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**UNIVERSITE D'AVIGNON ET DES PAYS DE VAUCLUSE**

# THESE

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de l'Université d'Avignon et des Pays de Vaucluse

**SPECIALITE : CHIMIE**

# Application des ultrasons aux procédés de transformation des produits agroalimentaires

**par**

*Daniella PINGRET*

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*To my mother.*

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# *Scientific Publications*

## *Publications*

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Emmanuel Petitcolas, Jean-Paul Canselier, Farid Chemat.
  First Investigation on Ultrasound-Assisted Preparation of Food Products: Sensory and Physicochemical Characteristics.
  *Journal of Food Science 76 (2): C287-C292,* ***2011.***

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Carine Le Bourvellec, Catherine M.G.C. Renard, Farid Chemat.
  Lab and pilot-scale ultrasound-assisted water extraction of polyphenols from apple pomace.
  *Journal of Food Engineering. 111(1): 73-81,* ***2012.***

- Daniella Pingret, Grégory Durand, Anne-Sylvie Fabiano-Tixier, Antal Rockenbauer, Christian Ginies, Farid Chemat.
  Degradation of edible oils during food processing by ultrasounds: electron paramagnetic resonance, physicochemical and sensory analysis
  *Journal of Agricultural and Food Chemistry, 60: 7761-7768,* ***2012****.*

- Karim Assami, Daniella Pingret, Smain Chemat, Brahim Y. Meklati, Farid Chemat.
  Ultrasound induced intensification and selective extraction of essential oil from *Carum carvi* L. seeds.
  *Chemical Engineering and Processing: Process Intensification,* ***2012, in press****.*

- Farid Chemat, Daniella Pingret, Timothy J. Mason.
  Sono-Soxhlet: In situ ultrasound assisted extraction of natural products.
  *Journal of Chromatography A, submitted.*

- Karim Assami, Smain Chemat, Daniella Pingret, Brahim Y. Meklati, Anne-silvie Fabieno-Tixier, Farid Chemat.
  The use of ultrasound extraction for flavoring olive oil with caraway.
  *Journal of the Science of Food and Agriculture, Submitted*

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Farid Chemat
  Degradation during application of ultrasound in food processing: A Review
  *Food Control, submitted.*

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Farid Chemat
  An improved ultrasound Clevenger for extraction of essential oils from orange peels
  *Journal of Chromatography A, submitted.*

## *Book Chapters*

- Daniella Pingret, Anne-Sylvie Fabiano-tixier, Farid Chemat.
  Chapter 5.19. Accelerated Methods for Sample Preparation in Food.
  *Comprehensive Sampling And Sample Preparation. Editor J. Pawliszyn.*
  *Elsevier, **2012**,* p. 441-455. Oxford: Academic Press.

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Farid Chemat.
  Chapter 4. Ultrasound-assisted extraction. *In: Natural Products Extraction: Principles and Applications. Editors M.A. Rostagno and J.M. Prado.*
  *Royal Society of Chemistry, **2012**, in press.*

## *Communications*

- Daniella Pingret, Gregory Durand, Anne-Sylvie Fabiano-Tixier, Farid Chemat. *Compréhension de la dégradation des huiles végétales traitées aux ultrasons.* 6èmes Journées Franco-Italiennes de Chimie, April 16-17, **2012**, Marseille, France. (oral communication)

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Farid Chemat. *Impact evaluation of ultrasound on processed vegetable oils*. CIGR Section VI International Symposium, April 18-20, **2011**, Nantes - France. (oral communication)

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Farid Chemat. *Etude de l'impact des ultrasons sur des huiles végétales*. Journée de l'Ecole Doctorale Sciences et Agrosciences, June 16, **2011**, Avignon – France. (poster communication)

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Farid Chemat. *Evaluation de l'impact d'un traitement ultrasonore sur des huiles végétales*. 22ème Journée de la Chimie SCF-PACA, Mars 11, **2011**, Toulon – France. (poster communication)

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Carine Le Bourvellec, Catherine M.G.C. Renard, Farid Chemat. *Optimized ultrasound-assisted water extraction of polyphenols from apple pomace*». Fruit and Vegetable Processing, April 18-21, **2011**, Avignon – France. (poster communication)

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Emmanuel Petitcolas, et Farid Chemat. *Clean extraction of polyphenols from dried apple pomace assisted by ultrasound*. Giornate Italo-Francesi Di Chimica - SCF, April 26-27, **2010**, Genova - Italy. (poster communication)

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Emmanuel Petitcolas, et Farid Chemat. 2010. *Ultrasound-Assisted Preparation of Food: Sensory and Physicochemical Characteristics*. 12th European Society of Sonochemistry Meeting, May 30 – June 3, **2010**, Crete - Greece. (poster communication)

*"The true value of things does not reside in the time they last,*
*but in the intensity with which they occur. That is why there are unforgettable*
*moments, inexplicable things and incomparable people."*

*Fernando Pessoa*

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## *SECOND PART*

# *General Introduction*

# Introduction Générale

De tous temps, les chimistes ont cherché à maîtriser ou à accélérer des réactions chimiques par un flux d'énergie d'ordre mécanique, thermique ou électromagnétique. Le simple fait de chauffer ou de mélanger un milieu réactionnel est un de ces moyens de contrôle. Les avancées technologiques ont fait naître des applications encore plus spécifiques qui sont maintenant utilisées dans notre quotidien. Les ultrasons de puissance, par exemple, participent à cette maîtrise, tout en réduisant au maximum la consommation d'énergie par la sélectivité de leurs actions mécaniques et thermiques qui interviennent au sein même du milieu. L'utilisation des ultrasons dans les procédés alimentaires pour le traitement, la préservation et l'extraction est de plus en plus courante. Ces derniers mettent en jeu à la fois des phénomènes physiques et chimiques qui diffèrent fondamentalement des procédés classiques d'extraction, de transformation et de conservation. Les ultrasons offrent donc un certains nombre d'avantage en termes de productivité, de rendement et de sélectivité, avec des temps de traitement optimisé, une amélioration de la qualité, une réduction des risques physiques et chimiques ainsi que le respect de l'environnement.

Depuis plusieurs années, les industriels de l'agroalimentaire sont à la recherche de nouvelles technologies de transformation et de préservation des aliments. Les nouvelles tendances alimentaires, la diminution ou la prohibition de certains additifs et le poids de la sécurité alimentaire incitent les chercheurs à trouver de nouveaux moyens pour améliorer les divers procédés agroalimentaires conventionnels. L'histoire a commencé en 1927 quand Richards et Loomis publièrent leur article ayant pour titre: « The chemical effect of high frequency sound waves: a preliminary survey ». Leurs travaux décrivaient les applications des ultrasons de puissance dans une gamme de procédés incluant l'émulsification et le nettoyage des surfaces. De nos jours, le nettoyage, le dégazage, le démoulage et la découpe par ultrasons sont devenus des opérations courantes dans les industries agroalimentaires.

Dans une première partie de ce mémoire nous présenterons les phénomènes physiques régissant la technologie des ultrasons, la façon dont les ultrasons sont produits ainsi que leurs cheminements jusqu'à la zone de traitement de l'échantillon. Nous développerons par la suite deux exemples d'applications des ultrasons, tout d'abord dans l'extraction de drèches de pommes puis dans la préparation de produits alimentaires.

Dans une seconde partie, nous nous sommes intéressés aux phénomènes de dégradation observés lors de nos expérimentations mais également à l'issu d'une étude bibliographique approfondie. Nous avons pour cela étudié la dégradation des huiles végétales après traitement ultrasonore et essayé de comprendre le phénomène ainsi que le mécanisme d'action de ces derniers afin de pouvoir l'éliminer ou le limiter.

# *First Part*

# First Part

## *Ultrasound-Assisted Processing for Food Applications*

### *Summary*

Ultrasound has been increasingly used in the food domain in processing, preservation and extraction processes. The effects of ultrasound arise from the cavitation phenomena caused by the irradiation of ultrasonic waves through the medium. The advantages of ultrasound are extensive, overcoming the shortcomings of conventional techniques on solvent and energy consumption, allowing a shorter time of treatment with a low cost of implementation. In the first chapter, the mechanism of ultrasound will be revised, as also the general uses of ultrasound in food processing techniques, followed by the uses in preservation and in extraction. In the second chapter, an application of ultrasound-assisted water extraction of polyphenols from apple pomace will be presented, highlighting the benefits of the technique compared to the conventional extraction. In the third chapter, an application of ultrasound to assist food preparation is presented, showing the potentials in the use of ultrasound in the direct preparation of food products.

# Chapter I

## *Applications of ultrasound in processing, preservation and extraction*

- Daniella Pingret, Anne-Sylvie Fabiano-tixier, Farid Chemat.
  Chapter 5.19. Accelerated Methods for Sample Preparation in Food. In: Comprehensive Sampling And Sample Preparation. *Editor J. Pawliszyn.*
  *Elsevier, **2012**, in press.*

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Valérie Tomao, Camille Viot, Farid Chemat.
  Chapter 4. Ultrasound-assisted extraction. In: Natural Products Extraction: Principles and Applications. *Editors M.A. Rostagno and J.M. Prado.*
  *Royal Society of Chemistry, **2012**, in press.*

## 1.1. Introduction

Ultrasound has been considered an innovative and promising technique of the 21st century, with numerous applications in the pharmaceutical, cosmetic, chemistry and alimentary fields since the second half of the 20th century.

Ultrasound can be applied for either laboratory experiments or industrial scale procedures and numerous industrial facilities have been successfully applying the technique in the food processing, preservation and extraction. Different ultrasonic devices have been developed and adapted for each application in order to obtain the best results. In this chapter, the review of ultrasound theory and main influencing parameters applied to food industry will provide a better understanding of the mechanism of action by which this technique is more advantageous over conventional procedures, with a review of the main used devices at laboratory and industrial scales.

The applications of ultrasound in the food processing are extensive and arise mainly from the physical effects of ultrasound. Sonication has been successfully applied to filtration, defoaming, degassing, cooking, cutting, freezing, drying, sterilization, emulsification and homogenization. The application of ultrasound allow the use of milder conditions over a shorter time of processing when compared to conventional techniques, which results in enhanced quality products with a lower cost.

From biochemical effects of ultrasound, numerous applications in food preservation have been developed by inactivation microorganisms, spores and enzymes. Sonication will rupture cell walls and denature enzymes and when combined to other procedures, the efficiency of inactivation processes will be improved.

Also, ultrasound is used in assisted extraction procedures in a wide range of matrixes for further direct or indirect applications of the target compounds. In this chapter the main matrixes are presented such as fruits and vegetables, herbs and spices and oleaginous seeds. The proposed benefits of ultrasound-assisted extraction in the food industry include enhancement of overall extraction rate and yield, possibility of use of alternative solvents, use of cheaper raw product sources, and enhancing extraction of heat sensitive compounds.

## 1.2. Ultrasound principle

Ultrasounds are mechanical waves that necessitate an elastic medium to spread over and differ from sounds by the wave frequency (Figure I.1). The audible frequencies to humans are comprised between 16 and 20 kHz, while ultrasound frequencies range from 20 kHz to 10 MHz. From this large range of frequency, two main groups are distinguished and both are used in the food industry: diagnostic and power ultrasound (Mason et al., 2005). The main physical parameters that characterize ultrasound are the power (W), the frequency (Hertz) and the wavelength (cm), from which the ultrasonic intensity (I) is calculated (W. $cm^{-2}$). Diagnostic ultrasound (also called high frequency ultrasound) range from 2 to 10 MHz ($I < 1$ $W.cm^{-2}$) and is used in several fields such as medical imaging or even for defect detection (bond inspection for plastics). Conventional power ultrasound (also called low frequency ultrasound) range from 20 kHz to 100 kHz ($I > 1$ $W.cm^{-2}$). An extended range is used in sonochemistry (20 kHz to 2 MHz) and in this range, ultrasound is able to produce physical and/or chemical effects into the medium in order to facilitate or accelerate chemical reactions or even for other applications in the industry (cutting, plastic welding). A low power, and high frequency ultrasound is a non-destructive way of gaining structural and/or chemical information on the used medium.

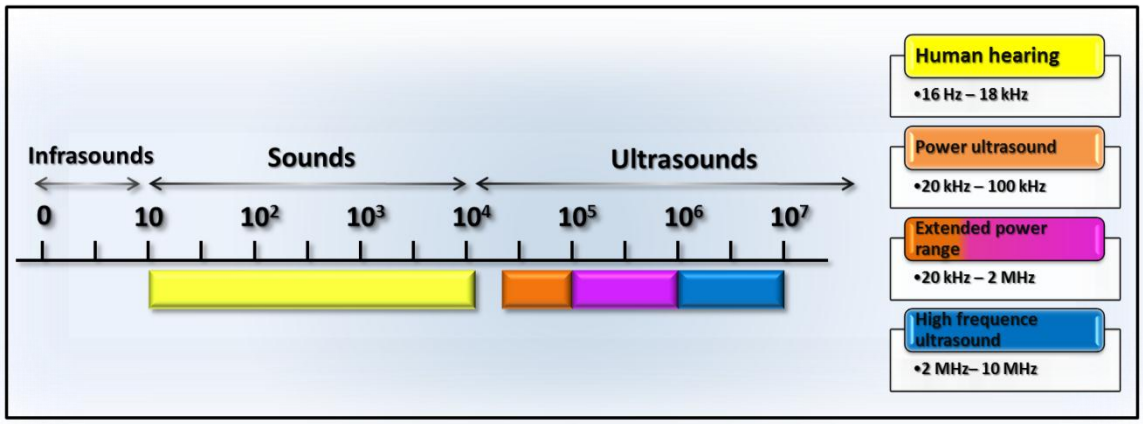


**Figure I.1.** Frequency ranges.

The major effects of ultrasound in a liquid medium are attributed to the cavitation phenomena, which are issued from the physical processes that create, enlarge and implode micro bubbles of gases dissolved in the liquid. The molecules that constitute the liquid medium are held together by attractive forces and as an ultrasound wave passes through an

elastic medium (Figure I.2), it induces a longitudinal displacement of those molecules, acting as a piston on the surface, resulting from a succession of compression and rarefaction phases (McClements, 1995). The molecules that form the liquid are temporarily dislodged from their original position and during the compression cycle they can collide with the surrounding molecules. During the rarefaction phase, a negative pressure will be exerted, pulling the molecules apart. The extent of the negative pressure depends on the nature and purity of the liquid. At a sufficient higher power, the attraction forces between them might be exceeded when the local pressure falls below the vapor pressure of the liquid, generating a void in the liquid. The voids created into the medium are the cavitation bubbles which are formed from dissolved gases (Suslick, 1989; Mason et al., 2005; Patist and Bates, 2008).

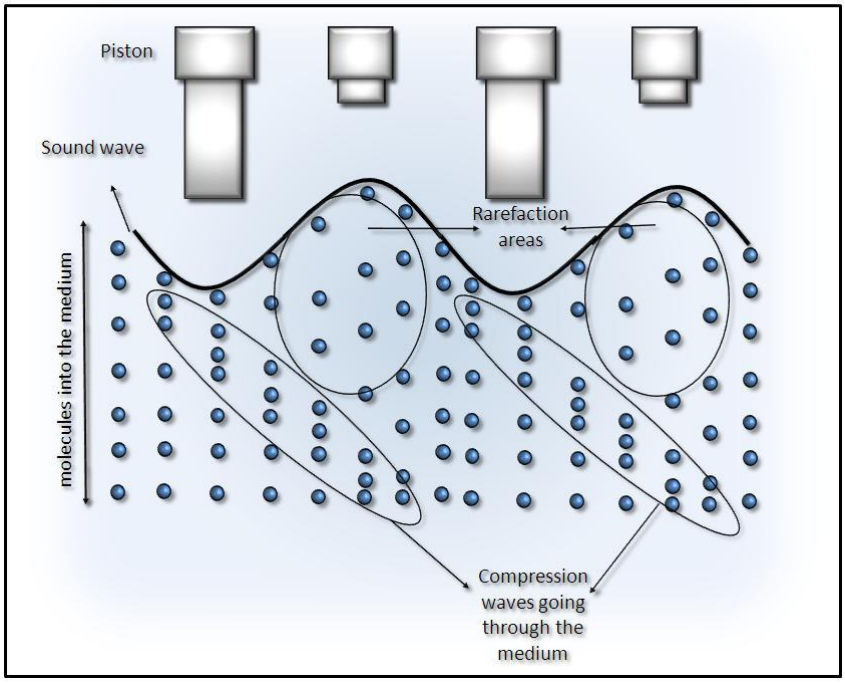


**Figure I.2.** Compression and rarefaction cycles induced by a sound wave.

In fact, theses cavitation bubbles are able to grow by rectified diffusion, since vapors (or gas from the medium) will enter the bubble during rarefaction phase and will not fully be expelled compression cycle. The negative transient pressures within the fluid, induces the growth of the bubbles and the production of simultaneous bubbles by the tensioning effect on the fluid (Mason et al., 2005). The following compression cycle provokes the decrease of the bubble size causing the partial absorption of the vapor, being repeated for many cycles until the size of the bubble is decreased in a manner that the bubble wall matches that of the applied frequency. When the size of theses bubbles reach this critical point they collapse during a compression cycle and, since heating is more rapid than thermal transport, a

transitory hot spot is created (Flint and Suslick, 1991; Suslick et al., 1999). The temperature and the pressure at the moment of collapse have been estimated to be up to 5000 K and 5000 atmospheres in an ultrasonic bath at room temperature, creating hotspots that are able to accelerate dramatically the chemical reactivity into the medium (Flint and Suslick, 1991; McNamara et al., 1999; Suslick et al., 2011).

When these bubbles collapse onto the surface of a solid material, the high pressure and temperature released generate microjets and shock waves directed towards the solid surface (Vinatoru, 2001). In the food industry, these microjets can be applied to the extraction of vegetal compounds (Figure I.3). The cavitation bubble generated close to the plant material surface (a) collapses during a compression cycle (b) and a microjet directed toward the surface is created (b and c). The high pressure and temperature involved in this process will destroy the cell walls of the plant matrix and its content will be released into the medium (d).

