intensive mixing for 1 min at different ethanol concentrations of ethanol in water ( $C_e = 0\%-50\%$ ). Here, open symbols for  $C_c^i = 4.74 \pm 0.09$  mg per 100 g DM and TPC<sup>i</sup> = 24.90  $\pm$  0.25 mg per 100 g DM correspond to the values obtained after initial washing of pomace in distilled water.

presented data correspond to the zero extraction time,  $t_c = 0$  (Figure S1). Addition of ethanol/water mixtures resulted in noticeable increase in  $C_c$  and TPC values even for short duration contact of pomace sediment with extraction solutions. Both the concentration of catechin and TPC increased with the increase in ethanol concentration. Observed initial extraction effects can reflect fast release of extracted polyphenols from the surface of pomace sediment.

Figure 2 compares extraction kinetics of catechin,  $C_c$ , and TPC at different concentrations of ethanol ( $C_e = 0$  and 30%) and times of ultrasound treatment ( $t_u = 0$  and 10 min). Application of ultrasound treatment and increase in concentration of ethanol clearly resulted in increase in the yield of polyphenols. In previous studies enhancing of extraction efficiency by ultrasound treatment was explained by the damage of cell membranes (sonoporation) and cell walls and by the development of ultrasonic capillary effect (Chemat *et al.*, 2017b).

The near-saturation levels of  $C_e$  and TPC were obtained at long extraction time ( $t_e \approx 60$  min). So, in follow-up experiments the value  $t_e = 60$  min was selected as optimum extraction time.

Figure 3 presents effects of time of ultrasound treatment,  $t_{us}$  on the values of  $C_e$  and TPC at different concentrations of ethanol,  $C_{e}$ . For the relatively long time of ultrasound treatment ( $t_u \ge 30$  min) the

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**Figure 2** Catechin concentration,  $C_c$ , and total polyphenols content, TPC, vs. extraction time,  $t_c$ , at different concentration of ethanol,  $C_e$ . Filled and open symbols correspond to the time of ultrasound treatment  $t_u = 0$  and  $t_u = 10$  min, respectively.

near-saturation level of  $C_e$  and TPC were obtained. The most pronounced effects were observed for higher concentration of ethanol,  $C_c = 30\%-50\%$ . So, the extraction protocols with  $t_u = 30$  min can be used for obtaining the saturation level of  $C_c$  and PTC. Figure 4 demonstrates the effects of concentration of ethanol,  $C_e$ , on the values of  $C_c$  and TPC at  $t_u = 30$  min. Increase in  $C_e$  resulted in increase in both the values of  $C_c$  and TPC, and their near-saturation levels were observed at  $C_e > 30\%-50\%$ . The obtained effects of ultrasound treatment and ethanol/aqueous extractant were in satisfactory correspondence with previously reported data on recovery of TPC from apple pomace (Virot *et al.*, 2010).

Figure 5 collects the obtained data on relative content of catechin in TPC (i.e. ratio  $R = C_c/\text{TPC}$ ) vs. TPC for all extraction protocols. The ratio  $C_c/\text{TPC}$ corresponds to the content of catechin in TPC and changes in R reflects the selectivity of extraction of catechin as compared to extraction of other phenolics. The inset to Fig. 5 shows  $C_c(\text{TPC})$  dependence. The protocols were designated as  $P_{c_e}^{l_u}$  and, for example,  $P_{10\%}^{l_0}$  corresponds to  $t_u = 10$  min and  $C_e = 10\%$ . The value of R obtained after initial washing of

The value of R obtained after initial washing of apple pomace in distilled water was rather high,  $R^i = C_c/\text{TPC}^i \approx 19\%$ . The shaded area at small values of TPC ( $\leq 50$  mg per 100 g DM) corresponds to the zero extraction time ( $t_e = 0$ ), that is, the data obtained immediately after adding of ethanol/water mixture and intensive mixing for 1 min (open symbols in Fig. 1). Note that in the shaded area the relative content of catechin was also rather high ( $\approx 15\%-31\%$ ).

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**Figure 3** Catechin concentration,  $C_e$ , and total polyphenols content, TPC, vs. the time of ultrasound treatment,  $t_u$ , at different concentrations of ethanol,  $C_e$ .



**Figure 4** Catechin concentration,  $C_c$ , and total polyphenols content, TPC, vs. the ethanol concentration,  $C_c$ , at  $t_u = 30$  min.

However, the further increase in TPC for longer  $t_e$  and  $t_u$  or high  $C_e$  resulted in decrease in R.

So, the initial portions of extracts was richer in catechin content as compared with last portions of extracts obtained using the long duration UAE and extraction in ethanol/water mixtures. Remarkably, that data for different applied protocols (i.e. for extraction conditions at different values of  $t_u$ ,  $t_e$  and  $C_e$ ) approximately fall into the same master curve. The experimental dependence  $C_e$ (TPC) can be well fitted with relatively

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**Figure 5** The relative contents of catechin in TPC,  $R = C_c/\text{TPC}$ , vs. TPC for different extraction protocols. Inset shows the concentration of catechin,  $C_c$ , vs. the total polyphenols content, TPC. The shaded area at small values of TPC ( $\leq$ 50 mg per 100 g DM) corresponds to the zero extraction time,  $t_e = 0$  min (Figure S1), for other protocols the total extraction time was  $t_e=60$  min. The dashed lines correspond to fitting using eqn (2).

high coefficient of determination  $(r^2 \approx 0.92)$  by the following power equation

$$C_c = a((\text{TPC} + 1)^b - 1),$$
 (2)

where  $a = 6.0 \pm 1.9$  mg per 100 g DM and  $b = 0.23 \pm 0.04$  are fitting parameters. The dashed lines presented in Fig. 5 correspond to fittings using eqn (2).

However, the mechanism of the observed extraction selectivity is not yet clear completely. The different sextraction processes in recovery of total polyphenols from apple pomace by ultrasound were identified (Pin-gret *et al.*, 2012). The extraction mechanism induced by ultrasound may include combination of many effects related with fragmentation, local erosion, sonocapillarity, capillary effect, detexturation and sonoporation (Chemat et al., 2017b; Khadhraoui et al., 2018). It can be speculated that manifestation of these effects is quite different in dependence on structure of flesh and peel tissues, ability of their swelling and rehydration and distribution of phenolic compounds inside these tissues. So, we can speculate the presence of different mechanisms for phenolic extraction from apple pomace. The apple pomace presents rather complex mixture of peels, mesh, seeds, cores and stems with dif-ferent contents of polyphenols and different abilities of extraction of them from constituents of pomace. Efficiency of extraction of different polyphenols can depend on the used extraction method. For example, extraction assisted by ultrasound in pure water

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allowed obtaining the high values of R ( $\approx 7\%$ -18%, Fig. 5) at relatively low level of TPC (≤200 mg per 100 DM, Fig. 3). Extraction in ethanol/water mixtures without treatment by ultrasound  $(t_u = 0 \text{ min})$  allowed obtaining smaller values of  $R (\approx 5\%, \text{ Fig. 5})$  at higher level of TPC ( $\approx 200-400$  mg per 100 DM, Fig. 3). Simultaneous application of UAE and using of ethanol/water mixtures allowed obtaining the higher level of TPC (≈850 mg per 100 g DM) at relatively small values of  $R \approx 2\%$ .

#### Conclusions

The extraction of polyphenols (catechin and TPC) from apple pomace was studied using ultrasound treatment (24 kHz, 150 W), ethanol/water mixtures (0%-50%) and moderate temperatures, 20-37 °C. The highest extraction efficiency of TPC was observed for time of ultrasound treatment  $t_u = 30$  min, concentration of ethanol of  $C_e = 50\%$  and total extraction time of  $t_e = 60$  min. The relative content of catechin in TPC ( $R = C_c/\text{TPC}$ ) was different in dependence of applied extraction protocol. The experimental dependence  $C_c(\text{TPC})$  for different applied protocols was fit-ted by power equation  $C_c = a((\text{TPC} + 1)^b - 1)$ , where  $a = 6.0 \pm 1.9$  mg per 100 g DM and  $b = 0.23 \pm 0.04$ are fitting parameters. The initial portions of extracts at small level of TPC ( $\leq$ 50 mg per 100 DM) were richer in catechin content ( $R \approx 15\%-31\%$ ) as commercial with last parameters. pared with last portions of extracts ( $R \approx 2\%$ ) obtained using long duration UAE and extraction in concentrated ethanol/water mixtures at high level of TPC(≈850 mg per 100 g). Therefore, simultaneous application of UAE and using of ethanol/water mixtures allows fine regulation of catechin extraction selectivity. In future studies, it is desirable to test these effects for other types of polyphenols. The potential towards the use of the UAE on a commercial scale for extraction of polyphenols from apple pomace has been demonstrated in the previous studies (Virot et al., 2010; Pingret et al., 2012; Jacotet-Navarro et al., 2016). We believe that the obtained laboratory-scale results on extraction selectivity will help for the scaleup of the UAE processes.

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#### **Conflict of interest**

The authors declare that they have no conflict of interest.

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#### **Supporting Information**

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Figure S1. The schema of extraction experiments.

Figure S2. Concentration catechin,  $C_c$ , in solvents vs. the fluorescence intensity I (au) at ethanol concentrations  $C_e = 0$  and  $C_e = 10\%$ .

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# **Supplementary Information**

Effects of ultrasound treatment and concentration of ethanol on selectivity of

phenolics extraction from apple pomace

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# Schema of extraction experiments

The general schema of extraction experiments is presented in Fig. S1. After preliminary washing and mixing for 1 min, the extraction in solvent was curried out and extraction time,  $t_e$ , was counted starting from this moment. The time of ultrasound-assisted extraction was  $t_u$  =0-30 min. Then conventional solvent extraction was continued for a time up to  $t_e$ =60 min at 20 °C.

Figure 1 The schema of extraction experiments.



Figure S1 The schema of extraction experiments.

# Phenolic content analysis

## Catechin

The concentration of catechin,  $C_c$ , in extract was estimated by fluorescence technique using the instrument Cary Eclipse Fluorescence Spectrofluorometer and 10 mm fused-silica cuvette

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(Agilent Technologies, Santa Clara, USA). The fluorescence spectra were obtained using the wavelengths 280 nm and 312 nm for excitation and emission, respectively (Wolfe, Wu, & Liu, 2003). For calibration the standard of catechin hydrate (≥0.98 %, Sigma-Aldrich, St-Quentin Fallavier, France) was used.



**Figure S2** Concentration catechin,  $C_c$ , in solvents versus the fluorescence intensity *I* (au) at ethanol concentrations  $C_e=0$  and  $C_e=10\%$ .

Figure S2 presents examples of calibration curves for the fluorescence of catechin for  $C_e=0$  and 10 %. For relatively small concentrations of ethanol ( $C_e\leq10\%$ ) the relationship between catechin concentration in solvent,  $C_s$  (mg mL<sup>-1</sup>) and fluorescence intensity *I* (au) was practically linear.

(1)

 $C_s \approx kI$ ,

Here,  $k=1.39\times10^{-5}$  mg/ml and  $k=0.72\times10^{-5}$  mg/ml are slopes for  $C_e=0$  and  $C_e=10\%$ , respectively.

For higher concentrations of ethanol ( $C_e > 10\%$ ) the extracts were diluted to the concentration  $C_e = 10\%$  and then analysed. The used fluorescence technique is express, precise

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and allowed determination of  $C_c$  in extracts using relatively small quantities of tested solutions.

## Total polyphenols

The total polyphenols content (TPC) was determined spectrophotometrically using the Folin– Ciocalteu method based on a colorimetric oxidation/reduction reaction of phenols (Singleton, Orthofer, & Lamuela-Raventós, 1999). Initially, 0.2 mL of diluted extract and 1 mL of Folin– Ciocalteu reagent (Merck, Darmstadt, Germany) (diluted 1:10 with water) were mixed and then 0.8 mL of Na<sub>2</sub>CO<sub>3</sub> (75 g L<sup>-1</sup>) (Prolabo, Fontenay-sous-Bois, France) was added. The sample was incubated for 10 min at 50 °C and then cooled for 10 min to room temperature, 20 °C. The absorbance was measured at 750 nm by the UV/Vis spectrophotometer (Thermo Spectronic Genesys 20, Thermo Electron Corporation, MA, USA). The calibration curve was obtained using gallic acid (Sigma-Aldrich, St-Quentin Fallavier, France). The results were expressed as mg of gallic acid equivalent (GAE) per 100 g of DM (hereinafter, mg/100 g DM).

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# **III.5** Conclusion

This chapter III is focused on the intensification of polyphenols extraction from apple products (flesh, peel and pomace) with ultrasound treatment. By comparing the obtained data, the extraction yields of polyphenols from apple products obtained with ultrasound-assisted extraction (UAE) are noticeable higher than the one obtained with conventional extraction (CE). The increase of ultrasound energy, intensity and treatment time resulted in the increase of polyphenol extraction values. Moreover, the results evidenced the possibility of fine regulation of extraction selectivity using different temperatures, UAE protocols, and ethanol/aqueous mixtures. The relative content of catechin in total polyphenol content, TPC, (R) presented the selectivity in extraction of phenolic compounds from apple products. For apple flesh, significant acceleration of catechin extraction was observed at higher temperature (T) and higher ultrasound energy (W). The values of R were noticeably higher with application of UAE as compared with CE. The catechin contents were noticeably higher for peel as compared to flesh and increased with the increase of W. The values of R depend on the type of tissue (flesh or peel), apple variety (green or red) and applied treatment protocols. For apple pomace, the highest extraction efficiency of TPC was observed at ultrasound treatment time,  $t_u$ =30 min, ethanol concentration,  $C_e$ =50% and extraction time,  $t_e$ =60 min. The initial portions of extracts at small level of TPC ( $\leq 50$ mg/100g DM) were richer in catechin ( $R \approx 15$ -31%), whereas the last portions obtained by using of intensive UAE and extraction in ethanol/water with high level of TPC (≈850mg/100g DM) were more exhausted in catechin  $(R\approx 2\%)$ . These obtained laboratory-scale results will help for the future scale-up of UAE processes. In the following, the mechanism of the observed extraction by ultrasound treatment is necessary to be studied.

# Chapter IV Cavitation Phenomenon during Ultrasoundassisted Extraction of Polyphenols

# **IV.1 Introduction**

Nowadays, UAE of antioxidant polyphenols from various food materials has been widely studied (Chemat et al., 2017b, 2011; Khan et al., 2010; Pingret et al., 2012; Rajha et al., 2014). Fewer studies are focused on the understanding of mechanisms leading to antioxidant polyphenol extraction enhancement due to the use of ultrasound (Chemat et al., 2017b; Mason and Lorimer, 2002; Suslick and Price, 1999).

Cavitation phenomenon generated by ultrasound treatment is widely regarded as the most important impact to increase extraction of valuable components (Chemat et al., 2017b; Mason and Lorimer, 2002). The cavitation phenomenon resulting from bubble collision can damage cell membranes of samples (Chemat et al., 2004), accelerate heat and mass transfer by disrupted cell walls of samples, and release the target compounds into the solvent (Roselló-Soto et al., 2015b). The shockwaves and mixing phenomena produced by cavitation can lead to increasing solid-liquid extraction yield. Bubbles explosion and shockwaves in solid/liquid mixtures can result in the fragmentation of biological particles, cell structure damage and cell membrane sonoporation (Suslick and Price, 1999). Particles fragmentation and cells destruction can increase effective surface area for solutes extraction. In addition, cavitation induces mass transfer improvement with increasing the depth and velocity of penetration of liquid into canals and pores (Malykh et al., 2003; Mason, 2015). Furthermore, previous studies have shown that cavitation would produce large amounts of heat and effectively improve convective heat transfer coefficient (LIMA and SASTRY, 1990; Sastry et al., 1989), which result in the extraction of the target compounds into the solvent (Singh and Heldman, 2001). Therefore, the cavitation phenomena produced by ultrasound leading to polyphenol extraction enhancement is interesting to be carried out for the purpose of:

1) Analyzing cells destruction by cavitation phenomena induced by US;

2) Analysis of correlations between disintegration degree of peel cells induced by US and efficiency of bio-compounds extraction from fruit peels;

3) Study of the effect of the carbon dioxide (CO<sub>2</sub>) concentration in gas water solvent on the polyphenols extraction efficiency introduced by ultrasound

# IV.2 Article IV Correlations between disintegration degree of fruit skin cells induced by ultrasound and efficiency of bio-compounds extraction Summary

In the first part of this chapter (details are presented in article IV Correlations between disintegration degree of fruit skin cells induced by ultrasound and efficiency of biocompounds extraction), cavitation phenomenon, the most important impact to increase extraction of valuable components by US treatment, was analyzed, include determination of cell wall disintegration index,  $Z_m$ , different extraction indexes (ionic content,  $Z_i$ , total polyphenols,  $Z_p$ ), and discussion of their correlations of different fruit peels (apple, persimmon, and banana). The total time of aqueous extraction was up to  $t_e=0-2700$  s and the US specific energy inputs, W, were in the interval between 0.033 and 0.299 kW.h/kg. The ionic, Z<sub>i</sub>, and total polyphenol, Z<sub>p</sub>, extraction indexes of the liquid extracts from different fruit peels were analyzed. The  $Z_m$  from microscopic images was calculated. The observed different behavior of Z<sub>i</sub>, Z<sub>p</sub>, and Z<sub>m</sub> indexes reflected different effects of cavitation phenomenon produced by US on damage cell membranes (so-called sonoporation) and rupture of cell walls. In extraction of ionic components the nearly linear  $Z_i(Z_m)$  dependencies were observed for all fruit peels with level of extraction grow in the row apple < persimmon < banana. In extraction of polyphenols the similar nearly linear  $Z_p$ ,  $(Z_m)$  dependencies were only observed for bananas and persimmons, but for apples this dependence was non-linear. Moreover, the order in a row of the level of extraction was depended upon the value of  $Z_m$ . The obtained data presented that the increase of US energy input caused the increase of values of  $Z_i$ ,  $Z_p$  and  $Z_m$  and the extraction efficiencies ( $Z_i$  and  $Z_p$ ) were related with  $Z_m$ .