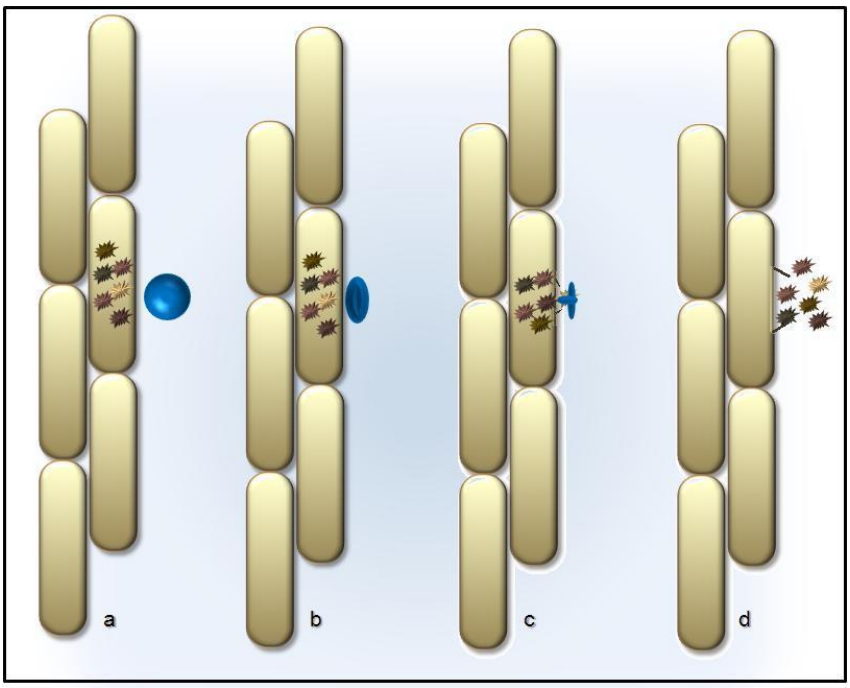


**Figure 1.3.** Collapse of cavitation bubble and release of plant material.

## 1.3. Influencing parameters

Important physical parameters related to Ultrasound-Assisted Extraction are presented in this section. Ultrasound power, temperature, and solvent type affect not only the extraction yield but also the composition of the extract and should thus be taken into consideration. The

food matrix and the target molecules for UAE should be carefully considered as a parameter also.

### *1.3.1. Solvent type*

Solvent choice is dictated by the solubility of the target analytes in the solvent but also by physical parameters such as viscosity, surface tension and vapor pressure of the medium. For cavitation bubbles to be effective, the negative pressure during the expansion cycle has to overcome the natural cohesive forces in the medium. The rise of viscosity increases these molecular interactions and therefore a significant increase of the cavitation threshold is observed. In the same way, a high surface tension decreases cavitation phenomena. Vapor pressure is also directly correlated with the temperature factor, which influences cavitation as well. Therefore, the solvent of choice for UAE should ideally have a very low vapor pressure and the ability to solubilize the molecules of interest (Flannigan and Suslick, 2010).

### *1.3.2. Temperature*

The temperature increase generates the rise of the vapor pressure and the decrease of the viscosity and surface tension, inducing more solvent vapors to enter the bubble cavity, reducing the pressure difference between the inside and outside of the bubble, which will collapse less violently and reduce sonication effects (Santos et al., 2008). As a consequence, at higher temperatures, cavitation can be achieved at lower amplitudes. However, the sonochemical effects of such bubbles may be reduced and the use of temperatures above a certain threshold might generate cavitation bubbles that grow very quickly, diminishing its efficacy. For extraction purposes, a higher temperature might result in a higher efficiency due to an increase in the number of cavitation bubbles and a larger solid-solvent contact area, as also an enhancement of solvent diffusivity with consequent enhancement of desorption and solubility of the interest compounds. However, this effect is decreased when the temperature is near the solvent's boiling point, since bubbles implosion might not induce sufficient energy shear forces to disrupt cell tissues (Palma and Barroso, 2002; Zhang et al., 2008; Esclapez et al., 2011). It is important to note also that a temperature control is imperative to prevent the degradation of thermolabile compounds.

### *1.3.3. Ultrasonic intensity*

Intensity can be expressed as the energy transmitted per second and per square meter of medium. This parameter is directly correlated with pressure amplitude of sound wave; with increase in the pressure amplitude, bubble collapse will be more violent. This last parameter influences directly the acoustic pressure generated when ultrasound are applied to an elastic medium. The increase of intensity parameter leads to an increase in the sonochemical effects. It is important to note that high amplitudes can lead to rapid deterioration of the ultrasonic transducer, which results in liquid agitation instead of cavitation and in poor transmission of the ultrasound through the liquid media. However, the amplitude should be increased when working with samples of high viscosity, such as oils (Santos et al., 2008).

### *1.3.4. Ultrasonic power and frequency*

Several studies show a great ultrasonic power causes major alterations in materials by inducing greater shear forces (depending on the nature and properties of the medium); however, in the food industry this parameter is usually optimized in order to use the minimum power to achieve the best results (Feng et al., 2010). Generally, the highest efficiency of UAE, in terms of yield and composition of the extracts, can be achieved by increasing the ultrasound power, reducing the moisture of food matrices to enhance solvent-solid contact, and optimizing the temperature to allow a shorter extraction time.

The most commonly used frequencies in sonochemistry are comprised between 20 and 40 kHz. With higher frequencies cavitation would be more difficult to induce, since the cavitation bubbles need a little delay to be initiated during the rarefaction cycle (Mason et al., 2005). The length of rarefaction phase (during which cavitation bubbles grow) is inversely proportional to ultrasonic frequency; therefore, at high frequencies larger amplitudes are required to generate cavitation. At low frequencies, the transient cavitation bubbles are relatively less numerous, although with high dimensions, which privileges the physical effects instead of the chemical ones.

### *1.3.5. Presence of dissolved gases*

Since cavitation bubbles are formed from gases (vapors) dissolved in the liquid, the absence of gases would dramatically difficult the creation of those bubbles. Dissolved gases into the solvent act as nuclei for a new cavitation bubble so this would increase the rate of cavitation bubbles formation. An increase in the vapor pressure and surface tension result in a decrease of the cavitation intensity with smaller number of cavitation bubbles that have consequently a larger diameter due to coalescence and/or growth with continued vaporization of the liquid medium (Chivate & Pandit 1995). If the external pressure is increased, then a greater ultrasonic energy is required to induce cavitation, that is, to break the solvent molecular forces. In addition, there is an increment in the intensity of the cavitational bubble collapse and, consequently, an enhancement in sonochemical effects is obtained (Santos et al., 2008). In another hand, as the creation of the cavitation bubbles is facilitated, they would grow faster and the solvent might undergo boiling: if the bubbles grow too fast, they would not have time to collapse and the liquid would boil without cavitation.

## 1.4. Instrumentation

All ultrasonic systems are composed of a transducer, which generates ultrasound. Although a wide range of transducer types is available, the purpose is the same. The generated ultrasound is irradiated by the emitter (also called reactor), which can also amplify the waves (Feng et al., 2010). The principal and most used emitters are the bath and the horn systems (or probe) and this last one if often attached to a horn tip known as sonotrode. Ultrasound equipments have been developed for both laboratory and industrial scales. For either application, the two most common types of ultrasound equipments (batch and probe systems) are used, although the intrinsic differences between those systems should be taken into account for better adaptation to the desired final purposes. Recently some continuous-flow apparatuses have been developed for both laboratory and pilot scale with mostly analytical chemistry applications. The coupling of ultrasonic equipment to analytical instruments provide considerably reduce costs by avoiding sample preparation steps such as concentration, filtration and derivatization before analysis.

### *1.4.1. Laboratory scale*

The first batch equipment developed is the ultrasonic cleaning bath, which is used for solid dispersion into the solvent (solubility of solid particles is increased as the particles size is reduced), for degassing solutions or cleaning small material by immersion (Figure I.4.A.). This type of equipment is easy to handle and has very low implementation cost, although presents some important shortcomings such as the declined power over time with attenuation of the intensity (which is dispersed in the water and glassware), decreasing the reproducibility and repeatability of experiments. Therefore, ultrasonic baths are less used for small quantity chemical reactions and most of the processes for with ultrasound is used in analytical chemistry are physical processes (filtration, homogeneization, cutting, etc.). Recently a new batch system reactor has been developed by R.E.U.S. (France) with capacities of 0.5 to 3 L operating at 25 kHz with an intensity of 1 $W.cm^{-2}$, being mostly used for extraction procedures (Figure I.4.B.). The inox jug is equipped of a double-mantle to circulate water for controlling the temperature.

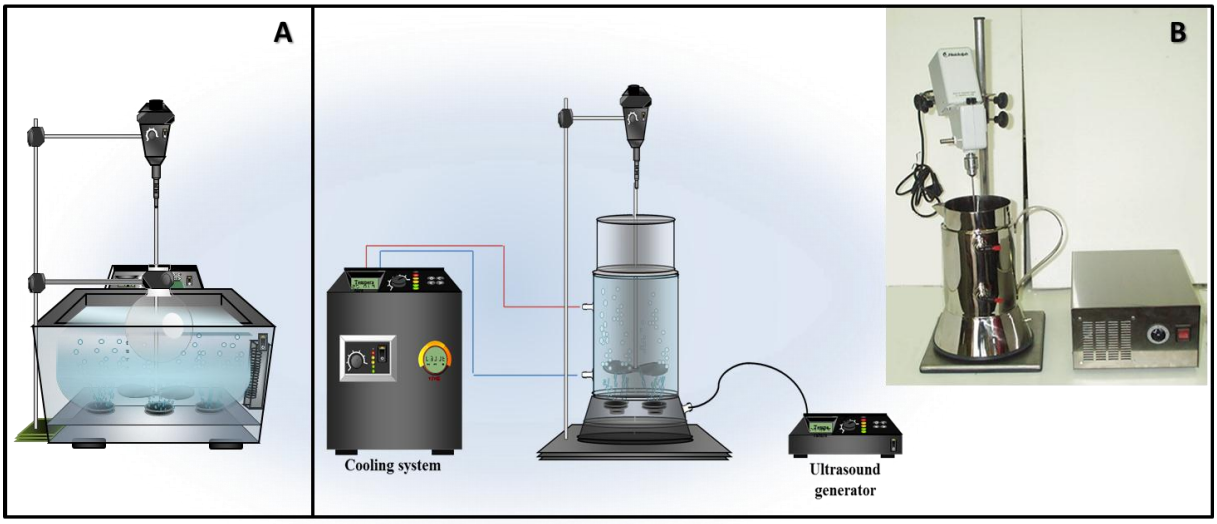


**Figure I.4.** Commonly used ultrasonic batch systems (**A.** US bath, **B.** US reactor scheme and picture).

For smaller volumes, the probe system (Figure I.5) is more adapted and is considered to be more powerful, since there is less dispersion of ultrasonic energy. The ultrasonic intensity is delivered by a small surface (the tip of the probe) and the fact of immersing the probe

directly into the reaction flask avoids attenuation. This system is widely used for chemical reactions but since the cavitation is concentred in a very small area, the temperature of the sample might rise rapidly; therefore, a temperature control method is often used (e.g. a double mantle reactor).

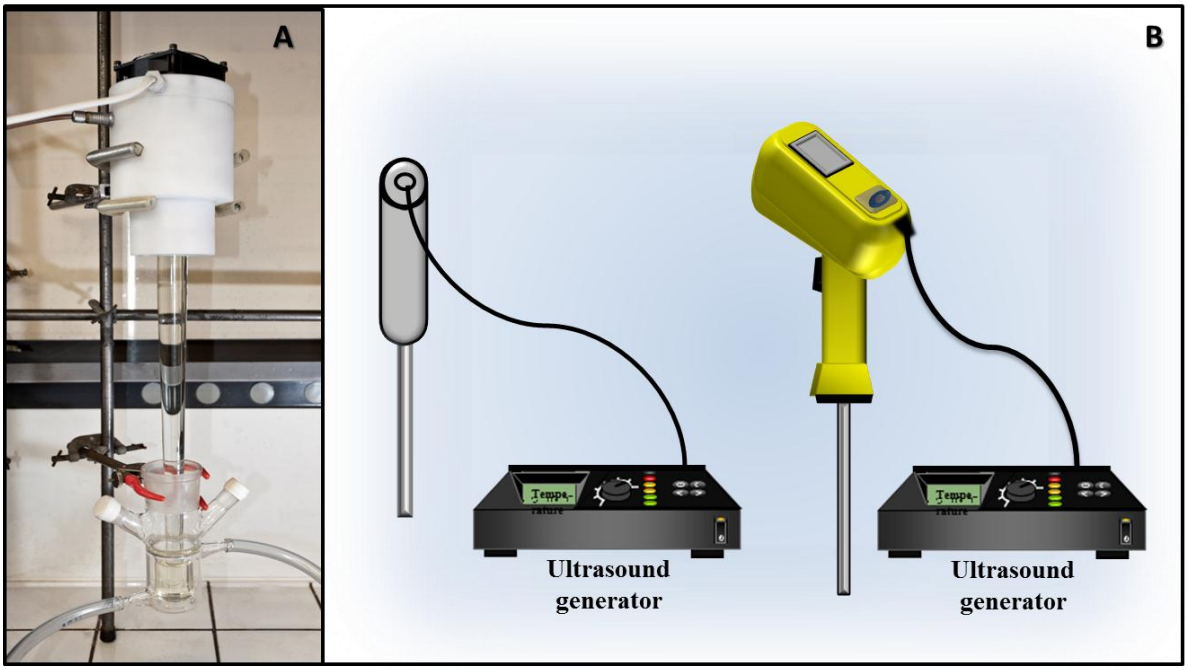


**Figure I.5.** Commonly used ultrasonic probe systems (**A.** quartz probe, **B.** titanium probes).

### *1.4.2. Industrial scale*

Both bath and probe systems (Figure I.6) are used industrially depending on the application and several types of ultrasonic devices have been developed for industrial uses or scale-up laboratory experiments by a large number of companies such as Hielscher, Branson, Vibracell and REUS, among others. The disposition of ultrasound transducer varies upon the device and to some, an agitation system is also used. Some continuous flow devices have also been developed for both probe and batch systems. REUS has developed reactors from 30 to 1000 L to which pump systems are coupled in order to fill the ultrasonic bath, to stir the mixture and to empty the system at the end of the procedure.

**Figure I.6.** Industrial scale ultrasonic devices (probe and bath systems).

## 1.5. Ultrasound in Food Processing

There is a great interest in ultrasound use in the food processing due to the fact that industries can be provided with practical and reliable ultrasound equipment. Nowadays, its emergence as green novel technology has also attracted the attention to its role in the environment sustainability. Ultrasound applications are based on the direct application to the product, coupling with the device and submergence in an ultrasonic bath. There are a large

number of potential applications of high intensity ultrasound in food processing and some are discussed below.

### *1.5.1. Filtration*

The main shortcoming of conventional filtration is the clogging of filters. The application of ultrasonic energy can increase the filtration flow by breaking the concentration polarisation and cake layer at the membrane surface without affecting the intrinsic permeability of membrane. Ultrasonically assisted filtration (generally referred to as acoustic filtration) has been successfully employed to enhance the filtration of industrial wastewater, which is generally considered difficult to process (Riera-Franco de Sarabia et al., 2000; Smythe and Wakeman, 2000; Kyllönen et al., 2006). Moreover, the optimised ultrasound intensity is very important to prevent the damage of filters. The use of ultrasound in combination with membrane filtration has also been investigated, with positive results (Kyllönen et al., 2005; Muthukumaran et al., 2005).

### *1.5.2. Defoaming*

The foam in liquid can cause product losses or reduce the production rate. High-intensity ultrasonic waves offer an attractive method of foam breaking since they avoid the need for high air flow, prevent chemical contamination and can be used in a confined environment (i.e., under sterile conditions), although a temperature control should be performed (Rodríguez et al., 2010). This fact makes it particularly appropriate for implantation in the food and pharmaceutical industries (Gallego Juárez et al., 2005). A system for ultrasonic defoaming has been developed based on a new type of focused ultrasonic generator. This new system has been successfully applied to control the excess of foam produced in high speed bottling and canning lines of carbonic beverages (Riera et al., 2006).

### *1.5.3. Degassing/Deaeration*

Degassing in an ultrasonic field is a highly visible phenomenon with the use of an ultrasonic cleaning bath filled with regular tap-water. It occurs when the rapid vibration of gas bubbles brought them together by acoustic waves and bubbles grow to a size sufficiently large to allow them to rise up through the liquid, against gravity, until they reach the surface (Laborde et al., 1998; Tervo et al., 2006).

In the food industry, this technique can be used to degas carbonated beverages such as beer (defobbing) before bottling (Brown and Goodman, 1965). In the processing of carbonated drinks, the purpose is to displace the air from the liquid surface in order to avoid organoleptic impairments of the product by bacteria and oxygen. Ultrasonically assisted degassing is particularly rapid in aqueous systems, but the removal of gas is much more difficult in very viscous liquids such as melted chocolate.

### *1.5.4. Cooking*

Ultrasound has the ability to improve heat transfer, which is a key requirement to avoid problems such as heterogeneous overcooking, and thus have been successfully used in cooking procedures. Some cooking procedures have been patented over the years, especially concerning the frying and cooking of meat with claimed reduction of energy consumption (Hausgerate, 1978; Pohlman et al., 1997). Ultrasound cooking results in greater cooking speed, moisture retention and homogeneity, suggesting that it could provide a new, rapid, energy-efficient method that may improve the textural attributes of cooked products (Mason et al., 2011; Pingret et al., 2011).

### *1.5.5. Cutting*

The use of ultrasound in cutting and slicing of products (Figure I.7) allow a better control of the depth of the blade, resulting in a cleaner cut with lower costs within a shorter time, being widely used in the agro alimentary industry. The vibration of the cutting blade

prevents the attachment of the food product to the used tool. Also, ultrasound-assisted cutting avoids the loss of products in the production line, increasing repetability with consequent better productivity, resulting in a final product with a more standard weight and dimensions. The most widespread application of ultrasound is in the cutting of fragile foodstuffs. It is used particularly in the case of fragile and heterogeneous products (cakes, pastry and bakery products) and fatty (cheeses) or sticky products (Arnold et al., 2009, 2011).

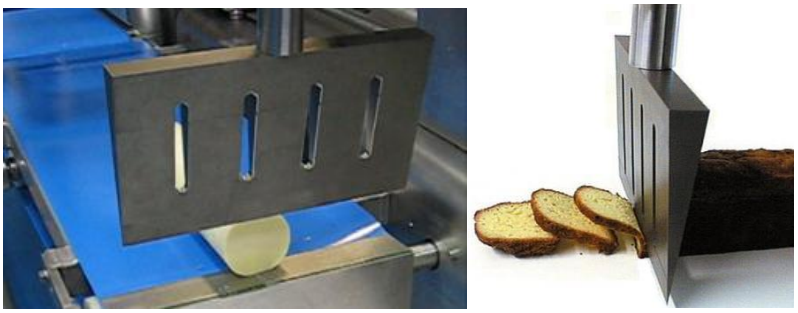

**Figure I.7** Ultrasound-assisted cutting.

### *1.5.6. Freezind and Crystallization*

Sonication is thought to enhance both the nucleation rate and rate of crystal growth in a saturated or supercooled medium by producing a large number of nucleation sites in the medium throughout the ultrasonic exposure. This may be due to cavitation bubbles acting as nuclei for crystal growth and/or by the disruption of seeds or crystals already present within the medium thus increasing the number of nucleation sites. Under the influence of ultrasound, conventional cooling provides much more rapid and even seeding, which leads to a much shorter dwell time (Acton and Morris, 1997). In addition, since there are a greater number of seeds, the final size of the ice crystals is smaller and so cell damage is reduced (Sun and Li, 2003).

Accelerated cooling is achieved by improving heat transfer, ultrasound influence besides the secondary nucleation, and the growth of the crystals as the form and size repartition of formed crystals (Li and Sun, 2002). A wide range of foodstuffs have been successfully frozen under the influence of ultrasound (Zheng and Sun, 2006; Luque de Castro and Priego-Capote, 2007; Kiani et al., 2011). It should also be noted that when ultrasound are

used to enhance crystallization of any kind, there is an additional benefit of helping to prevent encrustation of crystals on the cooling elements. This ensures efficient heat transfer throughout the cooling process.

### *1.5.7. Drying*

Diffusion at the boundary between a suspended solid and a liquid is substantially accelerated in an ultrasonic field and heat transfer is increased by approximately 30–60% depending on the intensity of the ultrasound (Povey and Mason, 1998). Due to the lower temperatures during dehydration and the shorter treatment times, food qualities such as flavor, color and nutritional value remain unaltered. The mechanism of this process involves a series of rapid and successive compressions and rarefactions in the food material induced by the ultrasound. With each contraction, a very small amount of water is expelled towards the surface of the product, and this water is evaporated by the hot gas stream (Juarez et al., 2001). A wide range of products have been examined, e.g. powdered milk (Boistier-Marquis et al., 1999), vegetables (Povey and Mason, 1998) and sugar crystals (Boucher, 1965).

### *1.5.8. Sterilization/ Pasteurization*

The use of ultrasound in pasteurization continues to be of great interest to the dairy industry. It has proved effective for the destruction of *E. coli*, *Pseudomonas fluorescens* and *Listeria monocytogenes* with no detrimental effect on the total protein or casein content of pasteurized milk (Cameron et al., 2009). The mechanism of microbial killing is mainly due to the thinning of cell membranes, localized heating and production of free radicals. Investigation on ultrasound effectiveness have also shown the inactivation of enzymes such as pectinmethylesterase, polyphenoloxidases and peroxidases responsible for deterioration of fruit & vegetable juice and various enzymes pertinent to milk quality (Vercet et al., 2002; Terefe et al., 2009; Tiwari et al., 2009; O'Donnell et al., 2010). In combination with heat, ultrasound can accelerate the rate of sterilization of foods, thus lessening both the duration and intensity of thermal treatment and the resultant damage. The advantages of ultrasound

over heat pasteurization include minimization of flavor loss, greater homogeneity of treatment and significant energy savings (Vercet et al., 2000).

### *1.5.9. Emulsification/Homogenization*

In the food industry, ultrasonic emulsification is attracting interest for products such as fruit juices, mayonnaise and tomato ketchup (Povey and Mason, 1998), in the homogenization of milk (Wu et al., 2001) and in aroma encapsulation (Mongenot et al., 2000), since it presents numerous advantages over conventional procedures, especially in terms of stability of emulsions (there is no need of tensioactifs) and reduction of energy consumption.

### *1.5.10. Miscellaneous Effects*

With low-power ultrasound, it is possible to accelerate fermentation processes and this has potential for a range of foodstuffs (Chisti, 2003). It is also possible to use ultrasound on foodstuffs to induce premature ageing. One of the main applications of ultrasonically aided oxidation is in alcoholic beverages for the ageing of fermented products such as wine and whisky (Chang and Chen, 2002). Indeed, a device has been patented for this purpose using a range of ultrasound between 20 and 80 kHz (Ho and Chiu, 2008). Another potential use of ultrasound in winemaking is for the improved dissolution of dyes and the tannins from the initial processing of the fruit.

## 1.6. Ultrasound in Food Preservation

Food processing combines the fundamentals of physics, chemistry, biology and microbiology to achieve the best in food preservation. Traditionally, heat treatment or in occurrence pasteurization and sterilization were the methods of choice, having the ability to destroy both microorganisms and enzymes (the latter being responsible for food deterioration). However the effectiveness of those methods is dependent on the treatment temperature and time, which leads to loss of nutrients, development of undesirable flavors,

colors and deterioration of the organoleptic properties of food. As a result new non-thermal technologies have been developed and the current challenge is to combine simultaneously mild thermal preservation techniques with new applications for microbial destruction, preserving at the same time the nutritional values and organoleptic characteristics (texture, color, taste), with a low consumption of energy, a competitive cost, an environmental friendliness and a high degree of safety.

Ultrasound processing answers to those requirements and it has proven to rupture cells, denature enzymes and to modify the metabolism of organisms. In combination with heat, ultrasonication can accelerate the rate of sterilization of foods, thus lessening both the duration and intensity of thermal treatment and the resultant damage. The advantages of ultrasound over heat sterilization include: the minimizing of flavor loss, greater homogeneity; and significant energy savings.

### *1.6.1. Microorganism inactivation*

Ultrasound treatment does not affect all microorganisms in the same way; therefore, the efficacy of the treatment will be affected by the type, shape or diameter of the microorganisms (Heinz et al., 2001). Larger cells are known to be more sensitive than the small ones, probably due to their larger surface area. In the same way, gram-positive bacteria are known to be more resistant than gram-negative ones, possibly because of their thicker cell wall which provides them a better protection against ultrasound effects (Drakopoulou et al., 2009). According to the literature differences in cell sensitivity could also be due to the more tightly adherent layer of peptidoglucans in gram-positive cells. Concerning the shape of the micro-organisms, cocci are more resistant than bacilli due to the relationship of cell surface and volume. Finally, spores are very hard to destroy compared to vegetative cells which are in phase of growth.

There are many examples of microorganisms inactivated using ultrasound. Some of these have been studied in culture media and others in food, using ultrasound either combined or alone and has proven to be effective against *Saccaromyces cerevisiae*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Listeria monocytogenes*, *Listeria innocua*, etc. (Ordoñez et al., 1987; Sala et al., 1995; Ciccolini et al., 1997; Lopez-Malo et al., 1999; Petin et al., 1999; Guerrero et al., 2001; Bermúdez-Aguirre et al., 2009).

Generally ultrasound or the combined processes provide a more effective inactivation in a reduced time compared to the conventional method, working at lower temperatures. The overall effects seem to be dependent upon ultrasound frequency and power (Mañas et al., 2000).

### *1.6.2. Spore inactivation*

Microbial spores are resistant to extreme conditions such as high temperatures and osmotic pressures, high and low pHs, and mechanical shocks. Those bacterial spores that survive heat treatment may severely restrict the shelf-life of thermally processed foods because of spoilage and poisoning. The endospores of Bacillus and Clostridium species are very resistant to extreme conditions. Inactivation of spores by sonication has been successfully applied to those species and the effect is dependent upon intensity and amplitude (Raso et al., 1999). Also, the increase of temperature during treatment results in greater rates of inactivation compared to thermal treatment alone. The pathogens *Bacillus cereus* and *licheniformis* spores have been found to be resistant to ultrasonic treatment alone (Burgos et al., 1972).

### *1.6.3. Enzyme inactivation*

To prevent denaturation, an enzyme has to keep its native conformation. Hydrophobic interactions, hydrogen bonding, van der Waals interactions, ion paring, electrostatic forces and steric constraints stabilise the three-dimensional molecular structure of globular proteins. For stabilisation of some food materials, enzymes must be inactivated or their activity reduced. In fact, the proteolysis caused by some enzymes like proteases can induce some defects of flavor and brown pigments. Enzyme inactivation can be easily achieved by heat treatment. However in some cases the high heat resistance of enzymes may be a problem as heat can negatively modify some food properties such as flavor, color or nutritional value. This is the driving force for the increased interest in an alternative method of enzyme inactivation: high power ultrasound. The effects of ultrasonic waves on proteins are very complex and sensitivity to ultrasound depends on the conditions of the treatment as well as on

the nature of the enzyme (McClements, 1995). Generally, ultrasonication in combination with other treatments is more effective in food enzyme inactivation and has been successfully applied to inactivation of enzymes in orange juice (Vercet et al., 1999) and processed tomatoes (Thakur et al., 1996; Vercet et al., 2002), lemons (Kuldiloke et al., 2007), fresh-cut apples (Jang and Moon, 2011), among others.

## 1.7. Ultrasound in Food Extraction

Despite its primary utilization in cleaning of surfaces and instruments, ultrasonic devices have been developed and largely used in the food industry. Because of the wide range of frequencies and power, ultrasound has different effects that allow it to be applied to different and various processes. Each process application has its particularities, but the general principle of ultrasound in those processes is based on mechanical and sonochemical effects that can be observed by the propagation of ultrasonic waves (Povey and Mason, 1998; Feng et al., 2010). Keeping in mind that the industry doesn't always aim for the highest yield, but the objective is to achieve a minimum consumption of non-renewable resources and expenses. Ultrasound are generally profitable in large scale applications, as results include a decrease in energy consumption, process time and enhancement of quality in the final product, and finally, the initial investments are rapidly paid back (Patist and Bates, 2008). The Table I.1 resumes the main applications of ultrasound in the three types of matrix explored in this section.

The application of Ultrasound-Assisted Extraction (UAE) in food processing technology is of major interest to the industrial increasing demand for a sustainable development, since it potentially enhances the extraction of components with a higher recovery, allows the replacement of organic solvents by safer (GREEN) solvents, reduces the quantity of solvent used and shortens the extraction time. This technique can be applied to a large range of products for the extraction of different compounds.

### *1.7.1. Fruits and vegetables*

In the food domain, the processing of fruits and vegetables is done in multiple steps for simple consumption or even for the extraction of interest molecules intended to direct or indirect applications in food industries or even other fields such as pharmaceutical and cosmetic. To those applications, those matrixes contain a wide range of secondary metabolites that can be extracted and purified for further applications such as lipids, phytochemicals, flavors, fragrances and pigments.

For instance, antioxidants issued from plants have numerous applications, being used either for health preventing reasons, as adjuvants in some formulations or with preservative purposes. In this scenario, antioxidants are able to prevent or slower the oxidation process by reacting preferably with the oxidizing agent instead of the target cells or molecules of interest. Ultrasound-Assisted Extraction (UAE) of antioxidants has been effectively applied to numerous matrices with great recoveries and optimum antioxidant activity (Khan et al., 2010; Londoño-Londoño et al., 2010; Santos et al., 2010; Pan et al., 2011).

Other compounds such as colorants (Chen et al., 2007; Sivakumar et al., 2011) and micro and macronutrients (Nascentes et al., 2001) have also been successfully extracted using ultrasound either coupled to other techniques or isolated. The extraction of antioxidants and carotenoids from orange, citrus peel and from tomatoes by UAE such as lycopene has been successfully optimized (Sun et al., 2011; Eh and Teoh, 2012; Konwarh et al., 2012).

### *1.7.2. Herbs and spices*

Several interest molecules extracted from herbs and spices are used in the food, cosmetic and pharmaceutical industries and various processes are used for this end. Among the used techniques, ultrasound has been successfully applied in the recovery of compounds from those matrices. A large range of herbs and spices have been submitted to Ultrasound-Assisted Extraction of compounds using conventional or green solvents.

**Table I.1.** Applications of ultrasound in the food processing.

| Matrix | Processing | Target compounds | References |
|---|---|---|---|
| ***Fruits and Vegetables*** | | | |
| Citrus peel | UAE bath | Flavonoids | Londono-Londono et al., 2010 |
| *Myrciaria cauliflora* | UAE bath | Antioxidants | Santos et al., 2010 |
| Pomegranate peel | Continuous and pulsed UAE | Antioxidants | Pan et al., 2011 |
| Orange peel | UAE | Polyphenols | Khan et al., 2010 |
| Green wattle bark, Marigold flowers, Pomegranate rinds, 4'o clock plant flowers and Cocks Comb flowers | UAE probe | Colorants | Sivakumar et al., 2011. |
| Red raspberry fruits | UAE probe | Anthocyanins | Chen et al., 2007. |
| Lettuce and cabbage leaves | UAE bath | Ca, Mg, Mn and Zn | Nascentes et al., 2001. |
| Tomato | UAE | Lycopen | Konwarh et al., 2012; Eh & Teoh, 2012 |
| Citrus peel | UAE probe | All-trans-β-carotene | Sun et al., 2011. |
| ***Herbs and Spices*** | | | |
| Pepper | UAE bath | Capsaicinoids | Barbero et al., 2008; Boonkird et al., 2008. |
| Caraway seeds | UAE bath | Carvone and limonene | Chemat et al., 2004. |
| *Rosmarinus officinalis* | UAE bath | Antioxidants | Albu et al., 2004; Paniwnyk et al., 2009. |
| *Mentha spicata* | UAE probe | Flavor compounds | Da Porto & Decorti, 2009. |
| Rice and maize wine | UA process | Accelerated aging | Chang & Chen, 2002. |
| Red and white wine | UAE bath | Volatile compounds | Cabredo-Pinillos et al., 2006; Hernanz et al., 1999. |
| Brandies and oak extracts | UAE bath | Volatile compounds | Caldeira et al., 2004. |
| ***Oleaginous Seeds*** | | | |
| Almond, apricot and rice bran | UAE | Oil | Sharma & Gupta, 2004; 2006. |
| Almond | UAE bath | Oil | Zhang et al., 2009. |
| Soybean | UAE bath | Oil | Li et al., 2004b; Zhang et al., 2008. |
| Flaxseed | UAE emulsifier | Oil | Li et al., 2004a. |
| *Isatis indigotica* Fort. | UAE bath | Oil | Li et al., 2012. |
| Viz and soybean | UAE/ microwave | Oil | Cravotto et al., 2008. |
| Sunflower, rape and soybean seeds | UAE Soxhlet | Oil | Luque-Garcia & Luque de Castro, 2004. |

Different capsaicinosids (noridihydrocapsaicin, capsaicin, dihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin) have been extracted from pepper (*Capsicum frutescens*) and by changing the extraction medium solvent in the Ultrasound-Assisted Extraction, selectivity is observed amongst those compounds (Barbero et al., 2008; Boonkird et al., 2008). The possibility of selecting the compound of interest by UAE was also observed for caraway seeds, where at low temperatures, a certain selectivity is observed for carvone extraction in detriment of limonene (Chemat et al., 2004). The UAE of rosemary shows carnosic acid is better extracted from dried material in ethanol, while rosmarinic acid is better extracted using methanol as solvent, from which extracts present better antioxidant activity (Albu et al., 2004; Paniwnyk et al., 2009).

Flavors and fragrances are complex mixtures of volatiles that are obtained from the secondary metabolism of aromatic plants (including herbs and spices), and usually are in low concentrations. Those substances generally consist of complex mixtures of mono and sesquiterpene hydrocarbons, and oxygenated materials biogenically derived from them (Da Porto and Decorti, 2009).

Another application of ultrasound for flavors extraction is the analysis of alcoholic beverages such as wine and brandy. To those beverages aromas are of great importance either for quality or appreciation parameters; therefore, ultrasound have been successfully used for aging of rice wine in 1 week or 3 days instead of classical 1 year aging (Chang and Chen, 2002). On the other hand, some volatiles are markers of quality for wine or brandy and thus, ultrasonic techniques were developed for extraction and analysis of those substances (Hernanz Vila et al., 1999; Caldeira et al., 2004; Cabredo-Pinillos et al., 2006).

### *1.7.3. Oleaginous seeds*

Fats and oils are a main source of energy used by the body and participate on the transmission of nerve impulse, maintain integrity of cell membranes, have a role in the cellular transport and are precursors of many hormones. From all sources of lipid, oilseeds are complex matrixes from which it is possible extract monoacylglycerols (MAG), diacylglycerols (DAG), triacylglycerols (TAG) and free fatty acids associated to other minor compounds such as pigments, sterols, alkaloids, etc. (Meireles, 2008).

The conventional methods for oilseeds extraction are hot or cold pressing, solvent extraction (Soxhlet) and eventually the combination of these. However, the press cake retains considerable amounts of oil and minor compounds and Soxhlet extraction might degrade fatty acids by the increased temperature and also uses hexane, a toxic solvent (Moretto and Fett, 1998; Sharma and Gupta, 2004).

In the past years, researchers have proved Ultrasound-Assisted Extraction (UAE) to result in great yields and high-quality of oils, allowing a faster extraction with great recoveries. Since the oilseeds present a hard shell of the cell wall and its breaking is crucial to oil extraction (Eggers et al., 1985; Shukla et al., 1992). Cavitation due to ultrasound is able to create more pores in those cells to allow a better contact with the extraction solvent, thus resulting in better yields with a reduced amount of solvents (Lou et al., 2010).

Numerous oleaginous seeds have been extracted under ultrasound. When used as a pretreatment before extraction, alone or in combination with other techniques such as autoclave, ultrasound have increased the oil yield for almond, apricot and rice bran, and scanning electron micrographs showed a destructuring of cell walls due to ultrasonic cavitation (Sharma and Gupta, 2004, 2006; Zhang et al., 2009). Flaxseed and soybean have also been extracted by ultrasound resulting in increases of oil yield when compared to conventional and microwave-assisted techniques (Li et al., 2004a, 2004b; Zhang et al., 2008). Ultrasound also have been used to valorize by-products such as *Isatis indigotica Fort.* seed oil (Li et al., 2012). The combination of extraction methods have also proven to be efficient, as in the case of microwave-assisted extraction of seaweeds and soybeans (Cravotto et al., 2008), and also in the case of the innovative Ultrasound-Assisted Soxhlet Extraction, which showed applicability not only for soybeans but also for rape and sunflower seeds (Luque-Garcia and Luque de Castro, 2004).

## 1.8. Conclusion

Ultrasound has been used for various processes in the chemical and food industry. In the extraction field, it has been implied as a "green technology" that, when compared to other extraction techniques, is much faster, less fossil energy consuming, allows the use of solvents more environmental friendly (which can be less toxic and/or polluting), and results in a more pure product of great yield. Ultrasound applications involve homogenization, emulsification, sieving, extraction, filtration, and crystallization.(2008) This technique has been applied to extract food components like aromas, antioxidants, pigments and other organic and mineral components from a variety of matrices. It presents numerous benefits and advantages, for instance, a better homogeneity achieved due to an effective micromixing and particle distribution; reduced thermal and concentration gradients; less extreme processing temperatures; more selective extraction, reduced equipment size, faster start-up, increased production and elimination/treatment of residues.