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# Correlations between disintegration degree of fruit skin cells induced by ultrasound and efficiency of bio-

# compounds extraction

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# Abstract

The ultrasound (US) assisted extraction of bio-compounds from different fruit skins (apples, bananas and persimmons) was studied. The aqueous suspensions of skins were treated by US with different energy inputs (0.033-0.299 kW·h/kg) and total time of aqueous extraction was up to 2700 s. The ionic,  $Z_i$ , and total polyphenol,  $Z_p$ , extraction indexes of the liquid extracts were analyzed. From microscopic images the cell wall disintegration index,  $Z_m$ , was determined. Increase in US energy input caused the increase of values of  $Z_i$ ,  $Z_p$  and  $Z_m$ . The correlations between extraction parameters and the disintegration index,  $Z_m$ , were discussed.

## **Keywords:**

Ultrasound-assisted extraction; Ionics, Polyphenols; Cell wall damage

Nomenclature			
$C_p$	specific heat capacity of water, kJ/(kg°C <sup>-1</sup> )		
d	damage degree		
$m_t$	total mass of sample, kg		
n	number of ultrasonic pulses		
$N_d$	number of damaged cells		
N <sub>t</sub>	total number of cells		
Р	total polyphenol content, mg/100 g DM		
t <sub>e</sub>	total duration of extraction, s		
$t_u$	time of each US pulse, s		
$t_w$	pause time between two US pulses, s		
$\Delta T$	temperature elevation, °C		
W <sub>US</sub>	US energy input, kW·h/kg		
$Z_i$	ionic extraction index		
$Z_m$	cell wall disintegration index		
$Z_p$	total polyphenol index		
σ	electrical conductivity, mS/cm		
Abbreviations			
CLSM	confocal laser scanning microscopy		
DM	dry matter		
FM	fresh matter		
FT	frozen-thawed		
GAE	gallic acid equivalent		
SEM	scanning electron microscopy		
U	untreated		
US	ultrasound		
#### **1** Introduction

Apples, bananas and persimmons are the first, second and fifth most widely produced and consumed fruits in the world [1–3]. These three kinds of fruits are rich in sugar, microelements and phenolic compounds, including catechin, epicatechin, rutin, phloridzin, gallic acid, chlorogenic acid, tannic acid, gentisic acid, protocatechuic acid (see Supplementary materials, S1) [4,5], and they have been recognized as an important and inexpensive source of polyphenols [6]. The texture of fruit skins is mainly defined by cell wall materials, pectin content and properties of microfibrils. Particularly, microfibril thickness can affect the mechanical strength of cell walls [7]. For example the apple cell walls can include 40% pectin, 20% cellulose, and 25% hemicelluloses (the dominant hemicellulose in apple parenchyma is xyloglucan) [8].

Fruit skins contain more significant amounts of phenolic compounds than fruit flesh and fruit juice [9]. They play an important role in maintaining human health, since they have a preventive effect against various types of diseases such as coronary heart disease, cancer, neurodegenerative diseases, gastrointestinal disorders and others diseases [10–12]. Nowadays the extraction of phenolic compounds from fruit skins has attracted great attention [13], and can be realized by conventional and alternative methods using enzymes, pressurized liquids, ultrasound, microwave, pulsed electric fields, supercritical fluids and other treatments [14–20].

Ultrasound (US) has been widely used for polyphenols extraction from different food materials (flesh, skin and pomace) [21–27]. The US induces cavitation, shockwaves and mixing phenomena leading to increasing solid-liquid extraction yield [28–30]. Bubbles explosion and shockwaves in solid/liquid mixtures leads to fragmentation of biological particles, cell structure damage, cell membrane sonoporation (Chemat et al., 2017). Particles fragmentation and cells destruction by collapsing cavitation bubbles increase effective surface area for solutes extraction.

Cellular particles damage induced by ultrasound has been widely studied using scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) [31–35]. For example, SEM was used to analyze the cell damage of an Opuntia skin matrix [36] and berry skins [37], and CLSM was used for detection of cell wall damage of a fried potato chip [38], etc.

This work is focused on the analysis of correlations between disintegration degree of fruit skin cells induced by US and efficiency of bio-compounds extraction from apple, banana

and persimmon skins. The analyses include determination of different extraction indexes (ionic content, total polyphenols), cell wall disintegration index and discussion of their correlations.

#### 2 Materials and methods

#### 2.1 Material

Commercial red apples (Gala), bananas (Cavendish) and persimmons (Ribera del Xùquer) were selected as the raw materials for this investigation. The samples of good and uniform quality (with near-spherical shape for apples and persimmons and near the same size for banana) were purchased at a local supermarket (Compiegne, France) and stored at 4 °C. All experimental data were collected within 10 days of the purchase of the samples. The skins (thin slices with lengths of 20 mm and widths of 10 mm) were removed from the bananas by hand and from the apples and persimmons with a razor blade. For preparation of the freeze-thawed products the samples were frozen at -20 °C for 12 hours and then thawed at room temperature ( $\approx$ 23 °C) for 12 hours. The moisture content was determined by drying at elevated temperature at 105 °C to a constant weight using MA 160 infrared moisture analyzer (Sartorius, Göttingen, Germany) and it was (on wet basis) 84%, 89% and 76% for apple, banana and persimmon fresh skins, respectively.

#### 2.2 Extraction experiments

The US-assisted and control extraction experiments with untreated (U) and frozenthawed (FT) skins were performed. Hereafter these experiments are denote as US, U and FT experiments. In all extraction experiments, the fruit skins (0.015 kg) were put into a glass beaker filled with distilled water (0.15 kg), i.e., the solid/solution ratio was 1:10. Extraction from U and FT samples was done at the room temperature  $T=23\pm3$  °C.

The US treatment was done directly in the glass beaker using an ultrasonic processor UP 400S (400 W, 24 kHz, Hielscher GmbH, Stuttgart, Germany). The titanium ultrasonic probe (H14, Hielscher GmbH, Stuttgart, Germany) with a tip diameter of 14 mm, and a length of 100 mm was used. The US treatment was done in pulsed mode with sequential application of n (=1-9) ultrasonic pulses [19]. After each US pulse with duration of  $\Delta t_u$ =180 s a pause with duration of  $\Delta t_u$ =120 s was applied. In order to avoid a temperature elevation during the application of US, the glass beaker was put in an ice/cold water box. The temperature of extraction was nearly room, T=23±3 °C.

In preliminary experiment with temperature elevation control the US power was determined as:

 $P = m_t C_p \Delta T / \Delta t_u = 0.108 \text{ kW}.$ 

(1)

Here  $C_p$  (=4.18 kJ/(kg°C<sup>-1</sup>)) is the specific heat capacity of water,  $m_t$  (165 g=150 g of water +15 g of skin) is the total mass of a sample,  $\Delta T$  is the temperature elevation.

The corresponding US energy input was calculated as  $W_{US}=(P/m_t)n\Delta t_u/3.6\cdot10^3=0.033n$  (kWh/kg). The maximum US energy input at n=9 was  $W_{US}=0.299$  kW·h/kg. The total duration of extraction experiments was up to  $t_e=2700$  s and they were done at a room temperature, T=23 °C.

#### 2.3 Analyses

The analyses include determination the extraction indexes (ionic content and total polyphenols) and cell disintegration index.

#### 2.3.1 Extraction indexes

The electrical conductivity,  $\sigma$ , and total polyphenol content, P, of extracts were measured during extraction experiments. The value of  $\sigma$  was measured using a conductometer (PCE-PHD 1, PCE Instruments France EURL, France). The value of P was determined using the Folin–Ciocalteu method based on a colorimetric oxidation/reduction reaction of phenols [39]. 0.2 mL of diluted extract and 1 mL of Folin–Ciocalteu reagent (Merck, Darmstadt, Germany) (diluted 1:10 with distilled water) were mixed. 0.8 mL of Na<sub>2</sub>CO<sub>3</sub> (75 g/L) (Prolabo, Fontenay-sous-Bois, France) was then added. The sample was incubated for 10 min at 50 °C and then cooled for 10 min at room temperature. For the control sample, 0.2 mL of distilled water was taken. The absorbance, A, was measured at 750 nm using a UV/Vis instrument (Thermo Spectronic Genesys 20, Thermo Electron Corporation, MA, USA). Gallic acid (Sigma-Aldrich, St-Quentin Fallavier, France) was used for the calibration curve. The results were expressed as mg of Gallic acid equivalent (GAE) per gram of 100 g dry matter samples (mg GAE/100g DM).

The ionic,  $Z_i$ ,[17,40], and total polyphenol,  $Z_p$ , extraction indexes respectively, were defined as

$$Z_{i} = \frac{\sigma_{US} - \sigma_{U}}{\sigma_{FT} - \sigma_{U}},\tag{3a}$$

$$Z_{p} = \frac{P_{US} - P_{U}}{P_{FT} - P_{U}}.$$
 (3b)

Here, lower case symbols correspond to U, US and FT experiments.

Figure 1 presents examples of temporal changes in the electrical conductivity,  $\sigma$ , (a) and total polyphenols content, P, (b) in extraction experiments for apples skins U, US, and FT. Remarkable that during extraction increase of  $\sigma$  and P was observed for all samples and was maximum for frozen-thawed skins. These skins were damaged by FT before starting extraction. It evidenced the long-term nature of extraction processes of bio-compounds from skins.

#### 2.3.2 Cell wall disintegration index

The cell wall disintegration was analyzed using the confocal laser scanning microscopy (CLSM) analysis performed with a ZEISS LSM 710 (ZEISS, Oberkochen, Germany). A single line excitation and multiple channel emission technique were used. For visualization of multispectral image data, the different channels were associated with a colors value on the display system. The RGB-color mode with three primary display colors (red, green and blue) was used. UV excitation was used with laser line at 405 and 488/543 nm, the fluorescence channel was given by the band pass filter (BP) 410–495 nm (blue), BP 495–564 nm (green) and longpass filter (LP) 563-685 nm (red).

The laser-induced fluorescence images from skin samples were taken with a cooled, integrating CCD camera (AT 200) mounted on a ZEISS LSM 710 microscope with an HBO 50 excitation lamp (Zeiss, Oberkochen, Germany). Samples were detected under identical conditions and with identical buffer solutions. Single x-y images as well as a series of x-z images were taken from all samples (image size x\*y\*z was 212.55  $\mu$ m\*212.55  $\mu$ m\*13.63  $\mu$ m), and the magnification times of images were 40.

Figure 2 presents examples of cell images for samples obtained after U (a) and US (b) extraction experiments for studied fruit skins. The data are presented for the maximum extraction time of  $t_e$ =2700 s. It corresponds to the US energy input,  $W_{US}$ =0.299 kW·h/kg in the US experiments. Cell walls were practically undamaged in skins obtained after U experiments (Fig.2a), whereas in skins obtained after US experiments the spaces between cells became larger (apple), and many cells became connected together (banana and persimmon) (Fig. 2b). Preliminary US extraction experiments with different US energy input (n=0-9,  $W_{US}$ =0-0.299 kW·h/kg) have shown that with a higher US energy input, the cell membranes and cell walls are damaged more severely, and more cells are connected to each other, and the spaces between cells become larger.

Processing of the CLSM images and calculation cell damage degree were done using the wholly automatic procedure supported with Matlab (Version 2011a, the Mathworks Inc., Mass, USA). Figure 3 shows the scheme applied for the processing and calculation of cell damage degree.

The following 5 steps were applied:

 Pre-processing. For presentation of the cell walls the RGB image was transformed into the blue image. To reduce possible noise, the median-filtering technique (a special case of filter called rank-statistic filter), which allowed the edges to be preserved while filtering out the peak noise, was used [41].

2) Segmentation. To remove background, the blue image was transformed into the gray level image and then to the binary image (called bi-level images, BW). The Otsu algorithm for edge detection is used and the threshold value was determined with using Sobel filter [42]. This filter is used in image processing for creation image emphasizing edges. It is based on the gradient approximation and convolving the image with a small, separable, and integer-valued filter.

3) Inversion and holes filling. The BW image was inverted, white became black, and black became white. Now, the black and white present cell walls and cell bodies, respectively. Then hole filling using a flood filling operation was applied [43]. The flood filling operation is performed recursively on all elements connected to the node of interest.

4) The edges of cell image were smoothed by eroding the image with opening and closing algorithms. Opening is erosion followed by dilation, closing is a dilation followed by erosion [44].

5) Corresponding pixels of each cells were found based on eight nearly label algorithm [45,46]. In this algorithm, the label of a pixel is influenced by the labels of its eight nearest neighboring pixels.

6) Calculation of cell numbers and determination of damage degree. To calculate the total number of cells,  $N_t$ , all the cells including the cells with incomplete edges (damaged cell walls) were labeled. The number of pixels in every labeled cell and the average number of pixels in all labeled cell were calculated. To calculate the number of damaged cells,  $N_d$ , the cells with number of pixels greater than the average value were identified. The damage degree was evaluated as  $d=N_d/N_t$ .

Finally, the cell wall disintegration index was calculated as

$$Z_m = \frac{d_{US} - d_U}{d_{FT} - d_U}.$$
(4)

Here, lower case symbols correspond to US, U and FT experiments.

Figure 4 presents example of temporal changes of the cell wall damage degree, d, in US, U and FT extraction experiments with apple skins. The damage degree, d, increased with increasing of extraction time,  $t_e$ , in U and US experiments, whereas in FT experiments the damage degree was approximately 0.91.

#### 2.4 Statistical analysis

All experiments and measurements of characteristics were repeated at least in 3-5 times. The mean values and the standard deviations were calculated. The error bars in figures correspond to the standard deviations.

### 3. Results and discussions

Figure 5 shows the temporal changes of ionic,  $Z_i$ , (a), and total polyphenol,  $Z_p$ , (b) extraction indexes, and cell wall disintegration index,  $Z_m$ , (c) for apple, banana and persimmon skins treated by US. The different tendencies in extraction kinetics of different components were observed. For examples, at long extraction time ( $t_e \approx 2700$  s), the ionic extraction index,  $Z_i$ , (Fig. 5a) nearly saturated at the levels of  $\approx 0.31$  and  $\approx 0.46$  for apple and persimmon skins, respectively, whereas the highest value of  $Z_i$  was observed for banana skins and it continuously increased even at  $t_e > 2700$  s. For banana skins the highest value of  $Z_p$  was also observed. The total polyphenol extraction index,  $Z_p$ , (Fig. 5b) nearly saturated at the levels of  $\approx 0.76$  and 0.62 for banana and persimmon skins, respectively. However, for the apple skins value of  $Z_p$  continuously increased even at  $t_e > 2700$  s.

For all studied skins the cell wall disintegration indexes,  $Z_m$ , continuously increased even at  $t_e>2700$  s and the highest disintegration was also observed for banana skins. The USinduced cell wall disintegration accompanied with intensive disintegration of skin tissues. The observed differences in behavior for different skins can reflect different effects of US on damage of skin tissues. Studies of the particle size distributions in liquid extracts revealed strong effects of US treatment on overall disintegration and fragmentation of skins (see Supplementary materials, S2 (fig. S1)).

The observed different behavior of  $Z_i$ ,  $Z_p$ , and  $Z_m$  indexes reflected different effects of US on disintegration of cell membranes (so-called sonoporation) and cell walls. The mechanisms of US action includes effects of erosion, fragmentation or induction of the US capillary

effects [24,28,47]. The efficiency of release of small molecules (e.g., ionic compounds) can be only determined by the damage of membranes, whereas the efficient release of polyphenols and other biocompounds with higher molecular weight or lower solubility in water [48] could also require high level of damage of cell walls.

Figure 6 presents the ionic,  $Z_{i}$ ,(a) total polyphenol,  $Z_{p}$ , (b), extraction indexes versus the cell wall disintegration index,  $Z_{m}$ , for skins of apples, bananas and persimmons. In extraction of ionic components the nearly linear  $Z_{i}$ ,( $Z_{m}$ ) dependencies were observed for all skins with level of extraction grow in the row applepersimmon<br/>banana (Fig. 6a). The observed behavior evidenced that US treatment resulted in damage of both the membranes and cell walls.

In extraction of polyphenols the similar near linear  $Z_i$ ,  $(Z_m)$  dependencies were only observed for bananas and persimmons. However, for apple skin this dependence was non linear (Fig. 6b). At small values of  $Z_m$  (<0.05) the level of extraction grow in a row apple<persimmon<br/>banana (Fig. 6a) and at higher cell disintegration the other row was observed: persimmon< apple<br/>banana. Overall, among the studied skins, the maximum levels of  $Z_i$ ,  $Z_p$ , and  $Z_m$  were observed for the banana skins. It may be explained by rather low skin hardness for the banana (for the studied samples the hardness grow in the row banana<apple<persimmon, see Supplementary materials, S3). The differences between apple and persimmon skins cannot be explained by skin hardness. Our hypothesis is that these effects reflect different localization and binding of polyphenols, their solubilization in cell vacuoles [49] as well as different fragmentation ability of skins under the influence of US treatment. Moreover, US treatment affected the thickness of skins, and effects of US treatment decreased in the row persimmon> banana> apple. This behavior may deflect the different effects of US on swelling and shrinkage ability of skins.

#### 4 Conclusions and final remarks

Effects of ultrasound treatment on extraction of bio-compounds (ionic,  $Z_i$ , and total polyphenol,  $Z_p$ , indexes in liquid extracts) and cell wall disintegration index,  $Z_m$ , for three fruit skins (apple, banana and persimmon) were evaluated. The total time of aqueous extraction was up to  $t_e$ =0-2700 s and the US specific energy inputs were in the interval between 0.033 and 0.299 kW h/kg. The observed different behavior of  $Z_i$ ,  $Z_p$ , and  $Z_m$  indexes reflected different effects of US on damage cell membranes (so-called sonoporation) and rupture of cell walls. Correlations between extraction efficiencies and cell wall disintegration degree were analyzed. In extraction of ionic components the nearly linear  $Z_i$ , ( $Z_m$ )

dependencies were observed for all skins with level of extraction grow in the row applepersimmon<br/>banana. In extraction of polyphenols the similar nearly linear  $Z_i$ , ( $Z_m$ )<br/>dependencies were only observed for bananas and persimmons, but for apple this dependence<br/>was non linear (Fig. 6b). Moreover, the order in row of the level of extraction was depended<br/>upon the value of  $Z_m$ . The maximum obtained values of  $Z_m$  were 0.087, 0.113, 0.155, for the<br/>apple, banana and persimmon skins, respectively. The highest extraction indexes  $Z_i$ ,  $Z_p$ , were<br/>observed for the banana skins that can be explained by the lowest hardness of this skin.