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# Chapter II

# *Ultrasound in the extraction of polyphenols from apple pomace*

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Carine Le Bourvellec, Catherine M.G.C. Renard, Farid Chemat.
  Lab and pilot-scale ultrasound-assisted water extraction of polyphenols from apple pomace.
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## 2.1. Introduction

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

Some of the polyphenols in the apple pomace present a high exploitable industrial potential as dietary or food antioxidant, exhibiting 2 to 3 times DPPH-scavenging activity and 10 to 30 times superoxide scavenging activity compared to vitamins C and E (Yinrong Lu and Foo, 1997; Y Lu, 2000). Polyphenols in apple pomace, in this case the methanolic extracts, also showed antiviral properties against *Herpes simplex* virus (Suárez et al., 2010). The safety of those polyphenols has also been evaluated and confirmed (Shoji et al., 2004). The content of phenolic compounds in the pomace is higher than the content in the juice and varies amongst different varieties of apples (Guyot et al., 1998; Guyot et al., 2003; Cetkovic et al., 2008; Van der Sluis et al., 2002; Price, 1999; Kolodziejczyk et al., 2009). The main polyphenol class in apple pomace is procyanidins. The hydroxycinnamic acid derivatives are mainly represented by chlorogenic acid (5'-caffeoylquinic acid). Phloridzin (major constituent of dihydrochalcones) was thought to be a specific component to apples (Mangas et al., 1999), however, further studies have shown this compound is also present in strawberries (Hilt et al., 2003). Compared to the apple fruit, apple pomaces are richer in procyanidins, due to interactions with the polysaccharides (Le Bourvellec et al., 2007), and in flavonols and dihydrochalcones due to their location in the peel and pips, respectively (Guyot et al., 1998); in addition, they contain lower concentrations of hydroxycinnamic acids and catechins.

Ultrasound has been used for various processes in the chemical and food industry. The technique using ultrasound is fast, consumes less fossil energy and permits the reduction of solvents, thus resulting in a more pure product and higher yields. This method has been

applied to extract food components such as aromas (Caldeira et al., 2004; Xia et al., 2006), antioxidants (Ma et al., 2009; Rodrigues et al., 2008; Wang et al., 2008; Virot et al., 2010), pigments (Fang Chen et al., 2007; Barbero et al., 2008) and other organic and mineral components from a variety of matrices. Ultrasound plays an important role as real potential sustainable technique for industrial applications for polyphenols extraction (Khan et al., 2010). The cavitation process that occurs during sonication causes the rupture of cell walls, consequently enhancing solvent contact with available extractable cell material (Vinatoru, 2001).

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

## 2.2. Experimental Section

### *2.2.1. Plant material and chemicals*

Apple pomace (Figure I.8) was obtained from Val-de-Vire Bioactives (Conde-sur-Vire, France) and kept in the dark until use. Standards of chlorogenic acid, (-) epicatechin and phloridzin, were purchased from Sigma Aldrich (St. Louis, USA). Other chemicals were of analytical grade and purchased from VWR International (Darmstadt, Germany).

### *2.2.2. Extraction Procedures*

In all extraction procedures, a 50 mM malate buffer in a pH 3.8 was used in order to mimetize fruit's conditions. To determine the optimal extraction conditions, the solid/liquid

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

**Figure I.8.** Apple pomace.

Ultrasound-assisted extractions (UAE) were performed in an ultrasonic extraction reactor PEX1 (R.E.U.S., Contes, France) with 14x10 cm internal dimensions and maximal capacity of 1 L, equipped with a transducer at the base of jug operating at a frequency of 25 kHz with maximum input power (output power of the generator) of 150 W (Figure I.9). The double-layered mantle (with water circulation) allowed the control of extraction temperature by cooling/heating systems. Considering the actual input power from the device is converted to heat which is dissipated in the medium, calorimetric measurements were performed to assess actual ultrasound power, calculated as shown in the equation 1 below (Toma et al., 2011).

$$\mathrm{P} = m.\,\mathrm{Cp}.\frac{d\mathrm{T}}{d\mathrm{t}} \qquad (1)$$

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

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

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

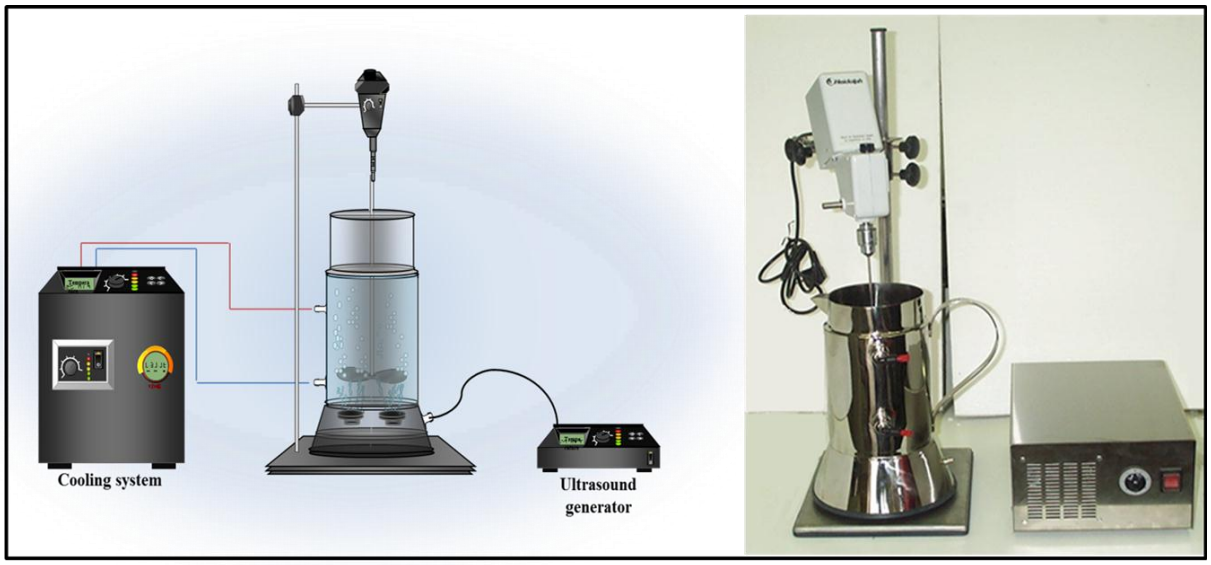



**Figure I.9.** Ultrasound apparatus used for extraction procedures (scheme and picture).

### 2.2.3. *Isolated compounds study*

In order to verify whether antioxidants present in the extracts undergo degradation during sonication, the following isolated compounds were submitted to ultrasound treatment:

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

### 2.2.4. *Total phenolics determination (TPC)*

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

### *2.2.5. Identification and individual quantification of phenolic compounds by HPLC-DAD*

Polyphenols were measured by HPLC after re-dissolution of the freeze-dried extracts in acidic methanol (1% acetic acid, v/v), or after thioacidolysis as described previously (Guyot et al., 2001), followed by filtration (PTFE, 0.45 µm). A Waters HPLC apparatus (Milford, MA, USA) was used, a system 717 plus autosampler equipped with a cooling module set at 4°C, a 600 E multisolvent system, a 996 photodiode array detector, and a Millenium 2010 Manager system. The column was a Purospher RP18 endcapped, 5µm (Merck, Darmstadt, Germany). The mobile phase was a gradient of solvent A (aqueous acetic acid, 25 mL/L) and solvent B (acetonitrile): initial, 3 % B; 0-5 min, 9 % B linear; 5-15 min, 16 % B linear; 15-45, 50 % B linear, followed by washing and reconditioning the column. HPLC peaks were identified on chromatograms according to their retention times and their UV-visible spectra by comparison with available standard compounds as described by Guyot et al. (2001). Quantification is performed by reporting the measured integration area in the calibration equation of the corresponding standard. Phloretin and phloretin xyloglucoside were calculated as phloridzin

equivalent, all flavonols were quantified against quercetin (molar responses, then their respective contents of glycosides are used to calculate concentrations in g/L or g/kg). Total flavonols and total polyphenols were the sums of the corresponding compounds, quantified by HPLC. The average degree of polymerization of flavan-3-ols was calculated as the molar ratio of all the flavan-3-ols units (thioether adducts plus terminal units) to (-)-epicatechin and (+)-catechin corresponding to terminal units.

### *2.2.6. Antioxidant activity: inhibition of linoleic acid peroxidation*

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

### *2.2.7. Experimental design*

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

mainly dependent on the ratio of solvent to sample, the extraction time, the temperature and the ultrasonic intensity.

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

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

Preliminary experiments allowed us to distinguish the variables implied in the model at five separated coded levels: -α (= -1.68), -1, 0, +1, +α (= +1.68). The limit values of each variable range were chosen as function of limitations of ultrasonic apparatus (minimum and maximum power available in the device), temperature of extraction for polyphenols (which might degrade above 40°C) and time of sonication. Values are presented on Table I.2 and involved a total of 20 experiments; including six replications at the centre point to evaluate experimental error measurement, and randomized to avoid effects of extraneous variables. Variables were coded according to the following Equation (3), where $X_i$ is the coded value, $x_i$, the real value of a variable, $\bar{x}_i$, the real value of a variable at the center point, and $\Delta x_i$, the step change:

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

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

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

Where: Y is the response variable TPC (mg of catechin equivalent per 100 g of dried apple pomace sample), $\beta_0$ is the average response obtained during replicated experiments of the CCD, $\beta_i$; $\beta_{ii}$; $\beta_{ij}$ are the linear, quadratic and cross-product effects, respectively, $X_i$ and $X_j$ are the independent coded variables. The results were analyzed using the Statgraphics XV® software.

### *2.2.8. Kinetics studies*

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

$$C_t = C_\infty(1 - e^{-kt}) \quad (3)$$

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

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

## 2.3. Results and Discussion

### *2.3.1. Solid/liquid ratio*

To determine the optimum solid/liquid ratio, total polyphenol compounds and the liquid absorbing capacity of the apple pomace were considered, as represented in Figure I.10. From this figure it is possible to observe that the optimum ratio was 150 mg of dry material/mL. For concentrations above 200 mg/mL the dry pomace absorbed all of the available liquid, increasing in volume. Since the ultrasound apparatus requires a minimum amount of free

solvent for extraction procedures, a combination of high TPC yields and higher amount of available solvent was chosen. The 150 mg/mL ratio results are corroborated by the values achieved by earlier studies such as Virot et al (2010) who used ethanol as extraction solvent of dry apple pomace.

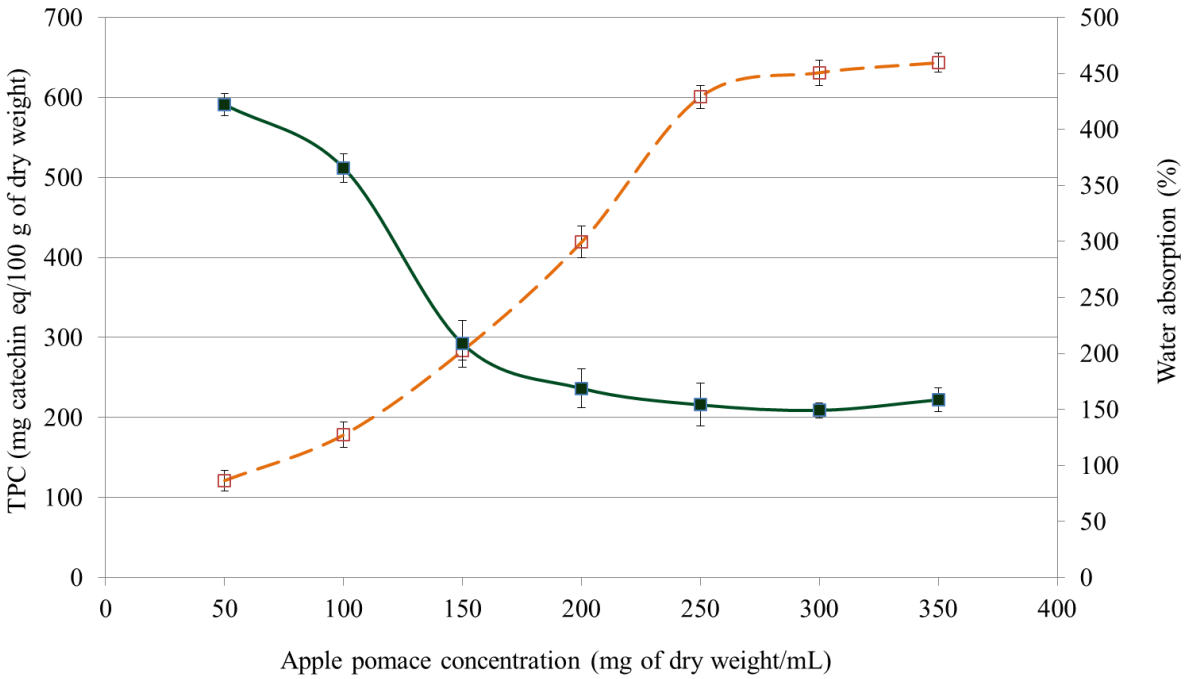



**Figure I.10.** Optimization of solid/liquid ratio for apple pomace extraction by water : polyphenol concentration in the extract (TPC) (□) and water absorption (■).

### *2.3.2. Experimental design studies*

Three key variables that affect extraction of phenolic compounds were studied in a central composite design: namely, ultrasonic intensity, temperature and sonication duration. Ultrasonic intensity ranged from 0.335 W/cm$^2$ to 0.764 W/cm$^2$. The chosen ultrasonic intensity limits were function of regulation limitations in the ultrasonic apparatus. Since appropriate temperature setting is necessary to avoid destruction of organic compounds as well as provide an efficient application of ultrasound (ultrasound effects are known to decrease with temperatures higher than 40-50°C), moderate temperatures were chosen with a

range of 9.9-40°C. Also, the increase in cavitation phenomena is directly proportional to the increase in the system temperature. However, at too high temperatures (higher than the ebullition point f the solvent in matter) a decrease in shock waves is observed, diminishing the effect of ultrasound (Lorimer and Mason, 1987). At last, polyphenols might undergo degradation at temperatures higher than 40°C, especially when combined to ultrasound (Kyi et al., 2005; Svitelska et al., 2004); therefore, a maximum temperature of 40 °C was chosen. Finally, the sonication time range chosen (from 5 to 55 minutes) was relatively short yet competitive with conventional extraction, showing a potential future industrial application. Since after a certain time cavitation bubbles do not continue to absorb energy to grow and collapse (Ozcan, 2006), and the usual time used for ultrasound-assisted extraction in the industry are usually not longer than 60 minutes (Chemat et al., 2011), 55 minutes was chosen as maximum limit. These three controlled variables were studied in a multivariate study with 20 experiments as shown in the Table I.2.

**Table I.2.** Variables involved in the Central Composite Design (CCD) and response obtained for TPC.

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

* UI: Ultrasonic intensity

**mg catechin eq/100 g MS

### *2.3.3. Results for TPC*

Coded experiments and responses obtained for each run of the central composite design are presented on Table I.3. The responses varied widely in function of parameters settings of experiments (from 257 to 460 mg of catechin equivalent per 100 g of dry weight). Significance and suitability of the design were then studied using a variance analysis (ANOVA, Table I.3). Statistical significance of each effect (including interaction terms, linear and quadratic $T^2$ effects) was tested by comparing the mean square against an estimate of the experimental error. Depending upon the degree of freedom (Df.) involved, F-ratio can be calculated (ratio of the mean squared error to the pure error). With a confidence level of 95%, F-ratio significance can be evaluated using the p-value column (significant effects have been typed in bold). Four effects were found significant at a 95% confidence level in the experimental domain studied. This observation can also be pointed out on a Pareto chart of standardized effects, presented on Figure I.11. Linear effects of the three key variables (UI, T, t) appear to be highly significant, as well as the quadratic effect of the sonication time ($t^2$).

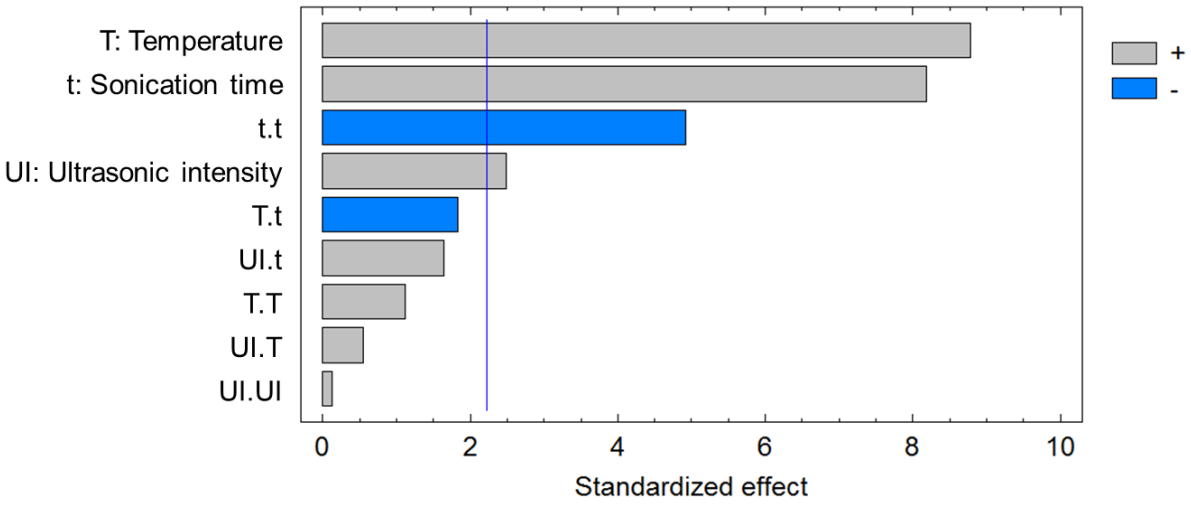



**Figure I.11.** Standardized Pareto chart of optimization multivariate study.

The lack of significance of the cross-product terms (UI.T, UI.t, T.t) suggests the absence of interactions between variables. The experimental data obtained from the CCD allowed us to determine an empirical relationship linking response studied (TPC) and key variables

involved in the model (in coded units). Thus, a second order polynomial equation was obtained:

$$Y = 154.0 + 0.98\ UI + 5.89T + 10.99t - 0.12t^2$$

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

**Table I.3**. ANOVA for TPC in the CCD.

| Source | Sum of squares | Df | Mean squares | f-Ratio | p-Value |
|---|---|---|---|---|---|
| UI: Ultrasonic intensity | 2966.43 | 1 | 2966.43 | 5.84 | **0.0362** |
| T: Temperature | 38446.5 | 1 | 38446.5 | 75.72 | **0.0000** |
| t: Sonication time | 33210.4 | 1 | 33210.4 | 65.41 | **0.0000** |
| $UI^2$ | 14.83 | 1 | 14.83 | 0.03 | 0.8677 |
| UI.T | 224.084 | 1 | 224.084 | 0.44 | 0.5215 |
| UI.t | 1225.13 | 1 | 1225.13 | 2.41 | 0.1514 |
| $T^2$ | 653.003 | 1 | 653.003 | 1.29 | 0.2832 |
| T.t | 1653.12 | 1 | 1653.12 | 3.26 | 0.1013 |
| $t^2$ | 11470.2 | 1 | 11470.2 | 22.59 | **0.0008** |
| Total error | 5077.52 | 10 | 507.752 | | |
| Total (corr.) | 95724.2 | 19 | | | |

*$R^2$ = 0.947 ; $R^2$ adj (ajusted for Df) = 0.899*

### *2.3.4. Optimization of ultrasound-assisted extraction*

A graphical representation can be introduced in order to visualize the significant relationship linking levels of variables and response studied (TPC). Figure I.12 depicts three-

dimensional plots, each plot highlighting the response behavior function of two variables with the third variable fixed to its central point. The most influential variables are the linear terms of sonication duration (t) and temperature (T): TPC increases linearly as sonication time and temperature increase. The same effect has been noticed with ultrasonic intensity (UI) but with a less predominant influence as observed in the Pareto chart. A slight influence of quadratic effect of sonication time ($t^2$) is also illustrated on these surfaces (presence of weak surface curvature when sonication time increases, Figures I.12a and I.12c Optimal settings for TPC maximization were 0.764W/$cm^2$ for ultrasonic intensity, 40 °C for temperature and 40 min for sonication duration. The TPC yield (555 mg of catechin equivalent per 100 g of dry weight) predicted by the model was verified experimentally using the optimized settings. Similar results were obtained by Virot et al (2010) for ultrasound-assisted extraction optimization using ethanol 50% (0.142 W/g, 40 °C and 45 minutes), while other techniques like pressurized fluid extraction and manual maceration resulted in optimized conditions of ethanol 60% and 102°C (Wijngaard and Brunton, 2009) and ethanol 56% at 80°C for 31 minutes or acetone 65% at 25 °C for 60 minutes (Wijngaard and Brunton, 2010), respectively. This shows the importance of optimizing parameters when a modification is done in one or various parameters such as solid/liquid ratio, temperature or solvent. Our extracts were obtained and optimized for water extraction, showing the viability of this procedure with great yields using water as solvent.