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#### **Figure captions**

Fig. 1. Examples of temporal changes of the electrical conductivity,  $\sigma$ , (a) and total polyphenols content, *P*, (b) in U, US and FT extraction experiments for apple skins.

Fig. 2. Examples of images for samples obtained after U (a) and US (b) extraction experiments for studied fruit skins (apple, banana, persimmons). The extraction time was  $t_e=2700$  s and in US experiment the energy input was  $W_{US}=0.299$  kW·h/kg.

Fig. 3. Scheme applied for processing of the images.

Fig. 4. Examples of temporal changes of cell wall damage degree, d, in US, U and FT extraction experiments for apple skins.

Fig. 5. Temporal changes of ionic,  $Z_i$ , (a), and total polyphenol,  $Z_p$ , (b) extraction indexes, and cell wall disintegration index,  $Z_m$ , (c) for skins of apple, banana and persimmon treated by US.

Fig. 6. Correlations between the ionic,  $Z_i$ , total polyphenol,  $Z_p$ , (b), and the cell wall disintegration index,  $Z_m$ , for skins of apples, bananas and persimmons.





# 1 Supplementary materials

2

# 3 S1 Main polyphenols in apple, banana and persimmon skins

- 4 The main polyphenols in apple, banana and persimmon skins are presented in Table 1.
- 5 Table S1. Main polyphenols in apple, banana and persimmon skins.

Apple skin			
	Content	Solubility in water or in	
Main polyphenols	mg/g DM	ethanol, mg/mL	Reference
(+)-catechin	0.99-4.42	ethanol: 50 mg/mL	
(-)-epicatechin	1.24-5.75	water: 34	
rutin	2.76-11.4	water: 125000	
phloridzin	0.71-2.42	water: 1	
chlorogenic acid	0.26-2.33	water: 40	[1]
	19.4±1.4		
gallic acid	mg/100g FM*	water: 11.9	[2]
Danana akin			
tonnia agid	2 22 28 14		
	2.25-58.14	water: 2860	
pyrocatecnol	3.10-23.11	water: 401	
gentisic acid	6./1-51.36	water: 22	
(+)-catechin	5.46-17.53	ethanol: 50 mg/mL	
protocatechnic acid	32.6-42.17	water: 18.2	
gallic acid	29.97-40.57	water: 11.9	
· · · · · ·		cold water: Sparingly	
caffeic acid	6.72-50.07	hot water: Freely soluble	
ferulic acid	6.51-16.88	water: 5.97	
cinnamic acid	7.03-12.97	water: 0.546	[3]
Persimmon skin			
epicatechin	$1.7{\pm}0.1$	water: 34	
ferulic acid	13.2±1.3	water: 0.546	
gallic acid	27.2±2.1	water: 11.9	
protocatechuic acid	$8.3 \pm 0.8$	water: 18.2	
vanillic acid	$0.7{\pm}0.08$	water: 1.5	
p-coumaric acid	82.6±7.8	ethanol: 50 mg/mL	[2]
*FM-fresh matter		-	

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#### 9 S2 Particle size distributions in liquid extracts

- 10 The particle size distributions in liquid extracts were analyzed using light scattering
- 11 method with Malvern Mastersizer X (Malvern Instruments S.A., France). The measurements
- 12 were done using the 45 mm lens and the optical pathway of 14.3 mm.



Fig.S1. Examples of the particle size distribution (below 2000  $\mu$ m) in the liquid extracts after U ( $t_e$ =2700 s) and US ( $t_e$ =300 and 2700 s) extraction experiments for apple (a), banana (b) and persimmon skins (c).

Fig. S1 presents the examples of the particle size distribution in the liquid extracts after 13 14 U ( $t_e$ =2700 s) and US ( $t_e$ =300 and 2700 s) extraction experiments for apple (a), banana (b) and persimmon skins (c). In absence of US treatment, the particle size distribution for the 15 apple skin consists of two maximums located at 138 µm and 550 µm. For banana and 16 persimmon skins the single maxima at 832 µm and 275 µm were observed, respectively. For 17 US ( $t_e$ =300 and 2700 s) extraction experiments the location of maxima for apple and banana 18 shifted to the smaller values of particle sizes. However, for persimmon skin the shifts of 19 maxima were unessential whereas US treatment affected the shape of the particle size 20 21 distributions. So, the US treatment can significantly influence the particle size distribution that reflect the damage of skin tissues induced by US. 22

#### 23 S3 Hardness tests for fresh skins

24 The hardness tests for fresh skins were performed in a Texture Analyser (model TA-XT

25 plus, Rhéo, Champlan, France). The samples were placed on the platform of the texture

analyzer and force deformation tests were carried out. The load force, F, was detected at deformation of 0.5 mm for all samples.



Fig. S2. Load force for fresh skins of apples, bananas and persimmons at deformation of 0.5 mm.

Fig. S2 presents the load force for fresh skin samples of apple, banana and persimmon at fixed deformation of 0.5 mm. Banana skin requires the smallest amount of force ( $F\approx10$ N). Apple skin and persimmon skin need a larger load force ( $F\approx20$  N and 25 N, respectively). Therefore, the hardness of the studied fruit skins decreases in the row persimmon> apple>banana. We assume that softer skin can be easier damaged during US treatment. Data presented in Fig. S1 evidenced that for softer banana and apple skins the US treatment more significantly decreased the particle size as compared to the stronger persimmon skin.

# 35 S4 Thickness of skins

The thickness of skins,  $\delta$ , was estimated using the scanning electron microscopy images (SEM) (Quanta FEG 250, Thermo Fisher Scientific, MA USA) at magnification of 50. The high voltage and mode of SEM was 20 kV and SE, respectively. The length and width of the extraction region for all samples was 5 mm and 3.5 mm, respectively.

- 40 The skins were fixed between two iron plates. For thinner samples, the edges of SEM
- 41 images were visually brighter because of the iron light reflection.



Fig.S3. Examples of SEM images for samples obtained after US extraction experiments for persimmon skin. The extraction time was te=0 and 2700s (US energy input was WUS=0 and and 0.299 kW·h/kg, respectively).

42 Fig. S3 presents examples of the SEM images for samples obtained after U (a) and US (b)

43 extraction experiments ( $t_e=0$  and 2700s,  $W_{US}=0$  and 0.299 kW h/kg) for persimmon skin.



Fig.S4. Effect of the ultrasound energy input on the relative thickness of skin samples,  $\delta/\delta_U$ , for studied fruit skin (apple, banana and persimmon). Here  $\delta_U$  is the thickness for U experiments.

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Fig.S4 presents the relative thickness of skins, δ/δ<sub>U</sub>, (here δ<sub>U</sub> is the thickness for U
experiments) versus ultrasound energy inputs (W<sub>US</sub>=0, 0.100 and 0.299 kW·h/kg) for studied
skins. For all skins, the values of δ/δ<sub>U</sub> decreased with an increase of W<sub>US</sub>. The effects of US
treatment decreased in the row persimmon> banana> apple.
Application of US treatment resulted in significant decreasing of the thickness of skins, δ.

The similar behavior was also observed for apple and banana skins.Surprisingly, the largest effect was observed for the persimmon skin with the strongest hardness of skin. This behavior may deflect the different effects of US on swelling and shrinkage ability of skins.

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# IV.3 Article V Effect of CO<sub>2</sub> concentration in gas water solvent on the polyphenols extraction from apple skins by ultrasound

# Summary

Cavitation bubbles generated by ultrasound are formed from gas dissolved in the liquid (Ashokkumar, 2011). Dissolved gas in the solvents will influence the efficiency of UAE by affecting cavitation phenomenon (Leong et al., 2011). Gases dissolve into the solvent can act as nuclei for a new cavitation bubble (Mason and Lorimer, 2002). When bubble size reaches a critical value, they will collapse near and onto the surface of a solid material result in fragmentation of friable materials, localized erosion, overall enhanced reactivity in the solvent and an increased mass transfer (Suslick and Price, 1999). Therefore, in the second part of this chapter (details are presented in article V *Effect of CO<sub>2</sub> concentration in gas water solvent on the polyphenols extraction from apple peels by ultrasound*), the effect of the carbon dioxide (CO<sub>2</sub>) concentration in gas water solvent on the polyphenols extraction gas water solvent on the polyphenols extraction from apple peels by ultrasound).

The apple peels were extracted with gas water solvents at different CO<sub>2</sub> concentration (0-7.05 mmol/L) compared to different conventional solvents (distilled water, 10% ethanol aqueous solvent and 10% methanol aqueous solvent) without and with ultrasound treatment (W=0.242 kW•h/kg) at room temperature. The extraction of TPC and the water holding capacity of apple peels extracted in different solvents with and without ultrasound treatment were compared. The TPC, TFC, PAC, pH, electrical conductivity ( $\sigma$ ) and antioxidant activity (DPPH) of extracts with gas water solvents at different concentration of CO<sub>2</sub> by ultrasound were detected. The extracted TPC and water holding capacity of apple peels both had a significant increase by ultrasound for all solvents, especially for gas water solvent. Since the cavitation phenomenon generated by ultrasound could increase the water absorption of the sample and the increase of water adsorption could subsequently contribute to the increase of extracted TPC. The CO<sub>2</sub> concentration in gas water solvent at 5.28 mmol/L was optimal for polyphenols (TPC, TFC, and PAC) extraction enhancement and antioxidant activity (DPPH) increase by ultrasound. Meanwhile, the pH and  $\sigma$  of liquid extracts increased with the increase of  $CO_2$  concentration in gas water solvents. These results adequately explained that the  $CO_2$ concentration in gas water solvents could influence polyphenols extraction of samples by ultrasound treatment, due to CO<sub>2</sub> gases dissolve into the solvent could act as nuclei for producing cavitation bubble by ultrasound, and the produced cavitation effect could finally increase polyphenol extraction of samples.

1	Effect of CO <sub>2</sub> concentration in gas water solvent on the
2	polyphenols extraction from apple skins enhanced by
3	ultrasound
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#### 19 Abstract

20 The effect of the carbon dioxide (CO<sub>2</sub>) concentration in gas water solvent on the 21 polyphenols extraction efficiency introduced by ultrasound was studied. The apple skins were extracted with gas water solvents at different CO<sub>2</sub> concentration (0-7.05 mmol/L) compared 22 23 to with different conventional solvents (distilled water, 10% ethanol aqueous solvent and 24 10% methanol aqueous solvent) without and with ultrasound treatment (W=0.242 kW•h/kg) 25 at room temperature. The extraction total polyphneol content (TPC) and the water holding capacity of apple skins extracted in different solvents with and without ultrasound treatment 26 27 were compared. The TPC, total flavonoid content (TFC), and proanthocyanidins content 28 (PAC), pH, electrical conductivity ( $\sigma$ ) and antioxidant activity (DPPH) of extracts with gas 29 water solvents at different concentration of CO<sub>2</sub> by ultrasound were detected. The extraction TPC and water holding capacity of apple skins both had a significant increase by ultrasound 30 31 for all solvents, especially was almost 1.6 and 2.4 times higher than the untreated samples for 32 gas water solvent (7.05 mmol/L CO2) respectively. The CO2 concentration in gas water 33 solvent at 5.28 mmol/L was optimal for polyphenols (TPC, TFC, and PAC) extraction enhancement and antioxidant activity (DPPH) increase by ultrasound. Therefore the gas 34 35 water solvent was effective for polyphenols extraction from apple skins by ultrasound.

36 Keywords: Ultrasound; Gas water solvent; CO<sub>2</sub> concentration; Polyphenols
 37 extraction; Antioxidant activity

38

#### 39 Introduction

40 Apple skin is the main solid part of waste products resulting from industrial 41 processing of apple juice or cider production [1]. Apple skin contains different polyphenols (chlorogenic acid, epicatechin, procyanidin B2, phloretin, and quercetin) and vitamin C [2,3]. 42 Apple skin is more reach in polyphenols than apple flesh. Many polyphenols have shown 43 44 strong antioxidant properties as oxygen scavengers, peroxide decomposers, and free radical 45 inhibitors [4,5]. Nowadays the extraction of polyphenols from apple skins attracts a great attention [6-8], as apple skin is considered as one of potential source of food antioxidants [9]. 46 47 In the past decades, the polyphenols extraction from apple was improved using microwave, pressurized liquids, ultrasound, pulsed electric fields, supercritical fluids [10-14]. 48 49 Particularly, ultrasound-assisted polyphenols extraction (UAE) from apple skins has been 50 widely investigated [15-17]. Acoustic cavitation produced by ultrasound was beneficial to 51 increase extraction efficiency due to the formation, growth and collapse of micro bubbles 52 inside a liquid phase, which could cause the rupture of cell walls, consequently enhance 53 solvent contact with available extractable cell material and accelerate diffusion [18-20].

54 For efficient recovery of polyphenols from apple with organic solvent (ethanol, 55 methanol, acetone) and their aqueous solutions were commonly used [21]. Nowadays, 56 combination of organic solvents and ultrasound-assisted polyphenols extraction from apple 57 skins has been reported. At optimum extraction conditions (ethanol concentration of 50%, temperature 50 °C, time of 30 min, ultrasonic power of  $\approx$  520 W), the maximum total 58 59 polyphenols content (TPC) value of  $13.26 \pm 0.56$  mg GAE/g from the unripe apple could obtain [22]. In addition, the yields of extraction polyphenols from apple pomace using 60 61 ultrasound (25 kHz, 150 W) and ethanol/aqueous solution (v/v=50%) increased more than 62 20% compared with conventional extraction [23]. Similar results were obtained for UAE optimization using ethanol 56% at 80 °C for 31 min or acetone 65% at 25 °C for 60 min, 63 respectively [24]. However, organic solvents extraction is not selective, and would result in 64 dangers by ultrasound, decrease of purity of extracts and it requires supplementary separation 65 procedures [25]. Since much safer and more effective solvents are essential to instead of 66 67 organic solvents for UAE.

Cavitation bubbles produced during ultrasound treatment are formed from gas
dissolved in the liquid [26]. Since dissolved gas in the solvents will influence the efficiency
of UAE by affecting cavitation phenomenon [27]. Gases dissolve into the solvent can act as

nuclei for a new cavitation bubble [19]. When bubble size reaches a critical value, they will collapse near and onto the surface of a solid material result in fragmentation of friable materials, localized erosion, overall enhanced reactivity in the solvent and an increased mass transfer [28]. Therefore we assume gas water solvents will enhance UAE. However, the gas water solvent and the impact of carbon dioxide (CO<sub>2</sub>) concentration in gas water solvent on the polyphenols extraction from apple skins by ultrasound was not yet elucidated.

77 This work is focused on the effect of CO<sub>2</sub> concentration in gas water solvent on the 78 polyphenols extraction from apple skins enhanced by ultrasound. The TPC of extracts and 79 water holding capacity of apple skins extracted in different solvents (distilled water, 10% 80 ethanol aqueous solvent, 10% methanol aqueous solvent, and gas water solvent with 7.05 81 mmol/L CO<sub>2</sub>) with and without ultrasound treatment were compared. The TPC, total 82 flavonoid content (TFC), and proanthocyanidins content (PAC), pH, electrical conductivity  $(\sigma)$  and antioxidant activity (DPPH) of extracts with gas water solvent at different 83 concentration of CO<sub>2</sub> (0-7.05 mmol/L) by ultrasound were detected. 84

#### 85 2. Materials and methods

## 86 2.1. Materials

Commercial red apples (Gala), and bottled gas water (Perrier) were selected as the 87 raw materials for investigation. Apples of good and uniform quality with near-spherical shape, 88 89 and bottles of Perrier (50 cl) were purchased from a local supermarket (Compiegne, France). 90 Apples and Perrier water were stored at 4 °C and room temperature respectively for further 91 experiments. The initial moisture content on wet basis for fresh apple skin was 84.34% 92 determined with MA 160 infrared moisture analyzer (Sartorius, Göttingen, Germany). The 93 concentration of carbon dioxide, CO2, in bottles of Perrier is 7.05 mmol/L since the concentration of bicarbonate in bottles of Perrier is 390 mg/L. 94

#### 95 2.2. Chemicals and reagents

Folin–Ciocalteu reagent, gallic acid, trolox, DPPH (2,2-diphenyl-1-picrylhydrazyl),
sodium nitrite, and catechin/quercetin 3-glucoside standard were purchased from SigmaAldrich (Saint-Quentin Fallavier, France). Sodium carbonate was obtained from VWR
(France). Vanillin and aluminium chloride were purchased from Acros Organics (Belgium).
Ethanol, methanol, hydrochloric acid (37%, 1M), and sodium hydroxide (1M) were
purchased from Fisher Scientific (France).

#### 102 2.3. Ultrasound experiments

103 The slice of apple skins with length and width of 20 mm and 10 mm, respectively, 104 was removed by a razor blade. Skin tissues (15 g) were mixed with 150 mL solvent (distilled 105 water, 10% ethanol aqueous solvent, 10% methanol aqueous solvent and gas water solvent 106 with different CO<sub>2</sub> concentration (0, 1.76, 3.53, 5.28, 7.05 mmol/L)) in a glass beaker. The 107 concentration of ethanol and methanol in solvents was selected as 10% in order to reach same density of solvents,  $\rho ~(\approx 0.979 \text{ kg/m}^3)$  as the gas water solvent with 7.05 mmol/L CO<sub>2</sub>. The 108 experiments were all performed at room temperature (~23 °C) and the solid solvent ratio was 109 110 1:10. Ultrasound treatment was done directly in the solvents with an ultrasonic processor UP 400S (400 W, 24 kHz, Hielscher GmbH, Stuttgart, Germany). The titanium ultrasonic probe 111 112 (H14, Hielscher GmbH, Stuttgart, Germany) with a tip diameter and length of 14 mm and 100 mm, respectively, was used. In order to avoid the temperature elevation produced by 113 114 ultrasonic pulses, the glass beaker was put in an ice/cold water box. Duration of every 115 ultrasonic pulse and duration of the pause between two pulses were 3 min and 2 min, 116 respectively, as previous research [17]. The total extraction time was 10 min without and with 117 ultrasound treatment. The total specific energy input of ultrasound, W, in ultrasound-assisted extraction was 0.242 kW•h/kg, which is determined by the following equation: 118

W = Pt /m

(1)

where P is the generator power (400 W), and t is the ultrasound treatment time (6 min=0.1 h),
m is the total mass of solution (0.165 kg).

121 After extraction in different solvents (distilled water, 10% ethanol aqueous solvent, 122 10% methanol aqueous solvent and gas water solvent with 7.5 mmol/L CO<sub>2</sub>), the TPC of the 123 liquid extracts and water holding capacity of apple skins with and without ultrasound 124 treatment were detected. For extraction in gas water solvent with different CO<sub>2</sub> concentration from 0 mmol/L to 7.05 mmol/L by ultrasound, polyphenols content (TPC, TFC, and PAC), 125 DPPH, pH and  $\sigma$  of extracts were detected. In addition the effects of the pH on phenolics 126 degradation (gallic acid, quercetin-3-O-glucoside and catechin) were analyzed with high 127 128 performance liquid chromatography (HPLC) detection.

# 129 2.4 Analysis

# 130 2.4.1 Total polyphenols content, TPC

131 Total polyphenol content (TPC) was determined using the Folin-Ciocalteu method based on a colorimetric oxidation/reduction reaction of phenols [29]. 0.2 mL of extracts 132 133 (distilled water as a blank), 1 mL of Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) (diluted 1:10) and 0.8 mL of Na<sub>2</sub>CO<sub>3</sub> (75 g/L) were added consecutively. The sample was 134 135 heated for 10 min at 50 °C and following cooled for 10 min at 4°C. The absorbance of 136 mixture was measured at 750 nm using UV/Vis instrument (Thermo Spectronic Genesys 20, 137 Thermo Electron Corporation, MA, USA). Gallic acid was used for the calibration curve. The 138 results were expressed as mg of gallic acid equivalent per 100 gram of dry matter (mg/100 g 139 DM).

## 140 2.4.2 Water holding capacity

A modified method was conducted to measure water holding capacity of apple skins [30]. The apple skins after extraction were wiped surface moisture with filter paper and subsequently dried with MA 160 infrared moisture analyzer. 1 g of the dried apple skin samples and 10 mL of distilled water was homogenised in a vortex for 1 min and left at room temperature for 24 h. Finally, the mass of residue was measured after removing supernatant with centrifugation at 1000rpm for 5 min (Z200, Hermle Labortechnik GmbH, Wehingen). The result was expressed as grams of water held by 1 g of fresh matter (g/1 g FM).

# 148 2.4.3 The optical images

149 The optical images of gas water solvents with different CO<sub>2</sub> concentration (0, 1.76, 150 3.53, 5.28, 7.05 mmol/L) before and after ultrasound treatment (W=0.242 kW•h/kg) were 151 directly observed using a reflection optical microscope (Leitz Orthoplan, Germany) at 152 magnification  $\times$  4. The microscope was coupled to a CCD camera (Sony, Japan) connected to 153 a computer. The gas water solvent was carefully got with a dropper, placed on a glass plate 154 and observed with a cold lamp. The software 'Archimed' was used to register images and 155 analyse the bubble size. In each experiment, 3 images from three different samples of one 156 solution were analysed.

# 157 <u>2.4.4 Total flavonoid content, TFC</u>

Total flavonoid content (TFC) was estimated according to the previous method [31]. 0.25 mL of extracts, 1.25 mL of distilled water and 0.75 mL of 5% NaNO<sub>2</sub> were added consecutively. After reaction for 6 min, 0.150 mL of 10% AlCl<sub>3</sub> was added into the mixture. Finally, 0.5 mL of 1 M NaOH was added and the total volume of mixture was added up to 2.5 mL with distilled water. Absorbance of mixture was measured at 510 nm. Results were expressed as mg of quercetin equivalent per 100 gram of dry matter (mg/100g DM).

# 164 2.4.5 Proanthocyanidins content, PAC

Proanthocyanidins (PAC) was determined using the vanillin assay as previous method [32]. 0.5 mL of extracts, 3 mL of 4% vanillin-methanol solution and 1.5 mL of HCl (37%) were added consecutively. Absorbance of mixture was measured after reaction for 15 min in dark at 500 nm. Results were expressed as mg of catechin equivalent per 100 gram of dry matter (mg/100g DM).

# 170 2.4.6 Antioxidant activity

171 The 2, 2-diphenyl-1-picrylhydracyl (DPPH) radical scavenging activity was used to 172 evaluate the antioxidant capacity of polyphenols [22,33]. The DPPH radical scavenging 173 activity assay was performed as previous method [34]. 0.05 mL of extracts and 1.45 mL of 174 DPPH solution (0.06 mM in methanol) were reacted for 30 min in dark at room temperature. 175 The decrease in absorbance of DPPH free radicals was read at 515 nm against methanol as a blank using a UV/Vis instrument. Trolox (0, 25, 50, 100, 250, 500 and 1000 mM/L) was used 176 177 as a standard antioxidant compound for calculation of the stand curve. The result was 178 presented in mmol of Trolox equivalents per litre (mM TE/L).

# 179 2.4.7 pH and electrical conductivity, σ

180 The pH and electrical conductivity,  $\sigma$ , (ms) of extracts and gas water solvents with 181 different concentration of CO<sub>2</sub> (0, 1.76, 3.53, 5.28, 7.05 mmol/L) were detected by a 182 multifunction pH meter (PCE-PHD 1, PCE Instruments France EURL, France).

# 183 2.4.8 The phenolic stability

- 184 The determination of phenolic compounds was performed using a Waters 717 HPLC
- 185 (Waters, France), equipped with Millenium 32 software, a degasser, a binary gradient pump,
- a Waters 717 plus thermoautosampler, a column oven, and a Waters 996 diode array detector.

187 The separation was carried out with a Aqua C18 column ( $150 \times 4.6$  mm; 0.5 µm particle size) 188 (Hypersil Gold, Torrance, CA, USA) at 30 °C. The elution gradient was performed according 189 to the previous method [35] with some modifications; the column was initially equilibrated with distilled water and acetic acid (98:2) as solvent A for x min. Polyphenols were eluted 190 with a three-stage linear gradient: from 92 to 76% of A in 20 min, from 76 to 60% of A in 10 191 192 min, and from 60 to 0% of A in 15 min with a flow rate of 1 mL/min. Acetonitrile and 193 distilled water (98:2) was used as solvent B. Absorbance of mixture was measured at 280 nm. 194 HPLC peaks were identified on chromatograms according to their retention times and their UV-visible spectra by comparison with available standard polyphenol compounds. 60 mg 195 196 galic acid, 8mg quercetin-3-O-glucoside and 30 mg catechin were dissolved in 20 mL 10% 197 acetonitrile aqueous solution at different pH (4.26, 5.47, 5.79, 5.91 and 5.93), respectively. Quantification was performed by reporting the measured integration area in the calibration 198 199 equation of the corresponding standard. Phenolics degradation was calculated with C/C<sub>0</sub>, C 200 was the phenolic content in different pH and Co is the initial content of gallic acid, quercetin-201 3-O-glucoside and catechin in 10% acetonitrile aqueous solution at pH=5.25, 3.54 and 4.17, 202 respectively (4 mg/mL, 0.4 mg/mL and 1.5 mg/mL, respectively).

## 203 2.5 Statistical analysis

All experiments and measurements of characteristics were repeated at least in triplicate. The mean values and the standard deviations were calculated. The error bars in figures corresponded to the standard deviations.

#### 207 3. Results and discussions

Fig. 1 shows the effect of the solvent nature (distilled water, 10% ethanol aqueous 208 solvent, 10% methanol aqueous solvent, and gas water solvent with 7.05 mmol/L CO<sub>2</sub>) and 209 210 ultrasound treatment on TPC. For all solvents, the extraction TPC was improved obviously by ultrasound (W=0.242 kW•h/kg). The extraction TPC with 10% ethanol aqueous solvent 211 and 10% methanol aqueous solvent were much higher than those with distilled water and gas 212 213 water solvent. For the control extraction, the extraction TPC with distilled water, 10% ethanol 214 aqueous solvent, and 10% methanol aqueous solvent were almost similar and higher than that with gas water solvent. The difference could be due to a water absorption further letting 215 solvent access and diffusion of polyphenols out of the apple skins. The solubility of 216 217 polyphenols could be higher in organic medium than in water solution, which can explain the

observed difference in TPC. Some previous studies have reported that the effectiveness of 218 219 recovery of the phenolic compounds in apples through extractions with organic solvents, such 220 as methanol, ethanol, acetone or solvents of these with water can be increased [36,37]. However for UAE, the extraction yield of TPC with gas water solvent was about 1.6 times 221 222 higher than the conventional extraction, which was more effective on enhance extraction with 223 distilled water (1.2 times higher for ultrasound-treated samples than the control samples). The results demonstrated gas water solvent was an effective solvent with assisted-extraction for 224 225 the recovery of polyphenols compounds in apple skins compared with different extraction solvents, because of dissolved CO2 in the solvent enhance cavitation phenomenon by 226 227 ultrasound [27].



Fig.1. Effect of the solvent nature and ultrasound on total polyphenols content, TPC. The ultrasound energy input, W, was 0.242 kW•h/kg. The concentration of  $CO_2$  in gas water solvent was 7.05 mmol/L. C and US correspond to conventional extraction and ultrasound-assisted extraction, respectively.

Fig. 2 compares the effect of the solvent nature (distilled water, 10% ethanol aqueous 228 solvent, 10% methanol aqueous solvent, and gas water solvent with 7.05 mmol/L CO<sub>2</sub>) and 229 230 ultrasound treatment on the water holding capacity of apple skins. Note that these results 231 were obtained after a solvent solid contact time of 10 min. There was no significant difference between the water holding capacity in the different solvents. These results show 232 that a contact time of 10 min cannot influence enough desorption and diffusion of water out 233 234 of the sample structure [38]. The water holding capacity was improved by ultrasound. The 235 maximum value (≈1.46 g /1g FM) with gas water solvent was around 2.4 times higher for 236 ultrasound-treated samples than the control samples, which was higher than that with other 237 solvents.

238This observation confirmed that water absorption was higher by ultrasound and could239contribute to explain the increase of TPC (Fig.1). The water absorption can favor solvent

access and diffusion of polyphenols out of the apple skins. The similar results of improvement of water holding capacity by ultrasound applied during meat brining or meat curing has also been shown [39–41]. The ultrasound sonocapillary effect can increase depth and velocity of penetration of liquid into cannals and pores of samples by ultrasound cavitation [42]. Therefore the water holding capacity of apple skins by ultrasound presented a significant increase, especially for extraction with gas water solvent.



Fig.2. Effect of the solvent nature and ultrasound on water holding capacity of apple skins. The ultrasound energy input, W, was 0.242 kW-h/kg. The concentration of CO<sub>2</sub> in gas water solvent was 7.05 mmol/L. C and US correspond to conventional extraction and ultrasound-assisted extraction, respectively.

Combined the results of extraction yield of TPC and water holding capacity of apple skins with different solvents (distilled water, 10% ethanol aqueous solvent, 10% methanol aqueous solvent, and gas water solvent with 7.05 mmol/L  $CO_2$ ) by ultrasound, the gas water solvent could be used instead of organic solvents on the polyphenols extraction from apple skins enhanced by ultrasound. Following the effect of  $CO_2$  concentration in gas water solvent on the polyphenols extraction from apple skins enhanced by ultrasound would be discussed.

252 Fig.3 presents the optical images of gas water solvents with different CO2 253 concentrations (0, 1.76, 3.53, 5.28, 7.05 mmol/L) before and after ultrasound treatment (W=0.242 kW • h/kg). In the absence of ultrasound, the bubble size increased with the 254 increase of CO<sub>2</sub> concentration in solvents. Immediately after the application of ultrasound, 255 the bubbles disappeared for all gas water solvents. This phenomenon maybe due to 256 application of ultrasound tended to degas a liquid [19]. Ultrasound will introduce the rapid 257 258 vibration of gas bubbles and bring these bubbles together. Then these bubbles will quickly 259 grow to a size sufficiently large to allow them to rise up through the water until to the solution surface then disappearing. 260





Fig.4. Effect of the CO<sub>2</sub> concentration in gas water solvent on the total polyphenol content (TPC), the total flavonoid content (TFC), and proanthocyanidins content (PAC), and the antioxidant activity (DPPH) by ultrasound (W=0.242 kW•h/kg).

282 Fig.5 presents the effect of the CO<sub>2</sub> concentration in gas water solution on the pH of gas water solvents and of apple suspension in gas water solvents with and without ultrasound 283 (W=0.242 kW•h/kg). In the absence of ultrasound, the addition of CO2 decreased the pH of 284 the gas water solvents up to about 5 for almost all tested CO<sub>2</sub> concentration. When ultrasound 285 286 was applied on these same solvents, the pH increased up to 6.4 for the highest CO2 287 concentration in gas water solvents. The pH in distilled water (CO<sub>2</sub> concentration=0 mmol/L) was higher than that in gas water solvents because of the reaction of CO<sub>2</sub> with H<sub>2</sub>O molecule 288 289 that forms H<sub>2</sub>CO<sub>3</sub>, which is a weak acid. With ultrasound treatment, the increase in pH might be attributed to the decomposition of HCO3<sup>-</sup> in solution into CO2 and OH<sup>-</sup>, which are alkaline. 290 291 Ultrasound may significantly improve the degradation rate of HCO3. The pH of apple 292 suspension in gas water solvents increased with the increase of CO<sub>2</sub> concentration in gas 293 water solution, both in the absence and in the presence of ultrasound. However, the pH of 294 solution was always higher by ultrasound, which might be attributed to the presence of more 295 components extracted from apple skins.



Fig.5. Effect of the CO<sub>2</sub> concentration on pH of gas water solvents and of apple suspension in gas water solvents without and with ultrasound treatment (W=0.242 kW•h/kg).

Fig.6 presents the effect of the CO<sub>2</sub> concentration in gas water solution on the 296 297 electrical conductivity, o, of gas water solvents and of apple suspension in gas water solvents with and without ultrasound (W=0.242 kW•h/kg). The values of o for gas water solvents 298 were almost the same with and without ultrasound. The  $\sigma$  increased from 0 to 1.75 ms with 299 the increase of  $CO_2$  concentration in gas water solvents. In addition, the  $\sigma$  of apple 300 suspension in gas water solvents was higher than the  $\sigma$  of the gas water solvents. This might 301 302 be due to the presence of nutrients such as minerals, vitamins, proteins and fatty acids, which 303 were released from apples [45].



Fig.6. Effect of the CO<sub>2</sub> concentration on electrical conductivity,  $\sigma$ , of gas water solvents and of apple suspension in gas water solvents without and with ultrasound treatment (W=0.242 kW•h/kg).

304 Fig.7 presents the effect of the pH on the degradation of gallic acid, quercetin-3-O-305 glucoside and catechin. Fast and high degradation rate was achieved with the increase of pH 306 for gallic acid, quercetin-3-O-glucoside and catechin. Previous studies showed the effects of the pH (3.5-6.5) on gallic acid degradation and pH=4.5-5 was the optimum level for 307 308 maximum yield of gallic acid [46,47]. Quercetin stability also decreased with the increase of 309 pH from 3.5 to 7 [48]. In addition, some studies also reported that catechin was quite stable in pH varying from 2 to 5 and degradation rate was slower with lower pH varying from 6 to 8. 310 At a pH greater than 8, catechin was degraded within several minutes [49]. Similar effects of 311 312 pH solution on phenol removal by electrical discharges have been observed [50]. Since the 313 pH of extracts liquid of apple skins in our study was approximately 4-6.5 (figure 7), polyphenols were quite stable during ultrasound extraction with the low degradation of 314

315 phenols ( $\geq 0.85$ ). Therefore, pH variation caused by gas water solvents with ultrasound 316 treatment made no difference on the polyphenols enhanced extraction from apple skins



Fig.7. Effect of the pH on the degradation of gallic acid, quercetin-3-O-glucoside and catechin (C/C<sub>0</sub>) (C<sub>0</sub> corresponds to the corresponding concentration at pH=5.25, 3.54 and 4.17, respectively).

#### 317 4. Conclusions and final remarks

The effect of gas water solvents on the polyphenols extraction efficiency by 318 319 ultrasound was studied. The extraction TPC from all solvents (distilled water, 10% ethanol 320 aqueous solvent, 10% methanol aqueous solvent and gas water solvent with 7.05 mmol/L 321 CO2) was improved obviously by ultrasound. The water holding capacity of ultrasound 322 treated sample in gas water solvent with 7.05 mmol/L CO<sub>2</sub> was almost 2.4 times higher than 323 the untreated samples, which was highest in all solvents. The water absorption was higher by 324 ultrasound and could contribute to the increase of TPC. The liquid extracts by ultrasound 325 exhibited a high phenolic compounds (TPC, TFC, and PAC) extraction and the antioxidant 326 capacity (DPPH) increase in gas water solvents with 5.28 mmol/L CO<sub>2</sub>. Meanwhile, the pH and  $\sigma$  of liquid extracts increased with the increase of CO<sub>2</sub> concentration in gas water 327 solvents. These results adequately explained that the CO2 concentration in gas water solvents 328 could influence polyphenols extraction by ultrasound, due to CO2 gases dissolve into the 329 330 solvent could act as nuclei for producing cavitation bubble by ultrasound, and the produced 331 cavitation effect could finally increase polyphenol extraction. Therefore the gas water 332 solvents could enhance polyphenols and active species production extraction efficiency by 333 ultrasound from apple skins.