### *2.3.5. Comparison and kinetic studies*

To evaluate the impact of ultrasound-assisted extraction in optimized conditions obtained from the response surface method, a comparison study was carried out between ultrasound and conventional extractions (Figure I.13; Table I.4). From Figure I.13, it is possible to observe that ultrasound-assisted extraction increased in TPC yield by more than 30% (420 and 555 mg of catechin equivalent per 100 g of dry weight for conventional and ultrasound-assisted extraction, respectively). The comparison shows a clear improvement of the extraction, which is attributed to ultrasonic cavitation, since this is the only variable of treatment that differs in both experiments.

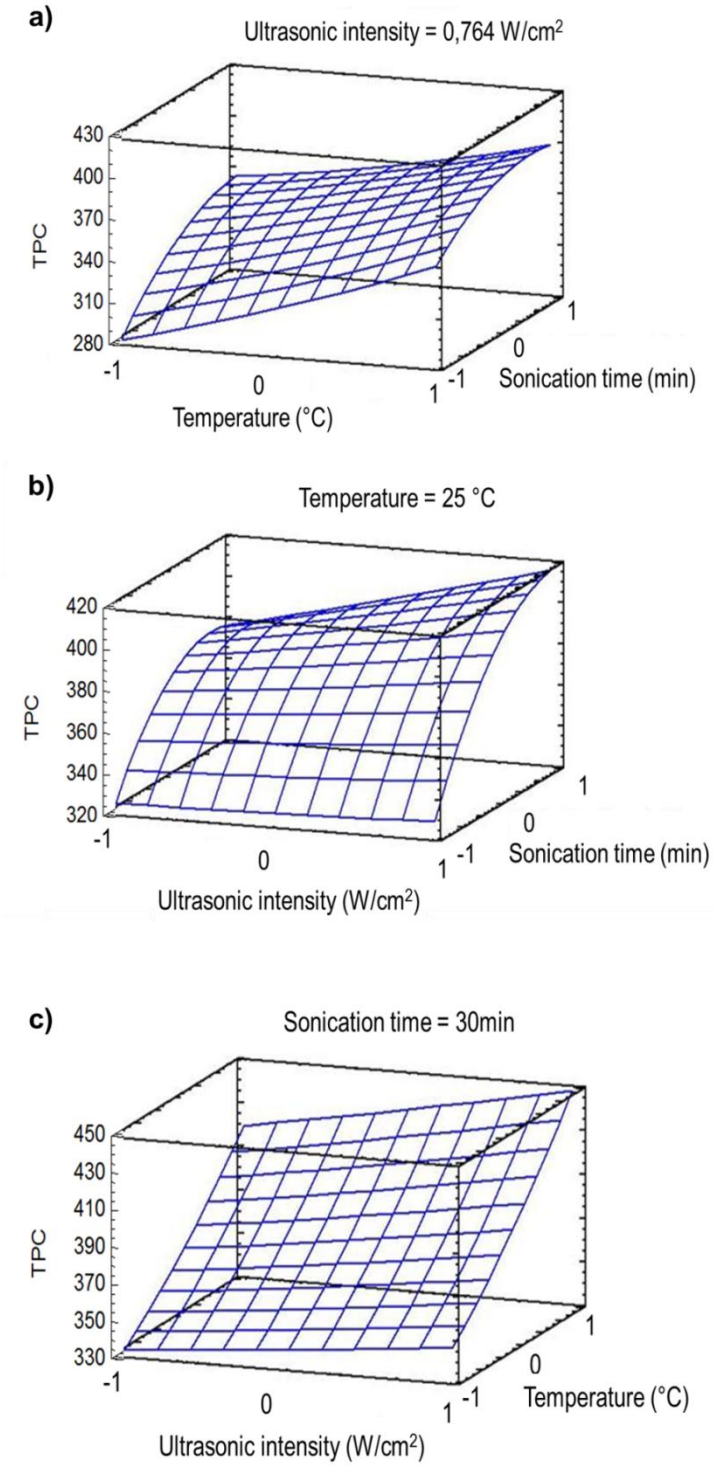



**Figure I.12**. Optimization of ultrasound-assisted apple pomace extraction by water: TPC investigation in the multivariate study (A) TPC as a function of ultrasonic intensity and sonication time, (b) TPC as a function of ultrasonic intensity and temperature, and (c) TPC as a function of temperature and sonication time).

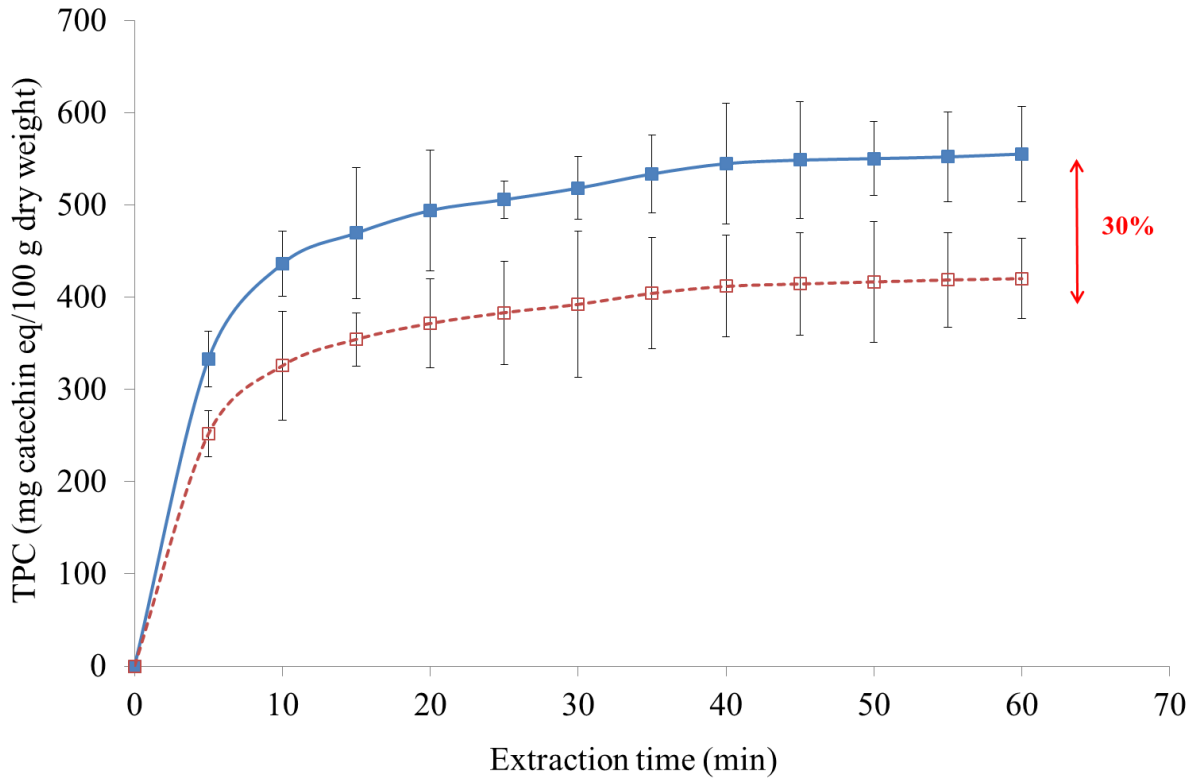



**Figure I.13.** Comparison between conventional (CE-□) and ultrasound-assisted extraction (US-■).

From the table I.4 it is possible to observe that extracts are rich in catechin monomers and phenolic acids, while residues are poor in phenolic acids and rich in procyanidins with a slight increase of the respective DP, which might be attributed to interactions between those compounds and the plant cell wall since the greater the DP, stronger the interaction (Le Bourvellec et al., 2007). The ultrasound extracts are richer in phenolic acids than the conventional extracts, mainly for PCQ. Also, monomers were better extracted than polymers, which was expected since the extraction was performed in an aqueous medium. In the case of the dihydrochalcones, since they are more present in the seeds, the grinding has a lot of influence. In our work, no grinding was used, which explains the greater amount in the residue compared to the extracts. Flavonols were not well extracted, which could be solved by a pre-treatment of apple peels. Phloridzine was not well recovered, probably due to its polarity, even though dihydrochalcones were better extracted by ultrasound than by the conventional technique.

**Table I.4.** Yields and polyphenol composition of apple pomace and its water extracts obtained by conventional and ultrasound-assisted optimized extraction. Yields are in % dry matter, and polyphenol composition in mg/kg of dry weight. The values in italics are the yields recorded for individual components.

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

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

Yields on ultrasound assisted extraction are greater for catechin, epicatechin and flavonols, which implies a partial destructuring of the apple epidermis, suggesting a not full destructuring of apple epidermis by ultrasound in this naturally resistant fraction. As for flavonols, yields are variable, possibly due to their solubility in the buffer. Ultrasound increase extract yield in 6 to 8% for dihydrochalcones and phenolic acids, although results suggest the bonds between polyphenols and polysaccharides were not broken, since polyphenols present a strong interaction with apple cell walls (Le Bourvellec et al., 2004, 2005, 2009). This amount of retention of polyphenols has already been observed in the literature (Kołodziejczyk et al, 2009).

Both extractions (conventional and optimized) follow first-order kinetics, with a fast period from 0 to 10 minutes and a slow period from 10 to 40 minutes of extraction as represented in Figure I.14 together with their respective coefficients. Indeed, the coefficients at the fast period are of 0.162 $min^{-1}$ for the ultrasound and 0.158 $min^{-1}$ for the conventional extraction; while for the slow period, the coefficients are of 0.088 $min^{-1}$ for the ultrasound and of 0.085 $min^{-1}$ for the conventional extraction. Since the difference between the coefficients for both equations were not significant, it is possible to conclude that the ultrasound treatment did not change the kinetics of the extraction, even though the extract yield for the ultrasound treatment is more important, which can be explained by the cavitation phenomena.

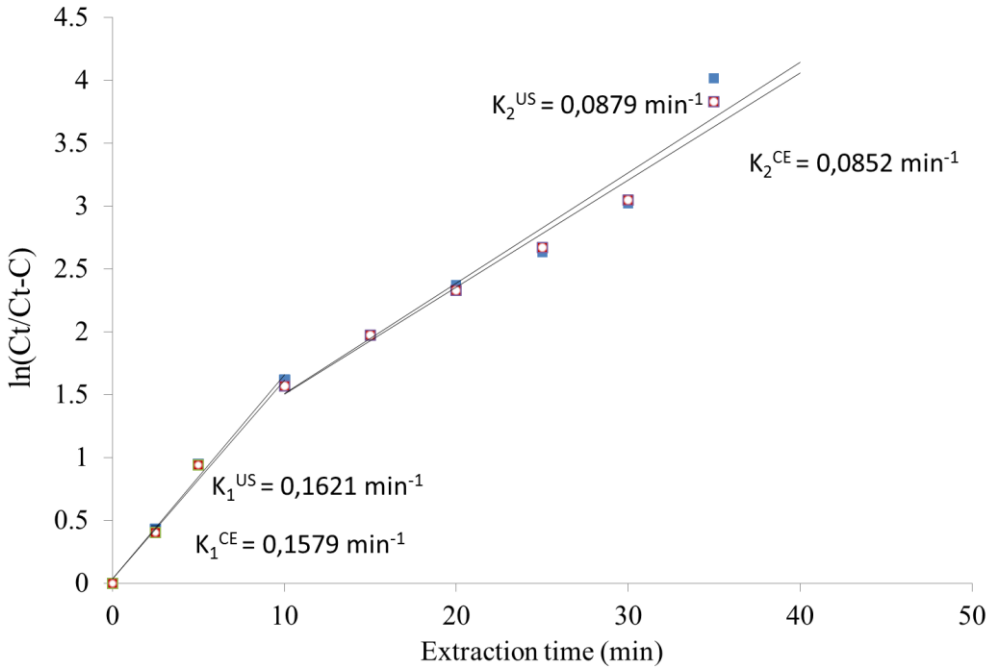



**Figure I.14.** Kinetics and respective constants for conventional (CE-□) and ultrasound-assisted extraction (US-■).

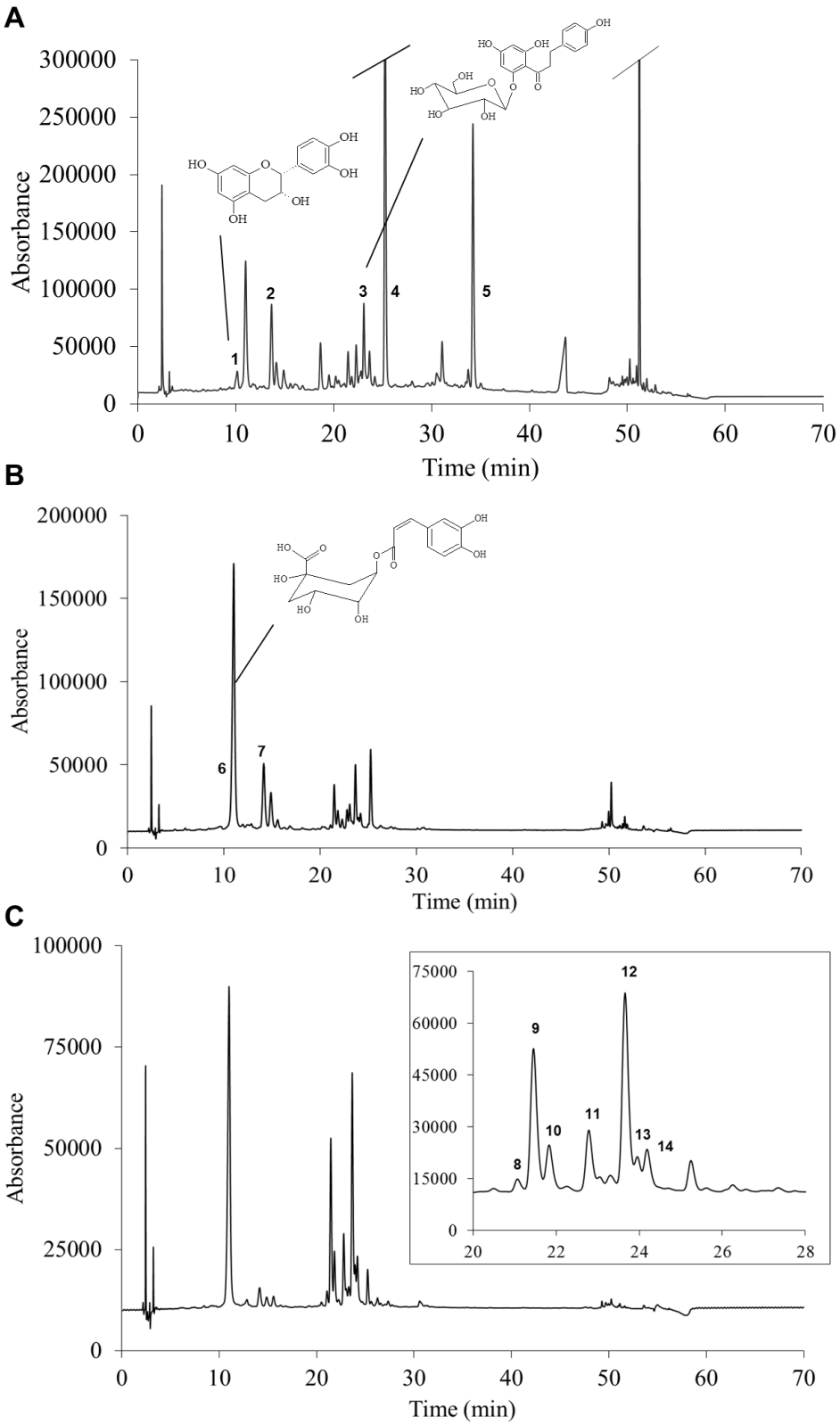
Some studies on the effects of ultrasound-assisted extraction using electronic microscopy (Veillet et al., 2010) showed that the cavitation phenomena is responsible for modifications on the plant material inducing disruption of the cells, due to the burst of the cavitation bubble on the surface of the matrix (Vinatoru, 2001). Studies can be done directly in the cell wall to verify the state before and after extraction, nevertheless due to the heterogeneity and complexity of our matrix (apple pomace), cytological or histological studies of these samples would not provide reliable statistical results.

### *2.3.6. Antioxidant Activity*

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

### *2.3.7. Ultrasound effects on extracted molecules*

In order to verify the innocuousness of ultrasound, three isolated compounds of apple pomace (namely (-)epicatechin, phloridzin and chlorogenic acid) were submitted to the optimized ultrasound extraction conditions. The degradation of these isolated products was evaluated comparing the initial mass to quantified final mass after treatment using HPLC-DAD (Figure I.15) against standards. These compounds were chosen due to their high concentration in the apple and/or importance of application, like phloridzin, which is mainly present in the apple's fruit. We observed no specific reaction products after ultrasound treatment. For chlorogenic acid 97.6% of the initial mass was quantified after US treatment, against 94.7% for epicatechin and 99.2% for phloridzin. This loss of 5% in weight can be due to experimental error.