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## **IV.4 Conclusions**

Chapter IV is focused on the cavitation phenomena generated by ultrasound treatment. The effects of ultrasound treatment on cell damage (cell wall disintegration index, Zm) and extraction of valuable components (ionic,  $Z_i$ , and total polyphenol,  $Z_{tp}$ , indexes in extracts) from fruit peels (apple, banana and persimmon) were analyzed. In addition, the influence of the carbon dioxide (CO<sub>2</sub>) concentration in gas water solvents on ultrasound-assisted extraction of polyphenols, including total polyphenol content (TPC), total flavonoid content (TFC), and proanthocyanidins content (PAC), from apple peels was studied. Based on the observation of the behavior of Z<sub>i</sub>, Z<sub>tp</sub>, and Z<sub>m</sub> indexes, US could damage cell membranes (sonoporation) and rupture cell walls. Furthermore, the efficiency of extraction of valuable components (Zi and  $Z_{tp}$ ) was depended upon the value of  $Z_m$ . For the extraction of ionic and polyphenol components, the linear relation between Z<sub>i</sub> and Z<sub>m</sub> was obtained for all fruit peels (apple, banana and persimmon). However, the relation of Z<sub>tp</sub> and Z<sub>m</sub> remained linearly unique for banana and persimmon peels not for apple peels. The obtained results demonstrated that cavitation phenomena generated by ultrasound could increase the extraction of valuable components from apple peels. This was because cavitation phenomena damaged cell membranes of samples and accelerated heat and mass transfer by disrupted cell walls of samples. In addition, gas water solvents could enhance cavitation phenomena to improve polyphenols extraction efficiency from apple peels. The reason was that CO<sub>2</sub> gases dissolved into the water solvent could act as nuclei for producing cavitation bubble by ultrasound. The experimental results found that the ultrasound treatment with CO<sub>2</sub> concentration in gas water solvent at 5.28 mmol/L was optimal for polyphenols (TPC, TFC, and PAC) extraction. Ultrasonic treatment can effectively intensify the extraction of polyphenols from apple products. However, in order to obtain higher concentration of polyphenols, purification process using different technologies following extraction will be needed.

# **Chapter V Purification of Polyphenols in Extracts**

# **V.1 Introduction**

After extracting polyphenols from apple products, purification of extracted polyphenols is commonly required (Peng et al., 2017). The purification methods for apple polyphenols are mainly organic solvent extraction, precipitation and crystallization (Dai and Mumper, 2010; Farías-Campomanes et al., 2013; Nawaz et al., 2006; Turkmen et al., 2006). However, these methods are relatively complex, long production cycles, high cost, and not environmentally friend. Recently, adsorption/desorption method using resin adsorbents and membrane technique (especially ultrafiltration and microfiltration) have been widely used for purification of extracted polyphenols from apple products (Borneman et al., 2001; Liu et al., 2013; Sun et al., 2013; Youn et al., 2004; Zhu et al., 2014), because of their advantages as few chemical substance addition, easy recycling, simple operation and easy to be realized on a large scale. The aim of this chapter is to investigate filtration of apple peel extracts for polyphenols purification.

# V.2 Article VI Ultrasound assisted purification of polyphenols of apple skins by adsorption/desorption procedure

## Summary

First of all, adsorption/desorption procedure with ultrasound treatment on the polyaromatic amberlite adsorbent XAD-16 was carried out to evaluate the feasibility of polyphenols purification (details are presented in article VI Ultrasound assisted purification of polyphenols of apple skins by adsorption/desorption procedure). Adsorption/desorption method using resin adsorbents was beneficial for the concentration and purification of phenolic compounds due to polyphenol substances could be adsorbed on the adsorbents with the strong noncovalent bonding and aromatic stacking and subsequently could be easily desorbed into organic solvents. However, the conventional adsorption/desorption procedures are time-consuming (Buran et al., 2014; Zagklis and Paraskeva, 2015). US treatment during adsorption/desorption procedures can reduce purification time and increase purification effect by accelerating mass-transfer and adsorption efficiency. US has been applied to improve adsorption of phenolic compounds, aromatic pollutants, dyes, anthocyanins, and metal ions (Ali et al., 2018; Breitbach et al., 2002; Dastkhoon et al., 2015; Ji et al., 2006; Jing et al., 2011; Juang et al., 2006; Midathana and Moholkar, 2009). Ultrasound-assisted purification of polyphenols from apple peel extracts by adsorption/desorption procedure based on using the adsorbent resins can be considered as the "green" purification technology with reduction of organic solvents and absorbents consumption, energy consumption, and purification time (Chemat et al., 2017a, 2017b).

Therefore in this part, the adsorption steps at different temperatures (T=25–40 °C) with application of US at different intensities (P=0–400 W) were studied. In addition, the effect of ethanol concentration in aqueous ethanol solution ( $C_e=0-96\%$ ) on desorption steps was analyzed. The isotherm of polyphenol adsorption, the adsorption kinetic curves and desorption kinetic curves were studied. The obtained data from adsorption/desorption experiment evidenced that the adsorption kinetics were fitted with stretched exponential law and evidenced that the presence of broad distribution of adsorption times that can depend on content of polyphenols in the solutions and applied power of sonication. The adsorption isotherm was fitted using the Freundlich isotherm model that also assumes the presence of adsorption on heterogeneous surface. Since the results demonstrated that the sonication significantly facilitated absorption. The effects of US power on the damage of XAD-16 were

discussed. The studies of desorption revealed the optimum desorption efficiency of polyphenols at 50% concentration of ethanol. Meanwhile, the desorption ratio, D, was positively affected by the sonication during the adsorption step. The significant higher adsorption/desorption efficiency (recovery) for polyphenols was observed with ultrasound treatment compared with the efficiency without ultrasound treatment. The highest adsorption/desorption efficiency (recovery) was observed for polyphenols as compared with proteins and soluble matter content, and it reached of about 30.6% (0 W) and about 68.9% (50 W) in absence and presence of sonication, respectively. The obtained data evidenced on a good perspective of application of adsorption/desorption procedure assisted by sonication for purification of polyphenols from apple peel extracts.

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## Ultrasound assisted purification of polyphenols of apple skins by adsorption/desorption procedure



Ultrasonic

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## ABSTRACT

Keywords. Polyphenols Apple skins Adsorbent XAD-16 Ultrasound Adsorption/desorption purification The ultrasound (US) assisted purification of polyphenols of apple skins by adsorption/desorption on the poly-aromatic Amberlite adsorbent XAD-16 was studied. The adsorption steps were done at different temperatures (T = 25-40 °C) with application of US at different intensities (P = 0-400 W). The desorption steps were tested in aqueous ethanol solution at different concentrations of ethanol ( $C_{et} = 0-96\%$ ). The isotherm of polyphenol adsorption was well described using the Freundlich model. The data on adsorption kinetics and static isotherm evidenced the presence of adsorption on heterogeneous surface with broad distribution of adsorption times that can depend on content of polyphenols in the solutions and applied power of sonication. The studies of desorption revealed the optimum desorption efficiency of polyphenols at 50% concentration of ethanol. The desorption ratio was positively affected by the sonication during the adsorption step. The highest adsorption/desorption efficiency (recovery) was observed for polyphenols as compared with proteins and soluble matter content and it reached of  $\approx$  30.6% (0 W) and 68.9% (50 W) in absence and presence of sonication, respectively. The effects of high US power on the damage of XAD-16 were discussed. The obtained data evidenced on good perspective of application of adsorption/desorption procedure assisted by sonication for purification of polyphenols from apple skin extracts.

#### 1. Introduction

The apple polyphenols present valuable bio-molecules with strong anti-inflammatory and beneficial effects to human health [1-4]. The content of polyphenols (e.g., procyanidins and flavonols) in apple skins is higher than in apple flesh or juice [5–7]. The concentrations of the main polyphenols in the apple skins were estimated as 0.99-4.42 mg/g dry weight (DW) for (+)-catechin, 1.24-5.75 mg/g DW for (-)-epicatechin, 2.76–11.4 mg/g DW for rutin, and 0.71–2.42 mg/g DW for phloridzin [8].

Different solvent extraction methods with assistance of ultrasound (US), high hydrostatic pressure, pulsed electric fields, and high voltage electrical discharges have been applied for recovery of polyphenols from apple products [9-15]. Commonly, purification of extracted polyphenols is required [16]. For the concentration and purification of phenolic compounds adsorption/desorption method using resin adsorbents have been applied [17-19]. This method includes adsorption of polyphenols from aqueous solution due to the strong noncovalent bonding and aromatic stacking and following desorption of polyphenols in organic solvents (e.g. in ethanol or methanol). For example, such techniques were applied for separation and purification of anthocyanins from mulberry [20] and blueberry [21], total polyphenols, chlorogenic acid and phlorizin from thinned young apples [22], and phenolic compounds from grape marc [23].

The adsorption/desorption procedures are time-consuming [21,23]. Ultrasound-assisted purification of polyphenols of apple skins by adsorption/desorption procedure based on using the adsorbent resins can be considered as the "green" purification technology with reduction of organic solvents and absorbents consumption, energy consumption, and purification time [24-26]. US treatment can be used to accelerate masstransfer and adsorption efficiency US has been applied to improve adsorption of phenolic compounds, aromatic pollutants, dyes, anthocyanins, and metal ions [27-33]. However, the adsorbent resins can be damaged under mechanical compression and ultrasonication [34], and therefore the US-assisted adsorption of food polyphenols requires thorough adjustment to avoid extracts contamination. The US assisted

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adsorption/desorption of food polyphenols have been rarely discussed in the previous works. We can only refer the recent work [35] concerning US assisted anthocyanins purification from blueberries using adsorbent resins.

This work studies purification of polyphenol extracts of apple skins using adsorption/desorption steps assisted by US. The poly-aromatic Amberlite XAD-16 was used as the adsorbent. The isotherms and kinetics of adsorption were studied at different US intensities (P = 0-400 W) and temperatures (T = 25-40 °C). The desorption from adsorbents saturated with bio-molecules was studied using ethanol water solutions ( $C_{eth} = 0-95\%$ ). The effects of sonication on purification of polyphenols were discussed.

#### 2. Materials and methods

#### 2.1. Materials

Commercial red apples (Gala) were selected as raw materials for the study. Apples of good and uniform quality (with near-spherical shape) were purchased at the local supermarket (Compiegne, France). The initial moisture content on wet basis for apple skin was 84.3% determined using MA 160 infrared moisture analyzer (Sartorius, Germany).

The hydrophobic poly-aromatic adsorbent Amberlite XAD-16 (Sigma Aldrich, France) was used in adsorption experiments. Commonly it is used for separation of large organic molecules up to 40,000 MW. According to manufacturer, the resin XAD-16 has surface area of  $900 \text{ m}^2/\text{g}$ , mean pore radius of 5.0 nm, pore volume of 1.82 mL/g and dry density of 1.08 g/mL [36]. To remove the impurities, porogenic agents and monomers trapped in the pores [21] the resin (10 g) was soaked in 95% ethanol (100 mL) for 6 h and rinsed with distilled water (until eluate was clear). Then it was soaked in 5% HCl (100 mL) for 6 h, rinsed with distilled water (until neutral effluent), soaked in 5% NaOH (100 mL) for 6 h, and rinsed with distilled water (until neutral effluent). Finally, it was dried in a convection oven UL50 (Memmert, Germany) at 70°C for 24 h. The final moisture content of resin was 80.39%.

#### 2.2. Methods

The schema of experimental study is presented in Fig. 1. It includes the preparation of polyphenols extract from apple skins, adsorption step from aqueous extract on adsorbent XAD-16 assisted by sonication and desorption step from the XAD-16 saturated by adsorbed bio-molecules in aqueous-ethanol solutions.

2.2.1. Preparation of aqueous extract of biomolecules from apple skins The slices (20 mm  $\times$  10 mm) from apple skins were prepared, dried in oven UL50 (Memmert, Germany) at 105 °C for 24 h, and grinded into powder using the grinder MCG2013B-16 (150 W, 50 Hz, Mandine, Ultrasonics - Sonochemistry 55 (2019) 18-24

France). Then the skin powder was put into a glass beaker filled with 50% ethanol aqueous solution with solid/liquid ratio of 1:10. The extraction was assisted using sonication applied with instrument UP 400S (400 W, 24 kHz, Hielscher GmbH, Stuttgart, Germany). The titanium ultrasonic probe H14 (Hielscher GmbH, Stuttgart, Germany) was used. The US treatment was done in pulsed mode with sequential application of n = 6 ultrasonic pulses [15]. After each pulse with duration of  $\Delta t_u = 3 \min$ , a pause with duration of  $\Delta t_w = 2 \min$  was applied. In order to avoid increasing temperature during US treatment, the glass beaker was put in an ice/cold water box to keep the temperature ( $\approx 25$  °C). The total time of US-assisted extraction was  $t_e = 30 \min$ .

The final extracts were centrifuged for 10 min at 4000 rpm (Laborzentrifugen 3–10, SIGMA, Osterode am Harz, Germany). The remove ethanol, the supernatant was concentrated at 40 °C in a vacuum rotary evaporator (Laborota 4001, Heidolph Instrument, Schwabach, Germany), then it was filtrated using filter paper (No.474, VWR, Geldenaaksebaan, Leuven) and stored at 4 °C until further experiments. In the prepared polyphenol extract the total polyphenols content, *TPC*, protein content, *PC*, and soluble matter content, °Brix, were 6.3 mg/mL, 0.58 mg/mL, 21.4%, respectively.

#### 2.2.2. Adsorption/desorption steps

The adsorption experiments were performed at 25–40 °C in a glass beakers by mixing of the resin (2 g) with polyphenol extracts (100 mL) at different concentrations of total polyphenols,  $C_0$  (0.01–6 mg/mL). The beakers were kept in shaking water bath SW22 (Julabo GmbH, Seelbach, Germany) at rate of 150 rpm. The total time of adsorption experiments was up to 1440 min.

In the adsorption step the probes of liquid extract were periodically taken, centrifuged using Galaxy Mini Centrifuge C1413-230EU (VWR, Paris, France) for 1 min at 6000 rpm, and analyzed for the total polyphenol content, *TPC*.

Adsorption capacity (mg/g dry resin) at equilibrium was calculated

$$Q = (C_o - C_e)V_a/M \tag{1}$$

where  $C_o$  and  $C_e$  are initial and equilibrium (final) concentrations of biomolecules (e.g., polyphenols) (mg/mL) in extract solutions, M is the mass of the resin (g) and  $V_a$  is the volume of the liquid medium (mL) in adsorption experiments.

In experiments with US-assisted adsorption, the preliminary pulsed sonication was applied at different US intensity, *P* (0–400 W), at the same manner as described in Section 2.2.1. The total number of pulses was n = 20, US treatment time,  $t_{us} = 60$  min, and total time of US-assisted adsorption was  $t_a = 100$  min. Then the adsorption in shaking bath was continued.

Desorption experiments were done using the resins previously saturated with bio-molecules in the adsorption step. The adsorbent (2 g) was put into the shaking glass beaker filled with aqueous ethanol solution (100 mL,  $C_{et} = 0-95\%$ ). Desorption experiments were performed



as

Fig. 1. The schema of adsorption/desorption steps in purification process.

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at 25 °C for 180 min. In the course of desorption the probes of liquid extract were periodically taken, centrifuged and analyzed for the soluble matter content, °Brix, and total polyphenol content, *TPC*. The desorption ratio, *D*, was calculated as

$$D = C_f V_d / [(C_o - C_e) V_a]$$
<sup>(2)</sup>

where  $C_f$  is the concentration of polyphenols (mg/mL) in the aqueousethanol solution, and  $V_d$  is the volume of the liquid medium (mL) in desorption experiments.

The adsorption/desorption efficiency (recovery), R, for phenolics, proteins and soluble matter content was calculated using the following equation:

$$R = C_f / C_i \tag{3}$$

where  $C_o$  and  $C_f$  are the initial (before adsorption) and final (after desorption) concentration of biomolecules, respectively.

#### 2.3. Analysis

The total polyphenols content, TPC, was determined using the Folin-Ciocalteu method based on a colorimetric oxidation/reduction reaction of phenols [37]. 0.2 mL of diluted sample and 1 mL of Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) (diluted 1:20 with distilled water) were mixed. Then 0.8 mL of Na<sub>2</sub>CO<sub>3</sub> (75 g/L) (Prolabo, Fontenay-sous-Bois, France) was added. The sample was incubated for 10 min at 50 °C and then cooled for 10 min at 4 °C in refrigerator. For the blank, 0.2 mL of distilled water was taken. The absorbance was measured at 750 nm using UV/Vis instrument (Thermo Spectronic Genesys 20, Thermo Electron Corporation, MA, USA). Gallic acid (Sigma-Aldrich, St-Quentin Fallavier, France) was used for the calibration curve. The results were expressed in mg/mL. The concentration of proteins was determined by means of Bradford method [38]. The details of analysis were presented in Technical Bulletin for Bradford Reagent (B 6916, Sigma-Aldrich). 7 standard solutions with various BSA (Bovine Serum Albumin) concentrations (0-25 µg/ml) were prepared for the standard curve. 1 mL of distilled water, 0.8 mL of Bradford reagent and 0.2 mL of diluted sample (distilled water for blank) were introduced into the tube successively, the mixture was mixed with a Vortex, and the absorbance was measured at 595 nm, then the concentration of protein was calculated using the standard curve. The results were also expressed as mg/mL. The soluble matter content, °Brix (g of DM/100 g solution, %), was measured by a digital refractometer (Atago, USA) at 25 °C.

The results of scanning electron microscopy (SEM), turbidity analyses, and Fourier transform infrared spectroscopy (FTIR) are presented in Supplementary Materials.

#### 2.4. Statistical analysis

All experiments and measurements were repeated at least in triplicate. The mean values and the standard deviations were calculated. The error bars in figures correspond to the standard deviations. TableCurve 2D\* (Systat Software Inc.) was used for data fitting.

#### 3. Results and discussion

Fig. 2 shows the adsorption capacity for polyphenols,  $Q_i$  versus the time,  $t_i$  at different initial concentrations of extracts,  $C_0$ . The values Q increased with adsorption time,  $t_i$  and saturated at long time of adsorption (t > 500-1000 min). The adsorption kinetics can be well fitted ( $R^2 \approx 0.999$ ) with the stretched exponential function:

$$Q = a \left[ 1 - exp(-(t/\tau_t)^{\beta}) \right]$$

where a,  $\tau_t$  and  $\beta$  are the fitting parameters.

The stretched exponential law represents the generalisation of the simple first order kinetics in presence of the distribution of adsorption



Fig. 2. Adsorption capacity for polyphenols,  $Q_i$  (TPC) versus the time of adsorption, t, and different initial concentration of polyphenols in extracts,  $C_0$ (0.01–6 mg/mL). Temperature is 25 °C, the symbols correspond to the experimental data and solid lines to the fitting with stretched exponential function (Eq. (3)).

times. The stretching parameter  $\beta$  characterizes the broadness of the distribution. The larger deviation of  $\beta$  from 1 is, the broader the distribution of the adsorption times. The mean adsorption time  $\tau$  is calculated as [39]:

$$t = \tau_t \beta \Gamma(\beta^{-1}) \tag{5}$$

where  $\Gamma$  is the Euler gamma function.

Fig. 3 shows the estimated mean adsorption time  $\tau$  (a), and stretching parameter  $\beta$  (b) versus the initial concentration of extract. The value of  $\tau$  noticeably increased with the increase of  $C_0$  (Fig. 3a), whereas stretching parameter,  $\beta$ , was in range of 0.7–1.1 and it gone through the maximum at  $C_0 \approx 0.5$  mg/mL.

The obtained data evidenced on the deceleration of adsorption processes with increase of  $C_0$  and presence of broad distribution of adsorption times at small values of  $C_0$  (< 0.5 mg/mL). Such distribution can reflect the distribution of binding energies at different adsorption sites for polyphenols on adsorbent. At small concentration, the



Fig. 3. Mean adsorption time,  $\tau$  (a), and stretching parameter,  $\beta$  (b), versus the initial concentration of polyphenols in extract,  $C_0$ . T = 25 °C.

20

(4)



Fig. 4. Saturation adsorption capacity,  $Q_{sc}$  (TPC) versus equilibrium concentration of polyphenols in extract,  $C_e$  (adsorption isotherm). Inset shows linear  $\ln Q_e$  versus  $\ln C_e$  dependence that corresponds to the Freundlich law, Eq. (6). The temperature was T = 25 °C.

polyphenols can be rapidly adsorbed on the most active sites, and at higher concentration, the adsorption involves also less active sites.

Fig. 4 shows the equilibrium isotherm for adsorption of polyphenols on adsorbent at T = 25 °C. The adsorption capacity, Q (mg/g dry resin), increased with increase of the equilibrium concentration of total polyphenols in solution,  $C_e$  (mg/mL). Inset shows lnQ versus ln $C_e$  dependence and its linearity corresponds to the validity of the Freundlich isotherm model:

$$Q = KC_e^{1/n} \tag{6}$$

The data fitting to this model gives the values of parameters  $K = 45.0 \pm 1.4$  and  $1/n = 0.64 \pm 0.02$  with correlation coefficient of  $R^2 = 0.990$ . The validity of the Freundlich isotherm model assumes the presence of adsorption on heterogeneous surfaces. The slope 1/n reflects surface heterogeneity, and it becomes closer to zero for more heterogeneous surface [40]. The surface heterogeneity is in correlation with observed distribution of adsorption times (Fig. 3).

Fig. 5 compares adsorption kinetics of polyphenols on adsorbent at different US intensities (P = 0.-400 W). Note that US treatment was done within initial 100 min. It can be seen that application of US treatment accelerated the adsorption capacity,  $Q_c$ . Inset to Fig. 5 shows  $Q_s$  versus *P* dependence. It can be seen that the most pronounced effects were observed when the US intensity increases from 25 to 100 W. The increase of *P* could increase the mixing intensity owing to turbulence created by the collapse of the cavitation bubbles. The positive impact of sonication on polyphenols adsorption can be explained by acceleration of the mass transport due to the effects of the cavitation bubbles [41].

Fig. 6 shows the evaluated mean time of adsorption,  $\tau$ , (a) and stretching parameter,  $\beta$ , (b) versus the US intensity, P (0–400 W). The value of  $\tau$  noticeably decreased with increasing of P, in absence of sonication it was  $\approx 340$  min and at  $P \ge 50$  W it was  $\le 40$  min. The stretching parameter,  $\beta$ , decreased with increasing of P and became rather small at  $P \ge 50$  W,  $\beta \le 0.5$ . The obtained data evidenced the presence of broad distribution of adsorption times in presence of sonication. Such distribution can be attributed to increasing the availability of different binding sites for polyphenols on the surface of adsorbent as the result of sonication.



**Fig. 5.** Adsorption kinetics of polyphenols on adsorbent at different US power (P = 0-400 W). Inset shows the saturation adsorption capacity of polyphenols on adsorbent,  $Q_m$ , versus the US power, P. The initial concentration of polyphenols in extract was  $C_o = 1$  mg/mL, the total time of US-assisted adsorption was  $t_a = 100$  min, and temperature was T = 25 °C.



**Fig. 6.** Mean time of adsorption, *r*, (a) and stretching parameter,  $\beta$ , (b) versus the US power, *P* (0–400 W). The initial concentration of extract was  $C_0 = 1 \text{ mg/}$  mL, the total time of US-assisted adsorption was  $t_a = 100$  min, and temperature was T = 25 °C.

Fig. 7 demonstrates the effects of temperature on the adsorption kinetics of polyphenols on adsorbent in absence (P = 0 W) and presence (P = 50 W) of sonication. The whole tendencies for the adsorption kinetics at P = 0 W and at P = 50 W were rather similar, i.e., increase in temperature always resulted in increase of adsorption capacity Q. Fig. 8 presents Arrhenius plots of the logarithm of the saturation adsorption capacity ln  $Q_s$  versus inverse absolute temperature, 1/T. The obtained data can be well fitted with high correlation coefficients (R2 > 0.998) using the following equation [42]:

$$Q_s = A e^{\Delta E / RT}$$
<sup>(7)</sup>

where A is the pre-exponential,  $\Delta E$  is the apparent activation energy and R (8.134 J/(mol·K)) is the universal gas constant. Note that the activation energy of adsorption,  $\Delta E$ , is a measure of the binding interactions strength.





**Fig. 7.** Adsorption kinetics of polyphenols on adsorbent at different temperatures (T = 23-40 °C) in absence (P = 0 W) and presence (P = 50 W) of sonication. The initial concentration of polyphenols in extract was  $C_o = 1$  mg/mL, and the total time of US-assisted adsorption was  $t_o = 100$  min.



**Fig. 8.** Natural logarithm of saturation adsorption capacity,  $\ln Q_{s_0}$  versus the reciprocal temperature, 1/T ( $K^{-1}$ ), in absence (P = 0 W) and presence (P = 50 W) of sonication. The initial concentration of extract was  $C_0 = 1$  mg/mL, and the total time of US-assisted adsorption was  $t_0 = 100$  min.

The obtained values of,  $\Delta E$ , were 7.41  $\pm$  0.51 kJ/mol and 14.14  $\pm$  0.49 kJ/mol at P = 0 W and P = 50 W, respectively. The range of  $\Delta E$  values corresponds to the physical adsorption [42].

The effects of sonication on absorption kinetics, adsorption capacity, and activation energy evidently reflected facilitation of adsorption processes in presence of sonication. Such effect can be explained by the acceleration of mass transfer process and changes in availability of active adsorption centres in the resin adsorbent in presence of sonication. Note that sonication could strength the affinity between polyphenols and resin adsorbents [20].

The US cavitation can increase the degradation of chemical compounds of natural products and lipid oxidation [43–45]. The lipid degradation originates from hydrolysis or oxidation, which can occur by auto-oxidation, photo-oxidation, or enzymatic-oxidation [43]. To avoid such effects during the US-assisted extraction the flow of argon to

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exclude  $O_2$  can be performed [45]. However, at high power or long time of sonication, the effects of US treatment on the structure of resin cannot be excluded [46]. Analysis of scanning electron microscopy images of resin adsorbent (XAD-16) revealed noticeable effects of sonication on the surface of resins (See Supplementary material S1). The granules of adsorbent before sonication had practically ideal near spherical shape with diameter of particles of 0.25–0.84 mm and rather smooth surface. The sonication resulted in visual degradation, the surface of granule became rougher and formation of large macrospores was observed. The observed phenomena can reflect local erosion, shear forces, sonoporation, fragmentation, capillary effect and detexturation [47]. Ultrasound cavitation can also produce cavitation bubbles on a surface that results in micro-jetting and generation of surface peeling, erosion and particle breakdown [26,48,49].

The intensive sonication at high power of P = 400 W can provoke disruption of the spherical particles into the small debrises. The turbidity data evidenced that these debrises can be removed by centrifugation of the samples after adsorption/desorption procedure (See Supplementary material S2).

The more detailed studies of desorption in aqueous ethanol solutions were performed for resins used in adsorption performed in absence (P = 0 W) and presence (P = 50 W) of sonication. The moderate power of P = 50 W was selected in order to exclude effects of disruption of the spherical adsorbent granules into the small debris. In all experiments the initial concentration of polyphenols in extracts was  $C_o = 1$  mg/mL and the total time of US-assisted adsorption was  $t_a = 100$  min. Fig. 9 shows desorption ratio for polyphenols, D, (Eq.(2)) versus the time of desorption, t, at different concentrations of ethanol ( $C_{et} = 0-95\%$ ) for P = 0 W (a) and P = 50 W (b). The most significant extraction of polyphenols was observed during the time of t < 120-180 min. The value of D increased with  $C_{et}$  below 50%, but further increase in  $C_{et}$  resulted in decrease of D.

The effects of ethanol concentration for the extraction time of 180 min are summarised in Fig. 10. The  $D(C_{et})$  curves gone through the maximums at  $C_{et} \approx 50\%$  where *D* values reached  $\approx 44.2\%$  and  $\approx 71.9\%$  in absence (*P* = 0 W) and presence (*P* = 50 W) of sonication, respectively. Therefore, the optimum desorption efficiency of polyphenols was observed using 50% ethanol aqueous solutions. This reflects the impact of ethanol on weakening of binding between molecules of polyphenols generally contain aromatic nuclei and their interactions with aromatic centres of resin adsorbent. The natural polyphenols generally contain aromatic nuclei and their interactions with of the concentration of ethanol. Moreover, the obtained data evidenced



**Fig. 9.** Desorption ratio for polyphenols, *D*, versus time of desorption, *t*, at different concentrations of ethanol ( $C_{et} = 0-95\%$ ) in absence (P = 0 W) and presence (P = 50 W) of sonication. The temperature was T = 25 °C.



Fig. 10. Effects of ethanol concentration, Cet, on the desorption ratio, D of polyphenols in absence (P = 0 W) and presence (P = 50 W) of sonication. The temperature was T = 25 °C.



Fig. 11. The recovery, R, of total polyphenols, TPC, proteins, and soluble matter, <sup>o</sup>Brix, in absence (P = 0 W) and presence (P = 50 W) of sonication. The temperature was T = 25 °C.

that desorption ratio D was also affected by the sonication during adsorption of polyphenols on resin adsorbent.

Fig. 11 compares the adsorption/desorption efficiency (recovery), R, of polyphenols, proteins and soluble matter content (°Brix) in absence (P = 0 W) and presence (P = 50 W) of sonication. The recovery of polyphenols reached the values of  $\approx 30.6$  and 68.9% in absence and presence of sonication, respectively. The significantly smaller effects were observed for recovery of proteins (  $\approx 8.4\%$  (0 W) and 10.8% (50 W)) and soluble matter content (°Brix) (  $\approx 5.6\%$  (0 W) and 4.4% (50 W)). Therefore, the applied adsorption/desorption procedure assisted by sonication allowed highest recovery of polyphenols and it can be used for their purification from other bio-molecules represented by proteins and soluble matter content. The analysis of FTIR spectra of samples (See Supplementary Materials S3) also evidenced that the applied adsorption/desorption procedure assisted by sonication can be useful for purification of total polyphenols of apple skin extracts.

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#### 4. Conclusions

The effects of sonication on adsorption/desorption purification of polyphenols of apple skins were tested using XAD-16 adsorbent. The adsorption steps were done at different temperatures (T = 25-40 °C) and assisted by the applied sonication at different intensities (P = 0-400 W). The desorption steps were tested in aqueous ethanol solution at different concentrations of ethanol ( $C_{et} = 0-96\%$ ). The stretched exponential law was used to fit adsorption kinetics. The data evidenced the presence of broad distribution of adsorption times that can depend on content of polyphenols in the solutions and applied power of sonication. The adsorption isotherm was fitted using the Freundlich isotherm model that also assumes the presence of adsorption on heterogeneous surface. The more detailed studies of the effects of sonication at relatively small power (P = 50 W) were tested to avoid the degradation of the adsorbent. The sonication significantly facilitated absorption kinetics, increased adsorption capacity and activation energy of polyphenols adsorption. In the desorption experiments the optimum desorption efficiency of polyphenols was observed for 50% concentration of ethanol. The desorption ratio D was positively affected by the sonication during the adsorption step. The highest adsorption/ desorption efficiency (recovery), R, was observed for polyphenols as compared with proteins and soluble matter content, and it reached of  $\approx$  30.6 (0 W) and 68.9% (50 W) in absence and presence of sonication, respectively. The obtained data evidenced a good perspective of application of adsorption/desorption procedure assisted by sonication for purification of polyphenols from apple skin extracts.

#### Acknowledgments

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1	Supplementary materials for the Manuscript
2	
3	Ultrasound assisted purification of polyphenols of apple skins by
4	adsorption/desorption procedure
5	
6	
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14	
15	S1 SCANNING ELECTRON MICROSCOPY IMAGES OF THE SONICATED ADSORBENT1
16	S2 TURBIDITY2
17	S3 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS
18	REFERENCES5
19	

## 21 S1 Scanning electron microscopy images of the sonicated adsorbent

The scanning electron microscopy (SEM) images with magnification of 100 were registered using SEM instrument Quanta FEG 250 (FEI, Holland) applied in an accelerating voltage of 20 kV. The microscope was coupled to a CCD camera (Sony, Japan) connected to a computer.



Fig. S1. SEM images of adsorbent Amberlite XAD-16 after sonication for 100 min at different US power, P=0-400W.

26 Figure S1 shows SEM micrographs of adsorbent Amberlite XAD-16 after sonication for 100 min at different US power, P=0-400 W. In absence of sonication (P=0 W) the adsorbent 27 consists of practically ideal spherical particles with diameter of 0.25-0.84 mm [1] and smooth 28 surface. Sonication resulted in degradation of adsorbent, and the surface of granules became 29 30 rougher. Moreover, the formation of large cavities and macrospores on the surfaces were observed. At the highest US power, P=400 W, the disruption of the spherical particles into 31 32 small debris was observed. The specific area of adsorbent can be increased due to the sonication and perturbation of the structure of adsorbent was increased. 33

### 34 S2 Turbidity

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Turbidity was measured with a XR turbidimeter (HACH Company, Loveland, USA). For
the turbidity measurement, the adsorption experiments with initial concentration of
2
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polyphenols  $C_o=1$  mg/mL were performed for 1500 min in presence of sonication during the first 100 min at different ultrasonic power, P=0-400 W.



Fig. S2. Turbidity, *T*, versus US power, *P*, for sample clarified by sedimentation in the Earth's gravity for 15 min (g-samples) or by centrifugation for 1 min at 6000 rpm (c-samples). Sonication was done for 100 min. The concentration of polyphenols was  $C_o=1$  mg/mL, the temperature was T=25 °C.

39 Then solutions were clarified by sedimentation in the Earth's gravity for 15 min (gsamples) or by centrifugation for 1 min at 6000 rpm (c-samples). Sedimentation in the Earth's 40 41 gravity allowed deposition of large undamaged adsorbent granules whereas application of centrifugation allowed deposition of small debris. Figure S2 shows the turbidity, T, versus 42 sonication power, P, applied in adsorption experiments. For g-samples, the turbidity 43 44 increased with increase of P and inverse situation was observed for c-samples. In absence of sonication the relatively large turbidity,  $T \approx 70$  NTU reflected the presence of large quantity 45 scattering bio-molecules in extracts. With application of sonication, these molecules can be 46 more effectively adsorbed by XAD-16 particles. For c-samples XAD-16 particles and their 47 3

debris removed from solution by centrifugation and turbidity decreases. For g-samples the increase of turbidity with P can be explained by increase of concentration of debris of XAD-16 particles in solution. Accounting for these observation the final centrifugation of the solutions after adsorption/desorption procedure is desirable in order to remove the debris of XAD-16 particles.

## 53 S3 Fourier transform infrared spectroscopy (FTIR) analysis

Fourier transform infrared spectroscopy (FTIR) analysis of solutions was performed
 using Nicolet<sup>TM</sup> iS5 (iD3 ATR, Thermo Scientific, TM, USA) instrument. The FTIR
 transmittance (%) was recorded between 600 and 4000 cm<sup>-1</sup>.



Fig. S3. FTIR bands in the spectral regions at 1400-2000 cm<sup>-1</sup> (a) and 2800-4000 cm<sup>-1</sup> (b) of initial extracts, and solutions after adsorption/desorption (A/D) steps for processing in absence (P=0 W) and presence (P=50 W) of sonication. The temperature was T=25 °C,  $C_o$ =1 mg/mL,  $C_{et}$ =50%.

Figure S3 shows the FTIR spectra of initial extract and solutions after adsorption/desorption steps ( $C_o=1 \text{ mg/mL}$ ,  $C_{et}=50 \%$ ) for adsorption processing in absence

59 (P=0 W) and presence (P=50 W) of sonication. The FTIR bands in the spectral regions at 60  $1400-2000 \text{ cm}^{-1}$  (a) and  $2800-4000 \text{ cm}^{-1}$  (b) are shown in more details. The peaks at  $\approx 1610-$ 61  $1650 \text{ cm}^{-1}$  can represent symmetrical and asymmetrical stretching vibration for the carboxyl 62 ion (COO-) which could be assigned to carboxylic acid ester or carbonyl groups [2,3]. The 63 broad bands at about 3400 cm<sup>-1</sup> can be attributed to bonded -OH groups, C-H stretching 64 bands and stretching vibration of O-H of aromatic ring [4,5]. 65 The intensity of peaks at about 1630 and 3300 cm<sup>-1</sup> decreased after

adsorption/desorption steps that reflected incomplete recovery of total polyphenol in full correspondence with the data presented in Fig. 11. For sonication assisted adsorption step, the peaks were more intensive that demonstrates that US-assisted adsorption could increase the purification polyphenols.