**A: Abs 280 nm:** (+)-catechin (**1**), (-)-epicatechin (**2**), phloretin xyloglucoside (**3**), phloridzin (**4**), epicatechin benzyl thioether (**5**); **B: Abs 325 nm:** 5'-caffeoylquinic acid (chlorogenic acid) (**6**), *p*-coumarinic acid (**7**); **C: Abs 350 nm:** rutin (**8**), hyperoside (**9**), isoquercetin (**10**), renoutrin (**11**), guajaverin (**12**), avicularin (**13**), quercitrin (**14**).

**Figure I.15.** C18 reverse phase HPLC-DAD chromatograms of ultrasound-assisted extracted apple pomace polyphenols at A: 280 nm; B: 320 nm; C: 350 nm.

### *2.3.8. Large scale ultrasound extraction*

While conventional procedures such as maceration are often time and/or energy consuming, ultrasound-assisted extraction provides numerous advantages from an industrial perspective. Ultrasound as a food processing technology has shown large commercial large scale application, with high returns on capital investment (with the break-even point about 4 months). Improvements in product efficiency, process enhancement and low maintenance cost are achievable on a commercial scale. Also, depending on the application, the required energy is comparable to other operation units currently utilized in the industry (Patist and Bates, 2008; Paniwnyk et al., 2009). Only 40 minutes in water (a green environmental solvent) are needed to recover polyphenols from apple pomace with higher yields compared to conventional extraction procedures. The recycling of an industrial byproduct such as apple pomace using a rapid technique consuming less energy is advantageous from an environmental point of view. For this purpose, a pilot study was performed in a 30 L extraction tank (Figure I.16) consisting of a quadruple output of ultrasound at 25 kHz and 4 x 200 Watts in the optimum conditions obtained from the previous experiments. Polyphenol yields in the ultrasound extraction were comparable to the lab scale experiments and 15% higher when compared to the conventional procedure using maceration (560 mg catechin equivalent per 100 g of dry weight for sonicated samples against 487 mg catechin equivalent per 100 g of dry weight for conventional ones).


**Figure I.16.** Large scale ultrasound apparatus (a), inside view of apparatus (b), UAE of apple pomace (c), liquid extract (d), and lyophilized extract (e).

## 2.4. Conclusion

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

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# Chapter III

## *Ultrasound in food preparation*

- Daniella Pingret, Anne-Sylvie Fabiano-Tixier, Emmanuel Petitcolas, Jean-Paul Canselier, Farid Chemat.
  First Investigation on Ultrasound-Assisted Preparation of Food Products: Sensory and Physicochemical Characteristics.
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## 3.1. Introduction

The emergence of developing countries means that the global food market is increasing continuously. This fact brings the urge to increase the scale of production and reduce manufacturing duration among food manufacturers. As a result of this demand, innovations in processing techniques, packaging, flavors, nutritive components and also quality and safety are increasing. Indeed, manufacturers need to use faster and more productive technologies in order to satisfy consumer requirements for a safe final product that retains all nutritional aspects.

Power ultrasound produces cavitation in liquid media, generating mechanical effects (intense pressure, shear forces) and temperature gradients within the material, which can disrupt its structure physically or promote chemical reactions (T. J. Mason, Paniwnyk, & Lorimer, 1996). In particular, ultrasound provides energy well adapted to the formulation of dispersed systems: emulsions (L/L), suspensions (S/L), aerosols (L/G or S/G) and foams, at least under certain conditions (Abismaïl, Canselier, Wilhelm, Delmas, & Gourdon, 1999). Although foaming is usually reduced during US emulsification regarding to mechanical stirring (Ultra-Turrax rotor-stator) process, one cannot conclude that US exerts a systematic antifoaming action, since US-assisted foam generation has been reported several times (Abismaïl et al., 1999; Lim & Barigou, 2005). In fact, the interactions caused by power ultrasound in dispersed (or colloidal) systems have been a subject of historical interest for physical chemists: emulsification and aerosol formation appear among the first applications of power ultrasound (Richards & Loomis, 1927). Early in 1928, Herbert Freundlich's works opened the "ultrasonic way" in the colloids field. At that time, this topic was the subject of a large number of papers, dealing for instance with the effect on colloidal media, viscosity reduction and thixotropic phenomena (Gillings, 1993). Ultrasound emulsification and, more generally, ultrasound effects in the formulation of dispersed systems have been already reviewed (Abismaïl et al., 1999; Gaikwad & Pandit, 2008).

The application of high intensity ultrasound (US) in the food industry has been a research subject for many years (Knorr, Zenker, Heinz, & Lee, 2004). This technique has been used in the cleaning process of equipments, homogenization, sterilization, heat and mass transfer, emulsification, dispersion, aerosol formation, defoaming, extraction (e.g. proteins), crystallization, freezing, degassing, filtration, drying, oxidation/reduction reactions, meat tenderization, recombined meat products, enzyme reactions or inhibition, cell disruption and

living cell stimulation (Dolatowski, Stadnik, & Stasiak, 2007; T. J. Mason et al., 1996; T.J. Mason & Paniwnyk, 1996; McClements, 1995; Povey & T. J. Mason, 1998; Torley & Bhandari, 2007). Since a lot of food products exist as dispersions (especially emulsions or foams) (Dalgleish, sans date; Dickinson & Patino, 1999; Friberg, Larsson, & Sjöblom, 2004; Xu, Nikolov, Wasan, Gonsalves, & Borwankar, 2003), there are numerous possibilities for applying this technology to the agro-food industry in order to meet demands for increasing production efficiency.

Ultrasound is presently used in a number of different sectors of the agro-food industry, *e.g.* processing (separation, homogenization etc.), preservation, extraction and sample preparation but to date there are very few examples of its use in food preparation. The optimized extraction of date syrup and the anti-microbial effects on the fruit was studied (Entezari, Hagh Nazary, & Haddad Khodaparast, 2004), as also the homogenization and fermentation of yogurt (Wu, Hulbert, & Mount, 2000). Regarding edible foams, a recent study shows that US improves the foaming properties of egg-white, and it results in crispier meringues from lighter and stiffer foam (Knorr et al., 2004).

The objective of this work was to compare sensory and physicochemical properties of three foam-type products prepared conventionally and with ultrasonic methods. Evaluations of texture, water activity and color, together with sensory analyses were performed for three types of food preparations, namely chocolate *Genoise*, sponge cake and chocolate mousse (viscosity was only determined for the chocolate mousse). These products were chosen for their semi-liquid state during preparation and for certain similarities in the processing steps. The effects of sonication on the different aspects of physicochemical parameters and sensory analysis are discussed. A literature search did not yield any reference to earlier reports on ultrasound-assisted preparation of food. This work was the first attempt to use an ultrasound device to assist food preparation, being a great contribution to molecular gastronomy.

## 3.2. Experimental Section

### 3.2.1. Materials and equipments

Ultrasound-assisted preparation (US) was performed in an inox PEX1 ultrasonic bath (R.E.U.S., Contes, France) with 14x10 cm internal dimensions and maximal capacity of 1 L, equipped of a transducer in the base of jug operating at a frequency of 25 kHz with maximum

input power (output power of the generator) of 150 W. The double-layered mantle (with water circulation) allowed the control of the temperature of the medium by cooling/heating systems such as an ultrasound "bain marie". The power dissipated in the medium, measured by calorimetry, was about 70W. For mixing/blending the products inside the bowl, a whisk (at constant speed) was used.

### 3.2.2. Food preparation

Three food products were prepared using conventional and ultrasound-assisted techniques, namely, chocolate Genoise, basic sponge cake and chocolate mousse. The products prepared without ultrasound are referred to as CV (for conventional) while those prepared with ultrasound are referred to as US. To better evaluate the influence of ultrasound in food processing, all preparations were carried out under the same conditions and the sonication during the mixing step of the process was the only difference between US and CV samples. The temperature was kept at 25°C during preparations as suggested by culinary procedures.

#### *3.2.2.1. Chocolate Genoise*

Drinking chocolate (35 g), sugar (20 g), 2 fresh eggs and a small amount of leavening agent were mixed with 20 g of water and 200 mL of cream and placed into the US bowl. Both mixtures were blended for 5min to homogenize the ingredients and then for 5 more minutes (the ultrasound, in the case of the US samples, was applied in these 5 last minutes). The mixture was transferred to an aluminum dish and placed in an oven at 180°C for 30min. Finally, the CV and US products were cooled down to room temperature before further analysis.

#### *3.2.2.2. Sponge cake*

Sugar (120 g) and 4 fresh eggs were put into the US bowl. The mixtures were blended for 5min until homogenization and again for 5 min (the ultrasound, in the case of the US samples, was applied in these 5 last minutes, cf 2.2.1.). Both products where then cooled down to room temperature. Next, 120 g of flour were put into the bowl and mixed slowly with a spatula before being transferred to an aluminum dish and placed in the oven at 180°C for 30 min. Finally, the samples were cooled down to room temperature before further analysis.

#### *3.2.2.3. Chocolate mousse*

Drinking chocolate (100 g), oil (30 g), sugar (30 g) and a small amount of gelatin powder were poured into 200 mL of pasteurized milk inside the US bowl. The CV mixture was blended for 5 min while the US mixture was blended for 3 min and sonicated for 2 min with ultrasound. The products were poured into small glass bowls and cooled down to 4°C for 4h before further analysis.

### 3.2.3. Analysis Methodology

The principal characteristics of the products were determined by physicochemical tests (namely texture, water activity and color) and sensory analyses. The assays were applied to all samples, except for viscosity, that was determined for the chocolate mousse only. The results of the physicochemical analyses reported below are the averages of 3 measurements.

#### *3.2.3.1. Texture*

Since texture is one of the most important sensory characteristics determining consumer preferences, this parameter was also measured by a reliable and practical technique based on the multi-parameter determination of texture characteristics to accurately predict sensory texture attributes (Montero-Calderón, Rojas-Graü, & Martín-Belloso, 2008). The texture of

the samples was determined by a typical texture profile analysis (TPA), which principle is the simulation of the chewing action (Figure I.17) and consequent evaluation of derived parameters (Bourne, 2002; Kim et al., 2009).

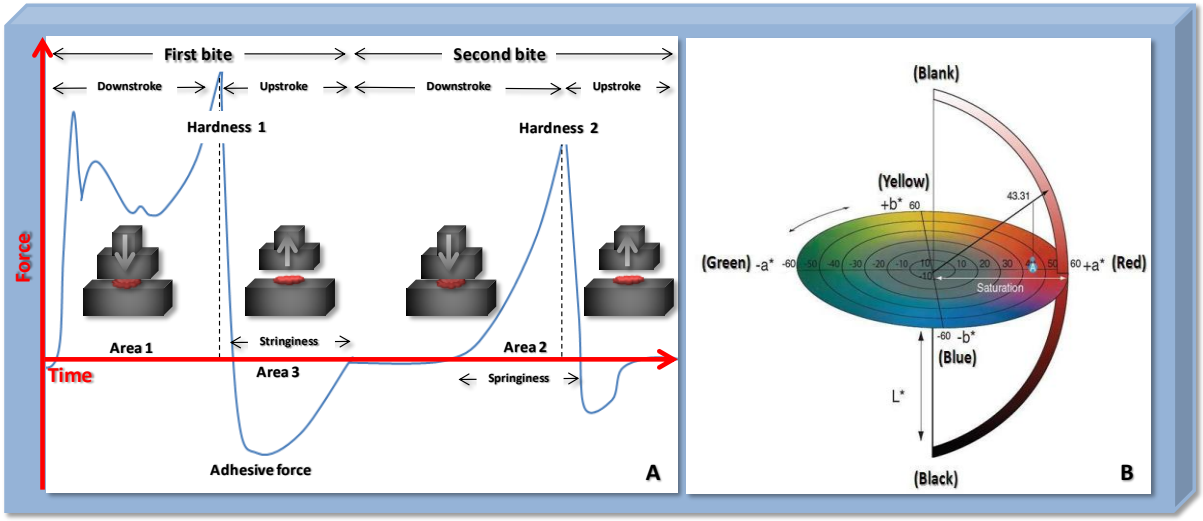


**Figure I.17.** General Texture Profile Analysis (TPA) curve **(A)** and CIE L*a*b* color assessment parameters **(B)**.

The instrument used was the TA3 probe of a QTS25 Texture Analyzer and the textural parameters considered were *hardness*, which measures the peak force of the first compression cycle and expresses the maximum force necessary to compress the sample and is expressed in Newton (N); *cohesiveness*, which expresses how well the product withstands a second deformation relative to how it behaved under the first deformation, measured as area of work during the second compression divided by the area of work during the first compression (dimensionless) ; *springiness*, which represents how well a product physically springs back after it has been deformed during the first compression, measured at the downstroke of the second compression by the distance of the detected height of the sample on the second compression (in mm), and *adhesiveness*, which corresponds to the work required to overcome attractive forces between the sample and the other surface, expressed in Newton (N) (Bourne, 2002; Chiabrando, Giacalone, & Rolle, 2009; Kim et al., 2009; Montero-Calderón et al., 2008).

The samples were placed in a 50 mL beaker and compressed as follows. First, the instrument height was checked to determine the initial height of the sample, followed by two consecutive compressions performed automatically at a test speed of 30 mm/min. Next, a

compression distance of 10mm was chosen, ensuring that the sample did not fracture before the second compression. Finally, the testing was carried out after the samples had been equilibrated to a standard temperature of 25 °C, for the initial taste panel training assessments.

#### *3.2.3.2. Water activity*

Water activity is a thermodynamic property that describes the energy status or escaping tendency of the water in a sample. It is closely related to the partial molar Gibbs free energy and indicates the availability of water present structurally or chemically in products (Van den Berg & Bruin, 1981). The $a_w$ value of a given medium is correlated with the deterioration of food stability due to the growth of microorganisms. For its measurement, pure water is used as a standard ($a_w = 1.0$) and the system must be in equilibrium at a defined temperature (Scott, 1957). Water activity (Equation 1) is defined as the ratio of the vapor pressure of water in a material to that of pure water at the same temperature. Relative humidity of air is defined as the ratio of the vapor pressure of water in the air to its saturation value. At equilibrium, the water activity of the sample is equal to the relative humidity of air surrounding the sample in a sealed measurement chamber. Multiplication of water activity by 100 gives the equilibrium relative humidity (ERH) in percent (1).

$$a_W = [(P^V{}_W)_{sy}].[(P^V)_W]^{-1} \quad (1)$$

Where $a_W$ represents water activity ($0.0 \leq a_W \leq 1.0$), $(P^V{}_W)_{sy}$ represents the vapor pressure of water in the system [35], $(P^V)_W$ represents the vapor pressure of pure water and ERH represents the equilibrium relative humidity of air at a given temperature. The water activity ($a_w$) of the food preparations was determined using an electronic water activity meter, Hygrolab 3 (ROTRONIC AG, Bassersdorf, Switzerland). The equipment was calibrated before testing. The variability of this meter was about ± 0.0015 $a_w$ and ± 0.1°C. It is important to notice that water activity is a function of temperature.

#### *3.2.3.3. Viscosity*

Viscosity is defined as the internal friction of a liquid or its tendency to resist flow. Dynamic viscosity is usually denoted by η and is calculated as shown in the Equation 2.

$$\eta = \sigma.\gamma^{-1} \quad (2)$$

where η is the viscosity; σ is the shear stress ($N.m^{-2}$) and γ is the shear rate ($s^{-1}$) (Bourne, 2002). According to the International Organization for Standardization (ISO), η is expressed in Pascal second (Pa**.**s). The viscosity in the samples was estimated at 25°C using a rotary HAAKE VT550 viscometer and a SV-DIN sensor. The equipment was calibrated at different temperatures. The sensor parameters were the shear stress and the shear rate.

#### *3.2.3.4. Color assessment*

The color of the products was measured using the CIE L*a*b* system (CIE, 1986), which is a simplified mathematical approximation to a uniform color space composed of perceived color differences. The lightness *L** (from 0 to 100) perceived by a standard observer is assumed to follow the intensity of a color stimulus according to a cubic root law. The colors of lightness *L** are arranged between the opponent colors green-red axis (*a**, from -128 to +128) and blue-yellow axis (*b**, from -128 to +128) along the rectangular coordinates *a** and *b**, as shown in Figure I.17. Any color represented in the rectangular coordinate system of axes *L**, *a**, *b** can alternatively be expressed in terms of polar coordinates with the perceived lightness *L** and the psychometric correlate of chroma (*C**) as shown in Equation 3 (Hill, Roger, & Vorhagen, 1997).

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (3)$$

The color assessment of the samples was made using a colorimeter Minolta CM, 3500 d, calibrated with distilled water. The samples were placed on 50 mm diameter sterile plastic Petri dishes. Three measurements were made for each food preparation and the mean value was reported. The results were expressed in *C**.

#### *3.2.3.5. Sensory analysis*

The influence of ultrasound on the sensory characteristics of the products was evaluated. Sensory analyses (Yantis, 1992) were conducted by a panel consisting of 18 graduate students from the University of Avignon, France. The subjects were seated in sensory booths with appropriate ventilation and lighting. The samples were presented to each panelist on white polystyrene plates. Subjects were instructed to place the stimuli on the tongue and rub the tongue against the palate. Tap water was supplied to the panelists for rinsing between samples. The following attributes were evaluated for the three products: creaminess, lingering taste, granularity, sweetness, egg taste, dryness, crumbliness, crunchiness, mellowness, cocoa flavor and melting property.

For overall quality, the scale range was from -3 to +3. On this scale, a score of 0 corresponded to an ideal perception, a score of -3 represented the weakest attribute and a score of +3 represented the strongest attribute. For each sample, the panelists gave their preferences on a hedonic scale (1 to 6). The sum of the points was calculated for each sample. The flavor was correlated by the ranges of each sample from which the number one was the least appreciated and the number six was the most appreciated. A differentiation test was made as follows. Two samples of the product had to be recognized out of six by the panelists. The objective was to know if samples prepared with and without ultrasound were significantly recognized or not. The percentage of identification (PI) of samples was calculated. This value represented the number of times that the samples were recognized out of 100 identifications.

## 3.3. Results and Discussion

### 3.3.1. Chocolate Genoise

#### *3.3.1.1. Physicochemical analysis*

The preparation steps are represented in Figure I.18. The CV sample presented more hardness, less springiness, more cohesiveness and less adhesiveness than the sonicated one (US). Also, $a_w$ was slightly increased by US (Table I.5).