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- 83

# V.3 Article VII Purification of polyphenols from apple skins by membrane electro-filtration: Effects of pore size, pressure and applied voltage Summary

In the second part of this chapter, dead-end electro-filtration with membranes was used to filtrate apple peel extracts (details are presented in article VII Purification of polyphenols from apple skins by membrane electro-filtration: Effects of pore size, pressure and applied voltage). In recent years, membrane technique has been widely used for purification of extracted polyphenols from apple products (Borneman et al., 2001; Vladisavljević et al., 2003; Zárate-Rodríguez et al., 2001; Zhao et al., 2015), such as recovering the polyphenols from apple juice in the dead-end mode (Borneman et al., 2001) and from apple cider in the crossflow mode (Zhao et al., 2015). However, membrane fouling can introduce changes in permeate and retentate quality (Ulbricht et al., 2009) as well as reduce the efficiency of purification of polyphenols (Cai et al., 2009; Duclos-Orsello et al., 2006; Jaffrin, 2008; Rai et al., 2006; Wakeman and Williams, 2002) during conventional membrane filtration procedures. The "force field-assisted methods" (including electrical, magnetic and sonic forces) have received much attention as novel techniques to modify the filtration performance and prevent fouling (Chen et al., 2007; Wakeman and Williams, 2002). The influence of electric field on the process of membrane filtration was studied (Liu et al., 1999; Tarleton, 1992). However, most previous studies in the field were focused on the reduction of membrane fouling and improvement of membrane flux.

Therefore in this part, not only the influences of membrane pore size ( $d_m$ =3.02-50 nm), transmembrane pressure ( $\Delta P$ =1-6 bar) and direct current (DC) voltage (U=5-15 V) on the purification behaviour, and compositions changes after filtration were investigated, but also the influence of electro-filtration parameters on the selectivity of polyphenols purification in membrane processes was discussed. This work studied the performance of the membrane electro-filtration process for separation of polyphenols extracted from apple peels. The potential of low voltage DC electro-filtration for improvement quality of apple peel extracts was also discussed. The sieving ratio R (total polyphenols  $R_{tf}$ , proteins  $R_{pr}$ , total solutes  $R_{ts}$ , colour intensity  $R_l$ ) of filtrates with different transmembrane pressures, different membrane pore sizes, different DC voltages was calculated. The obtained data from membrane electro-filtration experiment evidenced that the sieving ratio R ( $R_{tf}$ ,  $R_{pr}$ ,  $R_{ts}$  and  $R_l$ ) of filtrates almost linearly increased with the increase of transmembrane pressure. Utilization of ultrafiltration membrane with small size of pores (UH030,  $d_m$ =4.35 nm) allowed effective rejection of

proteins. In addition, the rejection of proteins was observed in the anode (+) space and the most significant effect was observed at the DC voltage of 5 V. Such effects were attributed to the large size of protein molecules and their positive charges. In addition, for polyphenols the value of the sieving ratio  $R_{tf}$  changed a little with  $d_m$ , and the DC filtration allowed the purification of polyphenols in the anode (+) space and obtaining larger volume of filtrates. The obtained data evidenced the possibility of selective purification of polyphenols compared with proteins with membrane electro-filtration.

1	Purification of polyphenols from apple skins by membrane
2	electro-filtration: Effects of pore size, pressure and applied
3	voltage
4	Lu Wang <sup>a</sup> , Nadia Boussetta <sup>a</sup> , Nikolai Lebovka <sup>a,b</sup> , Eugene Vorobiev <sup>a</sup>
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## 22 Abstract

23 24 25 26 27	Dead-end electro-filtration of polyphenols extracted from apple skin was studied. The influence of membrane pore size ( $d_m$ =3.02-50 nm), transmembrane pressure ( $\Delta P$ =1-6 bar) and direct current (DC) electric field ( $U$ =5-15 V) on the purification efficiency was investigated. The sieving ratios $R$ of selected membrane UH030 ( $d_m$ =4.35 nm) towards the polyphenols, proteins, total solutes, and colour intensity increased with increase of transmembrane pressure.
28	It was demonstrated that utilization of this membrane for the DC electro-filtration allowed
29	more effective rejection of proteins at the anode part of the filter. Such effects were attributed
30	to the large size of protein molecules and their positive charge. Obtained data evidenced the
31 32	possibility of selective purification of polyphenols by membrane electro-filtration.
33	Keywords: Polyphenols; Membrane; Electro-filtration; Pore size; Pressure
34	
33	
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## 36 1. Introduction

Apple skin is a low-cost and rich source of valuable polyphenols  $1^{-5}$ . The polyphenols 37 are beneficial to human health with strong anti-inflammatory effects and high ability to 38 reduce the risk of getting some diseases <sup>6-8</sup>. In recent years, different separation techniques 39 based on adsorption chromatography, precipitation, crystallization, and membrane filtration 40 have been used for purification of polyphenols 9-14. Membrane separation was widely applied 41 for purification of extracted polyphenols <sup>15-18</sup> in a dead end and cross-flow modes <sup>16</sup>. The type 42 43 and characteristics of membranes influence importantly on the permeate flux and efficiency of polyphenols recovery. Microfiltration (MF) and ultrafiltration (UF) membranes have been 44 used to recover phenolic compounds from different juices and extracts, e.g. grape 45 pomace extracts <sup>15</sup>, apple cider <sup>19</sup>, and apple juice <sup>16,19,20</sup>. Different types of MF and UF 46 membranes were used as polyethersulfone (PES), polyvinylpyrrolidone (PVP) 16, 47 48 polyvinylchloride (PVC)<sup>21</sup>, polypropylene (PP)<sup>22</sup>, and ceramics<sup>20</sup>. However, membrane fouling can reduce permeate flux and decrease the polyphenols purification efficiency <sup>23-27</sup>. 49 50 Recently, the "force field-assisted methods" (including electrical, magnetic and sonic forces) have received much attention as novel techniques to modify the filtration performance and 51 prevent fouling <sup>27,28</sup>. The influence of electric field on the process of cross-flow membrane 52 filtration was studied <sup>29</sup>. There were many studies on membrane electro-filtration using direct 53 current (DC) electric field <sup>30,31</sup>. Membrane electro-filtration is a process in which charged 54 particles (such as proteins, organic colloids, and bacteria) are driven from the surface of the 55 membrane by applying an electric field <sup>32</sup>, which can reduce membrane fouling <sup>36</sup> and 56 increase permeate flux <sup>33,34</sup>. Previous studies have demonstrated that the application of DC 57 electric field could improve biopolymers recovery <sup>35</sup> in dead-end electro-filtration, enhance 58 permeate flux in cross-flow electro-ultrafiltration of sucrose-pectin juice <sup>36</sup>, protein solutions 59 <sup>33</sup>, and drinking water <sup>37</sup>. 60

61 Efficiency of membrane electro-filtration and selectivity of solutes purification depends from the solution characteristics (e.g. electrical conductivity, viscosity, ionic charge), 62 type and characteristics of the membrane, and parameters of electro-filtration (e.g. voltage and 63 transmembrane pressure), which should be optimised. The influence of electro-filtration 64 65 parameters on the selectivity of polyphenols purification in membrane processes was never 66 discussed before. This work studies the performance of the membrane electro-filtration 67 process for separation of polyphenols extracted from apple skins. The influences of DC 68 electric field, membrane pore size and transmembrane pressure on the selectivity of

69 polyphenols purification were investigated. The potential of low voltage DC electro-filtration

70 for improvement quality of apple skin extracts was also discussed.

## 71 **2. Materials and methods**

72 2.1 Material

Commercial red apples (Gala) were selected as raw material. Apples were purchased from the local supermarket (Compiegne, France), had a good quality and uniform nearspherical shape. The initial moisture content on wet basis of apple skin was 84.3%. It was determined with MA 160 infrared moisture analyzer (Sartorius, Germany).

## 77 2.2. Preparation of extracts

The apple skins were cut to small slices of 20 x 10 mm by a knife. The skin cuts were dried in a convection oven (UL50, MEMMERT, Germany) at 105 °C for 24h and subsequently ground into powder by a grinder (MCG2013B-16, 150 W, 50 Hz, Mandine, France) for 30s. The skin powders (20g) were subjected to extraction in 50% ethanol aqueous solution (200 mL) with solid/solution ratio at 1:10.

The ultrasound treatment was done directly in the glass beaker with an ultrasonic 83 84 processor UP 400S (400 W, 24 kHz, Hielscher GmbH, Stuttgart, Germany). The titanium 85 ultrasonic probe (H14, Hielscher GmbH, Stuttgart, Germany) with the tip diameter 14 mm 86 and the length of prove 100 mm was used. The ultrasound treatment was realised in pulsed 87 mode with sequential application of n=6 ultrasonic cycles as in the study <sup>38</sup>. Every ultrasound cycle had duration of  $\Delta t_u=3$  min and a pause between two cycles was  $\Delta t_w=2$  min. In order to 88 89 avoid temperature increase produced by ultrasound treatment, the glass beaker was put in an 90 ice/cold water box to keep the temperature at room temperature ( $\approx 25$  °C). The total time of 91 ultrasound-assisted extraction  $(t_e)$  of apple skins was 30 min.

92 Then the extracts were centrifuged at 4000 rpm for 10 min (Laborzentrifugen 3-10, 93 SIGMA, Osterode am Harz, Germany) to separate supernatant and solid sediment. The 94 ethanol in supernatant was removed by concentrating in a vacuum rotary evaporator 95 (LABOROTA 4001, Heidolph Instrument, Schwabach, Germany) at 40 °C. Obtained aqueous 96 solution was filtrated with filter paper (No.474, VWR, Geldenaaksebaan, Leuven), diluted by 97 6 times and stored in a refrigerator at 4 °C for further experiments. The concentration of total 98 polyphenols, concentration of proteins, concentration of total solutes, and colour intensity of extracts were 1.043 mg/mL, 0.097 mg/mL, 4.633% and 0.769, respectively. 99

## 100 2.3. Electro-filtration procedure

101 Setup for electro-filtration experiments is presented in Fig. 1. Dead-end electro-102 filtration was performed with effective membrane area of  $1.81 \times 10^{-3}$  m<sup>2</sup>. For each experiment, 103 90 mL of apple skin extract was used. 4 types of hydrophilic polyethersulfone filtration 104 membranes (Microdyn-Nadir GmbH, Germany) with different molecular weight cut-off and 105 pore size (Table 1) were used. New membrane was used for each set of experiments. 106 Filtration pressure of 1, 4, 6 and 10 bar was supplied and continuously monitored by a 107 pressure gauge. The experiments were done at room temperature, T=25 °C.

108 In order to avoid increase in temperature and degradation of extracted components the

109 DC low voltage ranged from 5 to 15 V supplied with generator FLUKE 45 and Consort

110 EV261 was used. The filtrate in the cathode (-) and anode (+) spaces was collected in beakers

111 placed on an electronic balance and the mass of filtrate was recorded by computer software

112 (Service électronique UTC, Compiègne, France).



Fig1. Schema of experimental device.

113 Table 1. Specifications of used hydrophilic polyethersulfone filtration membranes114 (Microdyn-Nadir GmbH, Germany)

Type of membrane	Molecular weight cut-off	Pore size, $d_m$
MP005	45660 kDa	50 nm
UP150	150 kDa	10 nm
UH030	30 kDa	4.35 nm
UP010	10 kDa	3.02 nm
UP150 UH030 UP010	150 kDa 30 kDa 10 kDa	10 nm 4.35 nm 3.02 nm

115 The sieving ratio R was defined as ratio of different substances presented in solution

116 after and before filtration (concentration of total polyphenols  $C_{tf}$ , concentration of proteins

117	$C_{pr}$ , concentration of total solutes $C_{ts}$ , and colour intensity I). The rejection coefficients of
118	different substances were calculated as $K=1-R$ .
119	For instance, the rejection coefficient $K_{tf}$ and sieving ratio $R_{tf}$ of total polyphenols are:
	$K_{tf} = 1 - R_{tf} = 1 - C_{tf} C_{tf0} \tag{1}$
120	where $C_{tf}$ and $C_{tf0}$ are the concentration of total polyphenols in filtrate and initial feed
121	(mg/mL), respectively. Similarly, the rejection coefficient $K_{pr}$ and sieving ratio $R_{pr}$ of proteins
122	are:
	$K_{pr} = 1 - R_{pr} = 1 - C_{pr} / C_{pr0} \tag{2}$
123	where $C_{pr}$ and $C_{pr\theta}$ are the concentration of proteins in filtrate and initial feed (mg/mL),
124	respectively. The rejection coefficient $K_{ts}$ and sieving ratio $R_{ts}$ of total solutes are:
	$K_{ts} = 1 - R_{ts} = 1 - C_{ts} / C_{ts0} \tag{3}$
125	where $C_{ts0}$ and $C_{ts0}$ are the concentration of total solutes in filtrate and initial feed, respectively.
126	The colourants rejection was estimated by the modification of colour intensity. The rejection
127	coefficient $K_I$ and sieving ratio $R_I$ of colour intensity were estimated as:
	$K_{I}=1-R_{I}=1-I/I_{0}$ (4)
128	where I and $I_0$ are the colour intensity of filtrate and initial feed, respectively.
129	Volume ratio $(V_r)$ of solution was calculated as:
	$V_r = V_f V_0 \tag{5}$
130	where $V_f$ and $V_0$ are the volume of final filtrate after filtration and initial feed (m <sup>3</sup> ),
131	respectively.
132	The purity of the filtrates toward polyphenols, P, was calculated as:
	$P = C_{t/}(C_{t/} + C_{pr}) \tag{6}$
133	where $C_{tf}$ and $C_{pr}$ are the concentration of total polyphenols and proteins in filtrate (mg/mL).
134	respectively.
125	2.4 Chamical analysis
155	2.4. Chemical analyses
136	The concentration of total polyphenols was determined with the Folin-Ciocalteu
137	method based on a colorimetric oxidation/reduction reaction of phenols <sup>39</sup> . 0.2 mL of sample
138	with diluted 20 times (distilled water as a blank), 1 mL of Folin-Ciocalteu reagent (Merck,
139	Darmstadt, Germany) and 0.8 mL of Na <sub>2</sub> CO <sub>3</sub> (75 g/L) (Prolabo, Fontenay-sous-Bois, France)
140	were introduced into the tube successively. The mixture was heated at 50 °C for 10 min and
141	subsequently cooled at 4 °C for 10 min. The absorbance of mixture was measured at 750 nm
142	by UV/Vis instrument (Thermo Spectronic Genesys 20, Thermo Electron Corporation, MA,
143	USA). Gallic acid (Sigma-Aldrich, St-Quentin Fallavier, France) was used for calculating the
144	calibration curve. The results were expressed as mg/mL.
	6

The concentration of proteins was determined with Bradford method as the details 145 presented in Technical Bulletin for Bradford Reagent (B 6916, Sigma-Aldrich)<sup>40</sup>. 7 different 146 concentration of BSA (Bovine Serum Albumin) standard solutions (0-25 µg/mL) were used 147 for calculating calibration curve. 0.2 mL of sample with diluted 20 times (distilled water as a 148 149 blank), 0.8 mL of Bradford reagent and 1 mL of distilled water were introduced into the tube 150 successively. The mixture was mixed with a Vortex, following the absorbance of mixture was measured at 595 nm by UV/Vis instrument (Thermo Spectronic Genesys 20, Thermo Electron 151 152 Corporation, MA, USA). The results were also expressed as mg/mL.

The colour intensity of extracts with diluted 10 times was measured by the adsorbance
 of mixture at 420 nm (A<sub>420</sub>) by UV/Vis instrument (Thermo Spectronic Genesys 20, Thermo
 Electron Corporation, MA, USA) <sup>41</sup>.

The concentration of total solutes was measured by a digital refractometer (Atago,
USA) at room temperature (≈25 °C). The results were expressed in °Brix (g of DM/100 g
solution, %).

159 2.5 Statistical analysis

All experiments and measurements were repeated at least in triplicate. Data were expressed as mean ± standard deviation. A probability value (p value) of less than 0.05 was considered statistically significant. The error bars in figures correspond to the standard deviations.

#### 164 **3. Results and discussions**

165 *3.1 Filtration without electric field* 

Figure 2 shows the dependence of membranes sieving ratios  $R_{tf}$ ,  $R_{pr}$ , an  $R_{ts}$ , and  $R_{I}$ from the size of pores  $d_m$  in absence of electric field and at the pressure of  $\Delta P$ =6 bar. The smallest sieving ratio was observed at  $d_m$ <10 nm for proteins ( $R_{pr}$ ) (Fig. 2) due to their bigger molecular size. The sieving ratios of total polyphenols ( $R_{tf}$ ), total solutes ( $R_{ts}$ ) and colour intensity ( $R_I$ ) were also smallest for the membranes with  $d_m$ <10 nm. These results are in conformity with earlier publications <sup>36,42,43</sup>.





Fig.3. Effect of transmembrane pressure  $\Delta P$  on the sieving ratios of total polyphenols  $R_{tf}$ , proteins  $R_{pr}$ , total solutes  $R_{ts}$ , and colour intensity  $R_l$  obtained for the membrane UH030  $(d_m=4.35 \text{ nm})$  in absence of electric field

179 Ultrafiltration membranes have a zero rejection for the low molecular weight 180 compounds such as sugar and salts, while reject the compounds with high molecular weight 181  $^{41}$ . For example, the rejection coefficient of total polyphenols  $K_{tf}$ , (Fig. 3) behaved as 182  $K_{tf}$ =0.03 $\Delta P$ +0.672 and the obtained data were similar to the previous studies  $^{16,44}$ .

Figure 4 shows the effect of pore size,  $d_m$  (b) and transmembrane pressure,  $\Delta P$  (a) on the volume ratio,  $V_r$  defined by Eq. (5). The values of  $V_r$  are lower for the membrane UP030 with smallest pore size  $d_m$  =4.35 nm (Fig. 4a) and they increase linearly with  $\Delta P$  ( $V_r$ =0.041 $\Delta P$ +0.584) (Fig. 4b). More details on the effects of the membrane pore size and filtration pressure on the filtration characteristics are presented in Supplementary Materials, Sections S1 and S2, respectively.





For further discussing of electro-filtration effects we have selected the ultrafiltration membrane UH030 ( $d_m$ =4.35 nm) and transmembrane pressure of  $\Delta P$  =6 bar.

191 *3.2 Effects of direct current voltage* 

192 Figure 5 shows the effect of DC voltage on the membrane sieving ratios obtained at 193 the anode (+) and cathode (-) parts of the filter. Only small differences in the concentration of 194 total polyphenols (and sieving ratio  $R_{tf}$ ) were observed for the filtrates at the anode (+) and 195 cathode (-) parts of the filter (Fig. 5a). For proteins, the value of sieving ratio  $R_{pr}$  increases 196 linearly with U in the cathode (-) part, whereas small quantity of proteins was obtained in the 197 anode (+) part. Such selectivity can be attributed to the positive charge of protein molecules. 198 The isoelectric point (IEP) value of almost 70% protein molecules of apple is in the range of 3-6<sup>45</sup>. In this study, the pH of apple skin extracts was 5.21, which is higher than the IEP of 199 200 protein molecules. Therefore, protein molecules possess the positive charges and do not 201 attracted by the anode. For total solutes, the sieving ratio  $R_{ts}$  somewhat increased with U at the 202 cathode (-) part and was practically unchanged at the anode (+) (Fig. 5b). For the colour 203 intensity of filtrates, the value of sieving ratio  $R_I$  decreased with increase of U in both the 204 cathode (-) and anode (+) parts.



Fig. 5. The sieving ratio of (a): total polyphenols  $R_{tf}$  and proteins  $R_{pr}$ , and (b): total solutes  $R_{ts}$  and colour intensity  $R_t$  in function of DC voltage. Anode is indicated as (+) and cathode as (-). Ultrafiltration was realised with membrane UH030 ( $d_m$ =4.35 nm) under transmembrane pressure of  $\Delta P$  =6 bar.

Figure 6 shows the effect of the DC voltage U on the rejection coefficients and volume ratio  $V_r$ . The biggest part of proteins was rejected even without electric field application and the value of  $K_{pr}$  remained high at DC application (Fig. 6a). For total polyphenols, total



solutes, and colour intensity, the near-linear dependences of the rejection coefficient wereobserved

 $K_{tf} = -0.138U + 11.327, r^2 = 0.943$  (8)

 $K_{ts} = -1.125U + 31.199, r^2 = 0.987$ (9)

(10)

 $K_I = 0.531U + 23.694, r^2 = 0.998$ 

210 The value of  $V_r$  increased linearly with  $U(V_r=0.006U+0.885)$  (Fig. 6b). Thus the DC

filtration allowed better purification of polyphenols and obtaining larger volume of filtrates.More details on the effect of the applied voltage on filtration characteristics are presented in

213 Supplementary Materials, Section S3.



Fig.6. Effect of electric field on the rejection coefficient (total polyphenols  $K_{tfs}$  proteins  $K_{pr}$ , total solutes  $K_{ts}$ , and colour intensity  $K_l$ ) of filtrates in function of DC voltage (a) and the volume ratio,  $V_r$  (b) after filtration.

Figure 7 shows the effect of the DC voltage U on the purity of the filtrates toward polyphenols, P. P-values reflect the selectivity of polyphenols purification. The value of P obtained for the non-filtered extract was rather low,  $P=91.49\pm1.62\%$  (open symbol in Fig.7). After the simple membrane filtration without electric field application (U=0 V), the purity P increased significantly (up to 91.49%). Membrane electro-filtration permitted obtain even higher purity of filtrate P=99.87% (at U=5 V) than in previously reported data<sup>11</sup>. Thus the DC filtration was beneficial for the selectivity of polyphenols purification from apple extracts.



Fig.7. Effect of electric field on the purity of the filtrates toward polyphenols, P.

#### 221 Conclusions

The dead-end electro-filtration was studied for the purification of polyphenols extracted from apple skin. Ultrafiltration membrane with small size of pores (UH030,  $d_m$ =4.35 nm) allowed effective rejection of proteins. It was attributed to the large size of protein molecules and their positive charge. Obtained data evidenced the possibility of selective purification of polyphenols by electro-filtration process.

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361362Fig. 1. Schema of experiment device.363364Fig. 2. Effect of pore size, $d_m$ , of membranes on the sieving ratio (total polyphenols $R_{tf}$ ;365proteins $R_{pr}$ , total solutes $R_{ts}$ , and colour intensity $R_l$ ) of filtrates at fixed transmembrane366pressure, $\Delta P=6$ bar, and in absence of applied voltage, $U=0$ V.367368368Fig. 3. Effect of transmembrane pressure, $\Delta P$ , on the sieving ratio (total polyphenols $R_{tf}$ ;369proteins $R_{pr}$ , total solutes $R_{ts}$ , and colour intensity $R_l$ ) of filtrates for membrane UH030370 $(d_m=4.35 \text{ nm})$ in absence of applied voltage, $U=0$ V.371
<ul> <li>Fig. 1. Schema of experiment device.</li> <li>Fig. 2. Effect of pore size, d<sub>m</sub>, of membranes on the sieving ratio (total polyphenols R<sub>tf</sub>, proteins R<sub>pr</sub>, total solutes R<sub>ts</sub>, and colour intensity R<sub>l</sub>) of filtrates at fixed transmembrane pressure, ΔP=6 bar, and in absence of applied voltage, U=0 V.</li> <li>Fig. 3. Effect of transmembrane pressure, ΔP, on the sieving ratio (total polyphenols R<sub>tf</sub>, proteins R<sub>pr</sub>, total solutes R<sub>ts</sub>, and colour intensity R<sub>l</sub>) of filtrates for membrane UH030 (d<sub>m</sub>=4.35 nm) in absence of applied voltage, U=0 V.</li> </ul>
363364Fig. 2. Effect of pore size, $d_m$ , of membranes on the sieving ratio (total polyphenols $R_{tf}$ ;365proteins $R_{pr}$ , total solutes $R_{ts}$ , and colour intensity $R_l$ ) of filtrates at fixed transmembrane366pressure, $\Delta P$ =6 bar, and in absence of applied voltage, $U$ =0 V.367368368Fig. 3. Effect of transmembrane pressure, $\Delta P$ , on the sieving ratio (total polyphenols $R_{tf}$ ;369proteins $R_{pr}$ , total solutes $R_{ts}$ , and colour intensity $R_l$ ) of filtrates for membrane UH030370 $(d_m$ =4.35 nm) in absence of applied voltage, $U$ =0 V.371
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367 368 Fig. 3. Effect of transmembrane pressure, $\Delta P$ , on the sieving ratio (total polyphenols $R_{t/5}$ 369 proteins $R_{pr}$ , total solutes $R_{ts}$ , and colour intensity $R_l$ ) of filtrates for membrane UH030 370 $(d_m=4.35 \text{ nm})$ in absence of applied voltage, $U=0 \text{ V}$ . 371
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1	Supplementary materials for the Manuscript
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3	Purification of polyphenols from apple skins by membrane electro-
4	filtration: Effects of pore size, pressure and applied voltage
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14	
15	S1 EFFECT OF MEMBRANE PORE SIZE ON FILTRATION PERFORMANCE
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Table. S1. Parameter  $\alpha C_{cs}$ , and the membrane resistance,  $R_m$ , of apple skin extracts after filtration with different pore size of membranes,  $d_m$ .

Membrane	$d_m$ , nm	$\alpha C_{cs}$ , m/kg	$d_{\alpha Ccs}$	$R_m, m^{-1}$	$d_{Rm}$
MP005	50	$1.758*10^{12}$	$1.993*10^{11}$	2.142*10 <sup>12</sup>	$2.732*10^{11}$
UP150	10	2.759*10 <sup>12</sup>	2.023*10 <sup>11</sup>	$2.541*10^{12}$	1.996*10 <sup>11</sup>
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51 of  $\alpha C_{cs}$  is a measure of membrane fouling due to deposit formation, the higher value of  $\alpha C_{cs}$ 

52 presents the higher fouling of membrane.  $R_m$  is the membrane resistance, and smaller pore 53 size of membrane owns larger resistance to pass through macromolecular species. Figure S2 54 shows the effect of membrane pore size,  $d_m$ , on filtration performance ( $\alpha C_{cs}$  (a) and  $R_m$  (b)). 55 The results showed that the membrane fouling and resistance were both decreased with the 56 increase of  $d_m$ . Moreover the effect of membrane pore size,  $d_m$ , on membrane resistance,  $R_m$ , 57 was more noticeably than on the membrane fouling,  $\alpha C_{cs}$ .

### 58 S2 Effect of transmembrane pressure on filtration performance



Fig. S3. Filtration curves of apple skin extract with membrane UH030 by different transmembrane pressure,  $\Delta P$ : (a) Filtrate flux *J* vs. time *t*; (b) ratio of filtration time and filtrate volume t/V vs. filtrate volume V. Here the pore size of membrane,  $d_m$ =4.35 nm, the transmembrane pressure,  $\Delta P$ =1-10 bar.

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Table. S2. Parameter  $\alpha C_{cs}$ , and the membrane resistance,  $R_m$ , of apple skin extracts after filtration with different  $\Delta P_c$ .

$\Delta \mathbf{P}$ , bar	$\alpha C_{cs}, m/kg$	$d_{\alpha Ccs}$	$R_m, m^{-1}$	$d_{\text{Rm}}$
1	$2.813*10^{13}$	$7.006*10^{10}$	$2.313*10^{13}$	$2.312*10^{11}$
4	$1.138*10^{13}$	$1.506*10^{11}$	$4.740*10^{13}$	$1.152*10^{12}$
6	$5.751*10^{12}$	$8.447*10^{10}$	5.440*10 <sup>12</sup>	$6.323*10^{11}$
10	$6.526*10^{12}$	$1.279*10^{11}$	4.868*10 <sup>12</sup>	$1.690*10^{11}$

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Fig. S5. Filtration curves of apple skin extract with membrane UH030 by different voltage of direct current, U: (a) Filtrate flux J vs. time t; (b) ratio of filtration time and filtrate volume t/V vs. filtrate volume V. Here the pore size of membrane,  $d_m$ =4.35 nm, the transmembrane pressure,  $\Delta P$ =6 bar, the voltage of direct current, U=0-15 V.

Table. S3. Parameter  $\alpha C_{cs}$ , and the membrane resistance,  $R_m$ , of apple skin extracts after filtration with different voltage of direct current, U.

<i>U</i> , V	$\alpha C_{cs}$ , m/kg	$d_{\alpha Ccs}$	$R_m, m^{-1}$	$d_{Rm}$
0	$5.751*10^{12}$	$8.447*10^{10}$	5.440*10 <sup>12</sup>	$6.323*10^{11}$
5	4.132*10 <sup>12</sup>	$9.557*10^{10}$	3.452*10 <sup>12</sup>	8.863*10 <sup>10</sup>
10	$3.002*10^{12}$	8.686*10 <sup>10</sup>	9.647*10 <sup>11</sup>	$4.741*10^{11}$
15	3.124*10 <sup>12</sup>	$4.102*10^{10}$	7.260*10 <sup>11</sup>	$2.840*10^{11}$

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108         Membrane Cleaning by Different Ultrasonic Frequencies. Desalination 2010, 263 (1-           109         3), 133–138.           110           111	107	(3)	Cai, M.; Zhao, S.; Liang, H. Mechanisms for the Enhancement of Ultrafiltration and
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#### V.4 Conclusions

As discussed in Chapter V, the efficiency of polyphenols purification from apple peel extracts with adsorption/desorption process using ultrasound and with membrane electro-filtration was evidenced.

For polyphenols purification with adsorption/desorption process, the effects of temperature (T=25-40 °C) and ultrasound intensity (P=0-400 W) on adsorption capacity were studied. In addition, the effect of ethanol concentration ( $C_{et}=0.96\%$ ) in solution on desorption ratio was analyzed. The obtained results showed that the sonication during the adsorption step could significantly facilitate adsorption kinetics, increase adsorption capacity and activation energy of polyphenols adsorption, improve the desorption ratio and decrease purification time. The highest recovery of polyphenols in extracts reached 68.9 % with adsorption using ultrasound (P=50 W) and with desorption in aqueous ethanol solution at the concentration of ethanol ( $C_{et}=50\%$ ).

For polyphenols purification with membrane electro-filtration, the influence of membrane pore size ( $d_m$ =3.02-50 nm), transmembrane pressure ( $\Delta P$ =1-6 bar) and direct current (DC) electric field (U=5-15 V) on the purification efficiency was investigated. It was demonstrated that UH030 membrane used for DC electro-filtration prevents proteins passage to permeate. In addition, electro-filtration under DC voltage of 5 V and pressure of 6 bar permitted reduce filtration time and increase filtration flux. With this process parameters, effective purification of polyphenols (99.87%) compared with proteins from apple peel extracts was attained.

Obtained results evidenced a good efficacy of adsorption/desorption process assisted by sonication for polyphenols purification from apple peel extracts. Selective purification of polyphenols from apple peels with membrane electro-filtration was also demonstrated.

## **General conclusion and prospects**

Polyphenols of apple products are usually extracted with organic solvent, which leads environmental pollution and complicates extracts purification. Moreover, conventional extraction and purification technologies are time and energy consuming.

This thesis is devoted to the study of polyphenols extraction from apple products (apple flesh, peels and pomace) using ultrasound, and to the process optimization. The subsequent purification of polyphenols from apple peel extracts by adsorption/desorption and membrane filtration were also investigated.

The obtained results demonstrated that ultrasound can significantly increase extraction yields of total polyphenols and catechin compared to conventional extraction (CE). Interestingly, ultrasound assisted extraction (UAE) permitted more selective polyphenols recovery. The selectivity of catechin extraction (*R*) was depended on the type of apple tissue (flesh, peel or pomace), apple variety (green or red), extraction temperature, UAE protocols, and ethanol/water ratios. For studied apple products, the values of *R* were noticeably higher using UAE as compared to CE. For apple flesh and peels, the value of *R* was nearly constant for flesh of red apples (*R*=19%), and peel of green apples (*R*=16%). However for flesh of green apples and peel of red apples, the value of *R* was dependent on the ultrasound protocols. For apple pomace, using the UAE with low intensity and short duration leaded to more selective extraction of catechin (*R*≈15-31%). However, using intensive UAE and extraction in ethanol/water solution (concentration of ethanol, C<sub>et</sub>=50%) resulted in a high level of TPC (≈850mg/100g DM) and lower content of catechin (*R*≈2%).

The cavitation phenomena generated by ultrasound contribute to increase extraction of valuable components from fruit peels. Cavitation bubbles damaged cell tissue and accelerated heat and mass transfer. Cavitation phenomena were analysed in terms of the behavior of ionic solutes  $Z_i$ , total polyphenols,  $Z_{tp}$ , and cell disintegration  $Z_m$  indexes. The values of  $Z_i$  and  $Z_{tp}$  depended on the values of  $Z_m$  and presented linear relationship for banana and persimmon peels, while these dependencies were nonlinear for apple peels. In addition, the gas water solvents (CO<sub>2</sub> with concentration of 5.28 mmol/L) increased the extraction yields of polyphenols (TPC, total flavonoid content and proanthocyanidins content) by enhancing cavitation phenomena. The produced cavitation effect may increase the water absorption by samples, which contributes to better phenolics extraction.

Adsorption/desorption procedure using the polyaromatic amberlite adsorbent XAD-16 and assisted by sonication was beneficial for the purification of polyphenols from apple peel

extracts. The isotherm of polyphenol adsorption was well fitted using the Freundlich model. The adsorption kinetics was fitted with stretched exponential law. The data on adsorption kinetics and static isotherm evidenced the presence of adsorption on heterogeneous surface with a broad distribution of adsorption times that can depend on content of polyphenols in the solutions and applied power of sonication. Moreover, the obtained results showed that the sonication during the adsorption step could significantly facilitate absorption kinetics, increase adsorption capacity and decrease adsorption time. The studies of desorption process revealed the optimum desorption efficiency of polyphenols at ethanol concentration of 50%. The desorption ratio was positively associated with the sonication during the adsorption step. After adsorption and desorption processes, the highest recovery of polyphenols in apple peel extracts reached 68.9 % with sonication.

Dead-end electro-filtration with DC voltage of 5 V and pressure of 6 bar permitted effectively prevent proteins from passing through ultrafiltration membrane UH030 with size of pores ( $d_m$ =4.35 nm). The sieving ratio of total polyphenols,  $R_{tp}$  in filtrate increased linearly with the increase of transmembrane pressure and was independent from membrane pore size. This process permitted better purifying of polyphenols.

The US-assisted extraction is able to increase the extraction yields, improve the quality of the extracts and facilitate the subsequent separation and purification processes. Application of adsorption/desorption procedure assisted by ultrasound can effectively improve the purity of polyphenols and decrease purification time. Membrane electro-filtration permits obtain selective purification of polyphenols without using organic solvents. Generally, this research work provides a better understanding of polyphenols extraction and purification with ultrasound treatment.

The results obtained from this thesis raise some new questions and suggest some future prospects:

1. For the extraction:

1) Conduct a study on the combination of US with different treatment techniques (such as microwaves (MW), pulsed electric field (PEF), high voltage electrical discharges, supercritical  $CO_2$ , etc.) to find an optimal protocols of bio-compounds extraction from apple peels. For instance, MW application permits rapid heating the samples, which is beneficial to faster solvent penetration into samples, migration of dissolved molecules and better solubility of bio-compounds. As a consequence, US and MW can produce a synergistic effect for the extraction of bio-compounds.

2) Deepen investigation of different effects of US treatment, such as capillarity, detexturation, tissue fragmentation, local shear stress, sonocapillary effect and sonoporation. Cavitation phenomenon generated by ultrasound is often considered as a most important for plant tissue damage increasing extraction yield. However, during US-assisted extraction, other phenomena intensifying bio-compounds recovery occur, which needs their analysis.

3) Design an efficient ultrasound-assisted large-scale extraction with high extraction yields and low energy consumption for applications in industry.

2. For the purification:

1) Better understanding of phenolics adsorption/desorption mechanisms with resins is still needed.

2) A combination of US and other methods to improve the efficiency of purification during adsorption/desorption process will be interesting. For instance, US technology may be combined with vapour pressure and external electrostatic field.

3) Circulation and controllable temperature in membrane electro-filtration method need to be studied. During the membrane electro-filtration, the electrode chamber can be continuously flushed by circulating tap water. Although the flowing tap water can implement circular filtrating and keep a constant temperature, the tap water leads to the dilution of extracts and further decrease purification ratio. Therefore, it is significant to find solutions to implement circular membrane electro-filtration.

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