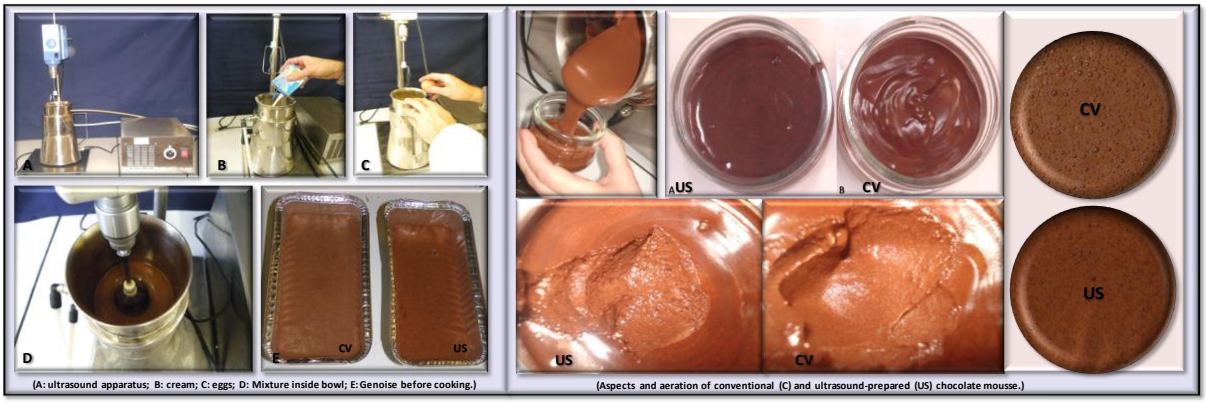


**Figure I.18.** Materials and steps for conventional (CV) and ultrasound-assisted preparation (US) of food and aspects of chocolate mousse.

There was a significant color difference between CV and US products, attributed to the fact that the US products were more aerated, since the ultrasound promotes a better homogenization regarding bubbles distribution and size (Figures I.18 and I.19).

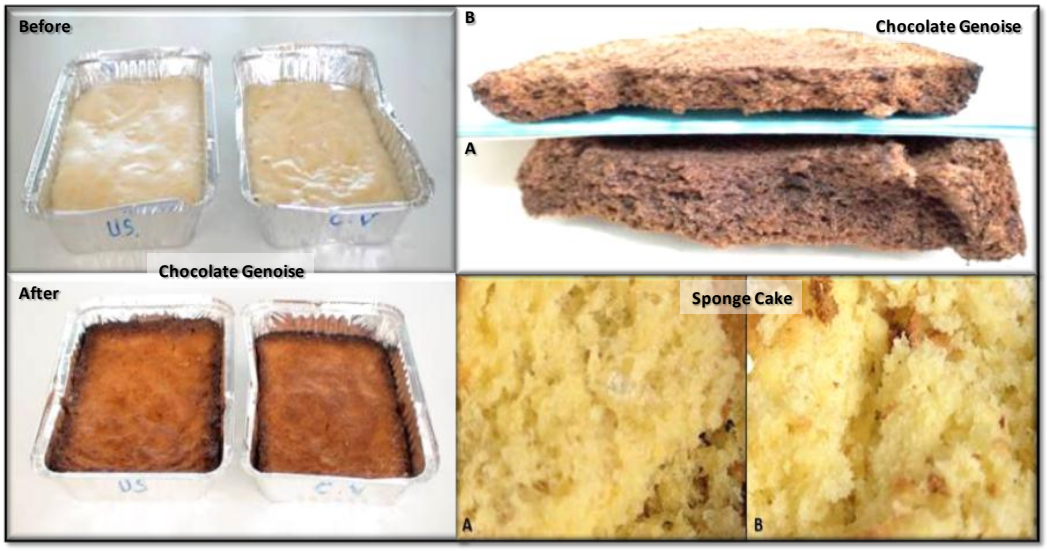


**Figure I.19.** Visual and internal aspects of conventional (**A**) and ultrasound-prepared (**B**) chocolate *Genoise* and sponge cake.

#### *3.3.1.2. Sensory analysis*

Neither the CV nor the US samples were recognized by the examiners but the sensory profile was better for the US product with a better appreciation of the attributes of sweetness, mellowness, crunchiness and cocoa flavor (Figure I.20 and Table I.5). In conclusion, the US samples were preferred by a majority of panelists.

### 3.3.2. Sponge cake

#### *3.3.2.1. Physicochemical analysis*

The CV sample was harder, less springy, less cohesive and less adhesive than the US sample. Also, $a_w$ was slightly increased by the ultrasound (Table I.5). There was a large difference of coloration between the two samples. As for the other products, the color was affected by the aeration/homogenization effect of the ultrasound (Figure I.19).

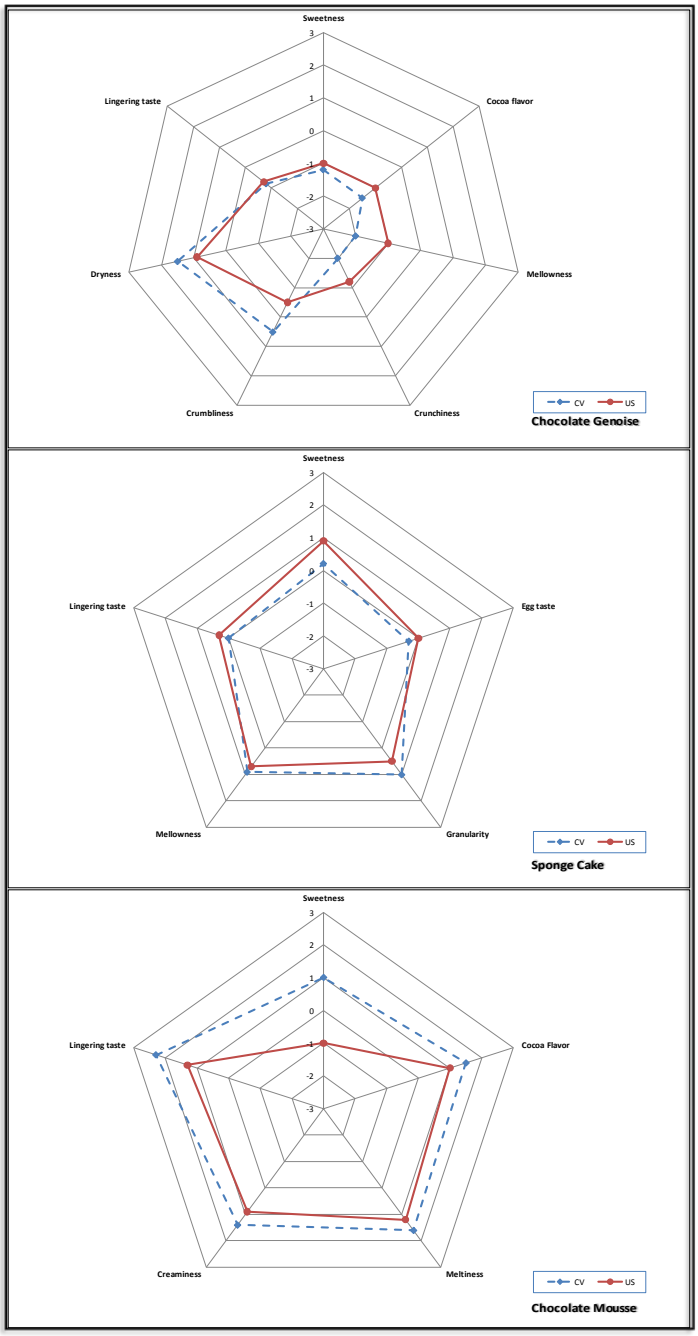


**Figure I.20**. Sensory profile of conventional (CV) and ultrasound-prepared (US) food samples.

### *3.3.2.2. Sensory analysis*

The percentage of identification (PI) was the same for both samples, so these values were not exploitable, but some attributes were more appreciated for the US samples such as

sweetness, egg taste (Figure I.20). The ultrasound product was slightly preferred by the panelists (Table I.5).

### 3.3.3. Chocolate mousse

#### *3.3.3.1. Physicochemical analysis*

The conventionally prepared sample (CV) was harder, less springy, less cohesive and more adhesive than the sample with ultrasound (US). Also, $a_w$ was slightly reduced by ultrasound (Table I.5). The coloration was different for CV and US samples (Table I.5 and Figure I.18). We observe that the chocolate mousse obtained by US was shinier, smoother, more aerated and presented a darker color. This color change may be assigned to the texture of the US sample, more specifically the size and the distribution of air bubbles producing different reflection properties. The viscosity was increased by the effect of ultrasound. In fact, the samples prepared with US were more homogeneous and denser than the CV samples (Figure I.18) because of the compression-rarefaction phenomenon occurring on the air bubbles in the product during sonication.

#### *3.3.3.2. Sensory analysis*

The attributes of the chocolate mousse were slightly changed by the ultrasound (Figure I.20). The CV samples were found to be more cocoa flavored, melting, creamy, lingering tastiness and sweet than the US samples. The identification percentage was larger for the US samples, meaning that they were recognized more easily than the CV samples. Additionally, the CV samples were largely preferred to the US samples due to the more pleasant taste attributed to the former one (Table I.5). Sensory experiments revealed that a short ultrasonic treatment of chocolate mousse is sufficient to generate a remarkable off-flavor. The panelists described the untreated chocolate mousse as typical, but the sample treated with ultrasound is described by different terms, mainly “metallic”, followed by “fishy”, “rancid” and “tallowy”. Some problems due to the use of ultrasonic systems have been raised by different teams (Chemat, Grondin, Shum Cheong Sing, & Smadja, 2004; Patrick, Blindt, & Janssen, 2004;

Schneider, Zahn, Hofmann, Wecks, & Rohm, 2006). Off flavors and oil degradation were observed due to the high intensity of the probe and the horn systems. In fact these two systems are very powerful because ultrasound are delivered on a very small surface, thus the intensity of ultrasound at the tip of the probe is very high (about 50–200 W/cm$^2$). The new device developed by REUS does not consist of a probe system, but the power is delivered on the whole base of the system which corresponds to a power of about 1 W/cm$^2$. As observed, at this power the samples are less subjected to oxidation, which makes the system more applicable to food processing.

**Table I.5.** Physicochemical and sensory analysis of conventional (CV) and ultrasound-prepared (US) food samples.

| Food samples | | Texture | | | | Physicochemical analysis | | | Sensory analysis | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Hardness (N) | Springiness (mm) | Cohesiveness | Adhesiveness (N) | $a_W$ | Coloration (C*) | Viscosity (Pa.s) | PI (%) | Values attributed by panelists |
| **Chocolate** | **CV** | 817 | 14.70 | 0.394 | 1284 | 0.713 | 3.40 | - | 0 | 26.5 |
| **Genoise** | **US** | 630 | 17.45 | 0.347 | 2961 | 0.723 | 5.27 | - | 0 | 56.0 |
| **Sponge** | **CV** | 1551 | 18.06 | 0.376 | 597 | 0.812 | 3.02 | - | 50.00 | 40.5 |
| **cake** | **US** | 286 | 26.34 | 0.465 | 735 | 0.870 | 5.36 | - | 50.00 | 43.5 |
| **Chocolate** | **CV** | 85 | 4.50 | 0.299 | 192 | 0.956 | 4.82 | 157.15 | 71.43 | 47.0 |
| **mousse** | **US** | 42 | 4.95 | 0.313 | 53 | 0.924 | 5.20 | 113.25 | 50.00 | 41.0 |

### 3.4. Conclusions

The physicochemical and organoleptic parameters of the three food products were changed when they were prepared with ultrasound (Table I.6). All products presented less hardness, while most presented more springiness (except for the mousse), more cohesive (except for the *Genoise*) and adhesiveness (except for the chocolate mousse). Ultrasound was also found to change the water activity of the products but not in a consistent way (generally it was increased, except for the chocolate mousse), making it difficult to draw a firm conclusion on those aspects. The color was changed in all samples and the viscosity of the chocolate mousse was decreased (Table I.5).

**Table I.6.** Summary of trends observed after ultrasound treatment.

| Parameter | Genoise | Sponge Cake | Mousse |
|---|---|---|---|
| Hardness | ↓ | ↓ | ↓ |
| Springiness | ↑ | ↑ | ↓ |
| Cohesiveness | ↓ | ↑ | ↑ |
| Adhesiveness | ↑ | ↑ | ↓ |
| Water Activity | ↑ | ↑ | ↓ |
| Viscosity | – | – | ↓ |
| Coloration | ↑ | ↑ | ↑ |
| PI (%) | = | = | ↑ |
| Preference | ↑ | ↑ | ↓ |

Ultrasound is known to provide a more homogenous mixing, so the distribution and size of particles are also more uniform, which can explain the viscosity decrease in US samples as well as the color difference, since different air bubbles distribution and size might result in different reflective properties. Sensory analysis showed that sonication modified some attributes such as sweetness, meltiness, dryness, crunchiness, mellowness and granularity and hence it was concluded that ultrasound application during preparation changed the flavor of the products. The formation of free radicals by ultrasound due to the breakage of molecular bonds would explain the physicochemical and organoleptic modifications on samples. Moreover, the variation in equilibrium points of the molecules and the formation of microjets could also be the origin of the greater color homogenization in the ultrasound-assisted prepared products. This homogenization might also likely result in better mass transfer between the different phases of the food. Furthermore, ultrasound generates energy due to the cavitation phenomenon, resulting in higher local temperature gradients. This energy can accelerate chemical reactions that might originate increased sweetness or other attributes perceived by the panelists.

In conclusion, ultrasound can be used to assist foam-type food preparations, resulting in more homogenous products that were not only well accepted by the panelists but even preferred to conventionally-prepared ones, as some organoleptic characteristics were improved. Since for the chocolate mousse some important changes were observed that conducted to less acceptance of the product, further studies should be conducted.

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# *Second Part*

# Second Part

## *Investigations on food stability during ultrasound processing*

### *Summary*

Although ultrasound has proven to be a very effective innovative technique of food processing, being applicable to many processes with numerous advantages over conventional techniques, the possible effects and consequences to products quality are often overlooked. In this part, some of the effects on food products induced by ultrasound are presented in the first chapter, evidencing the degradation of some compounds and the modifications in physicochemical parameters of food products. Then, the focus will be in the ultrasound effects on food products with high lipid content, followed by a brief theory on lipid degradation and analytical methods of oxidation estimation. In the second chapter, a more detailed study of lipid degradation induced by ultrasound is presented, with the study of different edible oils submitted to ultrasound treatment, followed by a comprehension of the mechanism of ultrasound induced degradation of lipids.

# Chapter I

## *Ultrasound effects on food products*

## 1.1.Introduction

Over the past few years, an increase on food demands either in terms of quantity or quality is observed, imposing modifications in the processing techniques. Some sample preparation methods carried out under severe conditions may give rise to chemical and physical changes that impair the organoleptic properties and reduce the content or bioavailability of some nutrients. Therefore, the food industry is constantly searching for emergent milder processing technologies capable of preserving chemical and physicochemical characteristics, together with sensorial and nutritional qualities as also the bioactivity of certain constituents.

Although ultrasound is able to produce benefic modifications in food parameters as presented in the first part of this work, the physicochemical effects of ultrasound treatment might also result in quality impairments of food products by the appearance of off-flavors, modifications in physical parameters and degradation of major and minor compounds. Due to the critical temperature and pressure conditions, allied to the formation of radicals during sonocavitation, some alterations in food components have been reported during ultrasound treatment.

Acoustic cavitation can produce radicals in the medium and molecules such as OH and H radicals accumulate in the surface of the cavitation bubble, which can be responsible for initiating formation of degradation products that can also trigger radical chain reactions and provoke substantial quality defects in those products (Makino et al., 1983; Riesz et al., 1985; Czechowska-Biskup et al., 2005).

Among the reported changes in food products submitted to ultrasound, color changes appear to be one of the most studied modifications, together with antioxidant activity and physicochemical characteristics such as viscosity, among others. Since food is a complex mixture of components, the fundamental study of isolated molecules is scarce in the domain and, in the development of new technologies; researchers tend to focus on the increase of yield and decrease of time and energy consumption, analyzing the principal characteristics of the product in detriment of the investigation constituents modifications.

An increasing number of reports in the literature concern modifications in high lipid containing food products. Lipid deterioration is of great economic importance in the production of lipid-containing food products. Oxidation of unsaturated lipids not only

produces unpleasant odors and flavors but can also decrease the nutritional quality and safety by the formation of secondary reaction products in foods. In food products, lipid autoxidation is often referred to as rancidity, which describes the off-flavors obtained by subjective organoleptic evaluation of the product (Hamilton et al., 1997). Oil oxidation can also destroy essential fatty acids and produce oxidized polymers and toxic compounds (Choe and Min, 2006). The lipid oxidation phenomenon depends on several complex reaction mechanisms, which are related to the lipid structure and the medium conditions in which the lipids are present. Some determining variables to lipids oxidative stability are the number and nature of present unsaturations, the type of interface between the lipids and oxygen, exposure to light and heat and the presence of pro- or antioxidants. The potential restrictions and/or uses of chemical effects generated by cavitation phenomena are shown in Figure II.1.

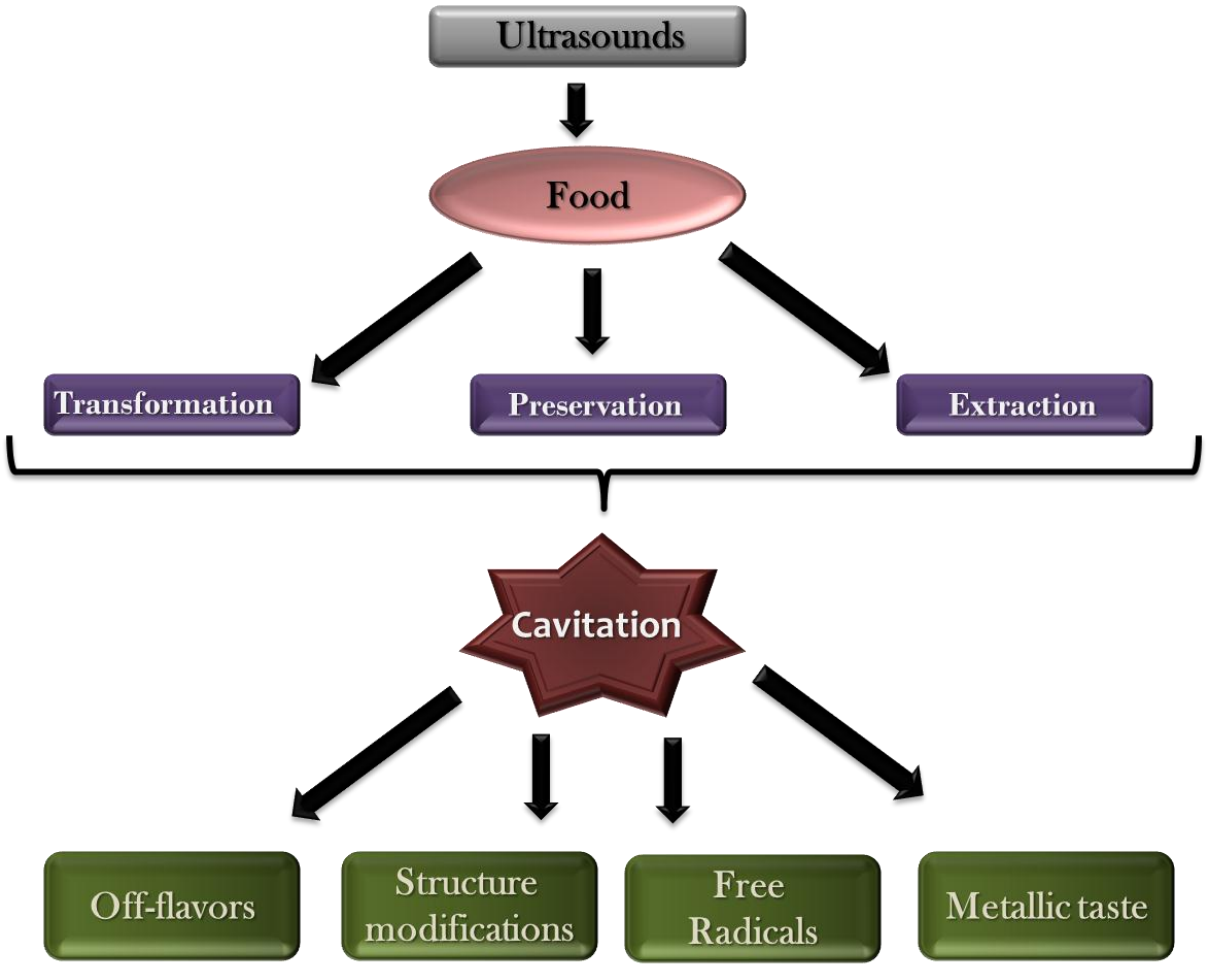



**Figure II.1.** Chemical effects generated by cavitation phenomena.

## 1.2. Impact of ultrasound on food products

Studies show some alterations in quality parameters of food products treated by ultrasound, which can result in safety impairments or less acceptance of the final product by the appearance of off flavors, change of color, decrease of sugar content, and/or modifications of minor compounds. The Table II.1 summarizes the effects on ultrasound-treated food products and experimental conditions used in the studies.

### 1.2.1. Color modifications

Color changes in a food product may affect the overall acceptability of the product for consumers. Several researchers have documented the effect of ultrasounds on the color of liquid and solid food products. Non-enzymatic browning in certain products model systems was studied with a continuous sonication system and brown pigments in milk treated by ultrasound increased with treatment time compared to heat-treated milk, which was probably caused by the Maillard reaction (Vercet et al., 2001). Browning and color changes were also observed in sonicated fruit juice, such as orange juice (Gómez-López et al., 2010; Knorr et al., 2002). The ultrasound treatment of glucose in an aqueous phase could yield glucosyl radical and polymers in the presence of oxygen, which could contribute to the formation of browning pigments (Kardos and Luche, 2001).

Besides a darkening of sonicated samples, some off-flavors were detected in apple and cranberry juices treated by ultrasound, together with a decrease in anthocyanin content, resulting in a lower acceptability of the products (Caminiti et al., 2011). Thermosonication of watercress (*Nasturtium officinale*) resulted in color changes as well, although the chlorophyll content did not present significant variations (Cruz et al., 2007). Darker colors were also observed in ultrasound-assisted preparation of chocolate mousse when compared to the conventional preparation due to the homogenous distribution of fat globules (Pingret et al., 2011).

**Table II.1.** Effects of ultrasound on sonicated food products.

| Food Matrix | Analyte | Experimental conditions | Observations | References |
|---|---|---|---|---|
| Apple and cranberry juice | Color, anthocyanin | 20 **A**, 750 **B**, probe **C**, 8 **D**, 43-58 **E**, color, anthocyanin content **F**, manothermosonication **G** | Darkening of sonicated samples, detection of off-flavors and decrease of anthocyanin content. | Caminiti et al. (2011) |
| Watercress (*Nasturtium officinale* ) | Color | 20 **A**, 125 **B**, probe **C**, 0-120 **D**, 82-92 **E**, color **F**, blanching **G** | Color changes (increase of the green color). | Cruz et al. (2007) |
| Crab shells and corn starch | Chitosan and starch | 360 **A**, 100 **B**, bath **C**, 0-90 **D**, 22 **E**, viscosimeter, UV **F**, rheological properties **G** | Degradation of chitosan and starch by hydrogen substraction. | Czechowska et al. (2005) |
| Corn hull heteroxylan | Xylan | 20 **A**, 100-200 **B**, bath **C**, 0.5 **D**, 70 **E**, $^{13}$C NMR, IR **F**, structure and properties study **G** | Viscosity changes and formation of new unsaturated structures. | Ebringerova & Hromadkova (1997) |
| Corn starch | Starch | 24 **A**, 400 **B**, probe **C**, 15-30 **D**, - **E**, viscosimeter, micrography **F**, rheological properties **G** | Destruction of the granular structure. | Jambrak et al. (2010) |
| Orange juice | Ascorbic acid | 20 **A**, - **B**, probe **C**, 0-60 **D**, 30 **E**, HPLC **F**, enzyme inactivation **G** | Browning of juice and lower ascorbic acid content. | Lee et al. (2005) |
| Tomatoes | Lycopen | 37 **A**, 140 **B,** bath **C,** 45 **D**, 47 **E**, HPLC **F**, extraction **G** | Isomerization of lycopen, with 14% increase of cis isomers and 76% decrease of trans isomers. | Lee-Sie and Teoh ( 2011) |
| Chocolate mousse | Color, lipids | 25 **A**, 150 **B**, bath **C**, 2 **D**, 25 **E**, color, sensory analysis **F**, food preparation **G** | Darker color of sonicated samples, decrease of viscosity and apparition of off-flavors. | Pingret et al. (2011) |
| Watermelon juice | Minor compounds | 20 **A**, 1500 **B**, probe **C**, 2-10 **D**, 25-45 **E**, HPLC **F**, microbial inactivation **G** | Ascorbic acid, lycopen and phenolics degradation. | Rawson et al. (2011) |
| Rabbiteye blueberries | Anthocyanins and total phenols | 850 **A**, 100 **B**, bath **C**, 180 **D**, 21 **E**, UV **F**, osmotic concentration **G** | Decrease in phenolics and anthocyanin contents. | Stojanovic and Silva (2007) |
| β-carotene | All-trans-β-carotene | 21-25 **A**, 950 **B**, Probe **C**, 10 **D**, 5 **E**, HPLC and IR spectroscopy **F**, - **G** | Degradation products include isomers 15-cis-β-carotene, di-cis-β-carotene and other compounds with C-O function group. | Sun et al.(2010) |
| Strawberry juice | Anthocyanins and ascorbic acid | 20 **A**, 1500 **B**, probe **C**, 0-10 **D**, 25 **E**, HPLC **F**, - **G** | Decrease in anthocyanin and ascorbic acid contents, suggesting an oxidation by pyrolise. | Tiwari et al. (2008) |
| Orange juice | Ascorbic acid | 20 **A**, 1500 **B**, probe **C**,0-10 **D**, 5-30 **E**, HPLC **F**, - **G** | Hydroxyl radical formation, interaction between free radicals and ascorbic acid. | Valdramidis et al.(2010) |
| Orange juice | Ascorbic acid, β-carotene | 20 **A**, - **B**, bath **C**, 0.2-0.5 **D**, 55-85 **E**, UV **F**, enzyme inactivation **G** | Decrease in ascorbic acid ans β-carotene contents. | Vercet et al. (2001) |

**A**: Frequency (kHz), **B**: Power (W), **C**: Type of ultrasound apparatus, **D**: Exposure time (min), **E**: Temperature (°C), **F**: Detection and analysis method, **G:** Process

**$^{13}$C NMR:** Nuclear magnetic resonance

**HPLC:** High-performance liquid chromatography

**IR:** Infra-Red spectroscopy

**UV:** Ultra-violet spectroscopy

### 1.2.2. Antioxidants modifications

Besides changes in organoleptic characteristics of food products, some minor components are affected by ultrasound treatment and might result in a lower acceptance by consumers or decay in quality. Some studies show degradation of antioxidants present in food products after sonication.

Degradation of lycopene from tomato ultrasound-assisted extraction has been observed. The authors proposed less degrading conditions by optimizing some parameters using an ultrasound bath and a mixture of hexane, acetone and ethanol, achieving only about 14% increase of *cis* isomers and 76% decrease of *trans* isomers, even though the extraction yield of sonicated samples was 26% higher than untreated samples (Eh and Teoh, 2011). Lycopene degradation was also observed in watermelon juice treated by ultrasound with the identification of oxidation compounds such as acetone, methyl-heptenone, laevunilic aldehyde and glyoxal. Also, ascorbic acid degradation and a decrease in total phenolics content were observed in the same samples and the suggested mechanisms for those reactions were pyrolysis and oxidation by $OH^{\bullet}$ radicals formed by cavitation (Rawson et al., 2011).

Antioxidant capacity of cyaniding 3-glucoside was evaluated before and after ultrasound treatment at 358 kHz resulting in a decrease of about 1/5 of the original value after 4h and the authors suggested that the molecule was hydroxylated during sonication (Ashokkumar et al., 2008).

The release of anthocyanin and phenolics was reported for rabbiteye blueberries processed by ultrasound-assisted osmotic dehydration, presenting a higher loss in the ultrasound treated sample when compared to samples treated by osmotic dehydration only. It was proposed that the cell rupture surface caused by cavitation might contribute to the release of anthocyanin and phenolics in those samples (Stojanovic and Silva, 2007).

Studies show degradation of flavonols such as anthocyanin and ascorbic acid observed after sonication. Despite an initial slight increase observed in the beginning of the treatment time (representing extraction of pigments from suspended pulps), anthocyanin presented a decrease of 3.2% compared to the initial value detected by HPLC in sonicated strawberry juice (Tiwari et al., 2008).

Degradation of both L-ascorbic acid in distilled water and of all-trans-ß-carotene were reported (Portenlänger and Heusinger, 1992; Sun et al., 2010), and the first one was attributed

to the generation of $H^{\bullet}$ and $OH^{\bullet}$ radicals. Degradation of oxidation-sensitive nutrients, such as thiamin and riboflavin in milk, as well as ascorbic acid and carotenoids in orange juice were analyzed after a continuous ultrasound treatment (Valdramidis et al., 2010). This type of treatment showed no effect on the concentration of thiamin and riboflavin in milk. In contrast, the contents of ascorbic acid and carotenoids in orange juice decreased by around 10% after the continuous ultrasound treatment.

Although ascorbic acid content in sonicated orange juices were lower compared to pasteurized samples due to sonodegradation, the storage study showed the content in both untreated and pasteurized samples decayed rapidly, while the content in sonicated samples presented a less pronounced degradation over time (Lee et al., 2005).

As for manothermosonication treatment of juice samples, carotenoids and ascorbic acid presented a slight decrease; and although sonicated milk presented higher browning indexes, there were no significant changes in thiamin and riboflavin contents in those conditions (Vercet et al., 2001)

### 1.2.3. Polysacharides modifications

Viscosity changes and the formation of new unsaturated structures were observed after sonication of water-soluble corn hull xylan and the authors suggest the changes in molecular properties of samples are due to a recombination of radicals formed in both saccharide and aromatic components induced by sonication of polysaccharides (Ebringerová and Hromádková, 1997). Viscosity changes were also observed in ultrasound-assisted preparation of chocolate mousse (Pingret et al., 2011).

Sonochemical degradation is observed in ultrasound treated chitosan and starch in aqueous solution by the formation of OH-radicals (which subtracts hydrogens from the molecule of origin) combined to mechanochemical effects. This degradation can be decelerated by the addition of *tert*-butanol (Czechowska-Biskup et al., 2005).

Sonicated corn starch presents a distortion of the crystalline region in granules prior to a reversible hydration of the amorphous phase, resulting in a destruction of the granular structure. A viscosity and consistency coefficient decrease were also observed, since

ultrasound facilitates the water uptake in the corn starch granules and those effects seem to be power dependent (Jambrak et al., 2010).

## 1.3. Impact of ultrasound on lipid containing food products

Besides degradation of minor compounds, modifications in color and physicochemical parameter of sonicated food products, some studies show the appearance of off-flavors and degradation of fats in high lipid containing food products treated by ultrasound. The Table II.2 summarizes the effects of ultrasound on high lipid containing food products as also the experimental conditions used in those studies.

### 1.3.1. Emulsification/crystallization

Emulsification has the objective of dispersing one immiscible liquid into another in the form of droplets, generating systems with generally minimal stability. The emulsification process requires a certain amount of energy input, since the reduction of the droplets size involves supplementary shear forces to induce the viscous resistance to absorb most of the energy during agitation (Abismaïl et al., 1999). Emulsification procedures using ultrasound is one of the earliest applications of the technique in the food industry. The cavitation bubbles implosion on two immiscible liquids results in an efficient mixing of the two layers, generating more stable emulsions when compared to conventional techniques (Mason et al., 1996). Among the advantages of emulsification under ultrasound over conventional procedures, the possibility of reducing fat globules size with homogenous distribution in reduced time is observed. Different types of apparatuses have been developed for emulsification purposes in the food industry using lower surfactant concentrations or even performing the process in the absence of this component with great results (Reddy and Fogler, 1980; Abismaïl et al., 1999; Coupland and Julian McClements, 2001; Pérez-Serradilla et al., 2007; Leong et al., 2009). Nevertheless, when present, surfactants accumulate in the interface of the cavitation bubble and might undergo degradation by radicals produced from decomposition of water that are also formed in the bubble (Makino et al., 1983).

In dairy products such as milk, the homogenous reducing of fat droplets size by emulsification prevents the fats from rising to the surface of the liquid as a distinct layer, providing inactivation of microorganisms at the same time (Piyasena, 2003). Mayonnaise treated by ultrasound, by the other hand, show excellent white color, resulting from smaller droplets size (Patist and Bates, 2008). However, during emulsification and processing of vegetable oils, a metallic and rancid odor has been detected only for insonated oil and foods. Some off-flavor compounds (for instance hexanal and hept-2-enal) resulting from the sono degradation of sunflower oil were identified (Chemat et al., 2004a, 2004b). Studies on the crystal structure of sonicated palm oil showed kinetics is affected and appears to be highly dependent upon ultrasound intensity (Patrick et al., 2004).

### 1.3.2. Homogenization

Homogenization is usually defined as a physical treatment by which the substance or mixture of substances is made uniform (Luque de Castro and Priego-Capote, 2007). In the food industry, homogenization is one of the most important steps in the processing of milk, increasing the stability and size of fat globules and preventing the phase separation (Wu et al., 2001).

The use of ultrasound for stirring, mixing or agitating systems without altering its chemical characteristics has been extensively studied, either at the laboratory or industrial scale, especially in the processing of milk, yogurt and ice cream with great results (Mason et al., 1996; Wu et al., 2001)

Nevertheless, milk homogenized by ultrasound presented an increase in total volatiles yield over sonication time, with the presence of secondary products such as: benzene, toluene, 1,3–butadiene, 1-buten-3-yne, 5–methyl–1,3–cyclopentadiene and a series of aliphatic 1-alkenes, whose pyrolytic origin was attributed to high localized temperatures associated with cavitation phenomena (Riener et al., 2009a, 2009b). Other compounds such as pentanal, hexanal and heptanal indicate free radical-induced lipid oxidation of unsaturated fatty acid hydroperoxides.

**Table II.2.** Effects of ultrasound treatment on high lipid containing food products.

| Food Matrix | Analyte | Experimental conditions | Observations | References |
|---|---|---|---|---|
| Olive oil | Fats | 20 **A**, 400 **B**, Probe **C**, 5 **D**, - **E**, spectrophotometer **F**, oxidative stability **G** | Oxidation due to ultrasound treatment was used for oxidative stability measurements. | Cañizares-Macias et al.(2004). |
| Sunflower oil | Fatty acids, volatiles | 20 **A**, 150 **B**, probe **C**, 0.5-30 **D**, 20 **E**, GCMS **F**, - **G** | Increase of peroxide value, decrease of polar compounds and appearance of off-flavors. | Chemat et al.(2004a) |
| Sunflower oil | Fatty acids, volatiles | 20-47 **A**, 450 **B,** probe **C**, - **D**, 60 **E**, GC, UV spectroscopy and GC/MS **F**, emulsification **G** | Detection of volatile compounds from degradation. | Chemat et al.(2004b) |
| Milk | Volatiles | 24 **A**, 200 **B**, probe **C**, 2-16 **D**, 15-25 **E**, SPME, GC/MS **F**, microbial inactivation **G** | Identification and increase of pentanal, hexanal, heptanal, octanal, 2-butanol, 2,2,4 trimethyl pentane. | Chouliara et al (2010) |
| Soybean seed oil | Fatty acids | 19-300 **A,** 80 **B**, probe **C**, 30-60 **D**, 45 **E**, GC/MS and sensory evaluation **F**, extraction **G** | Slight oxidation with decrease in the relative percentage of unsaturated fatty acids, irrespective of the degree of unsaturation. | Cravotto et al (2008) |
| Kiwi seed oil | Fatty acids | - **A,** 80 **B**, probe **C**, 30 **D**, 50 **E**, GC/MS and sensory evaluation **F**, extraction **G** | Detection of off-flavors and presence of oil oxidation compounds. | Cravotto et al (2011) |
| Olive oil | Fatty acids, minor compounds | 20 **A**, 750 **B**, probe **C**, 13-43 **D**, 30-70 **E**, HPLC and SPME/GC/MS **F**, bleaching **G** | Increase of peroxide value and acid value, losses in α-tocopherols and minor changes in fatty acids composition. | Jahouach-Rabai et al.(2008) |
| Thyme oil | Volatiles | 40 **A**, 240 **B**, bath **C**, 10-120 **D**, 30 **E**, GC/MS **F**, extraction **G** | Changes in the volatiles composition of the essential oil. | Kowalski and Wawrzykowski (2008) |
| Soybean Oil | Fatty acids | 20 **A**, 90-180 **B, p**robe **C,** 0.5-3 **D**, 25 **E**, GC/MS **F**, extraction **G** | Increase in saturated fatty acids, decrease in unsaturated fatty acids and an oxidation rate of 3.4%. | Li et al (2004) |
| Isatis indigotica oil | Fats | 40 **A**, 600 **B, b**ath **C,** 43.8 **D**, 49 **E**, GC/MS **F**, extraction **G** | Minor alterations in physicochemical properties of the oil. | Li et at. (2012) |
| Flaxseed | Fatty acids | 20 **A**, 600 **B**, probe **C**, 5-20 **C**, - **E**, GC/MS **F**, extraction **G** | Little effect on fatty acid composition, peroxide levels increased and generation of free radicals. | Metherel et al.(2009). |
| Palm and sunflower oil | Fatty acids, volatiles | 66 **A**, - **B**, probe **C**, 15 **D**, - **E**, microscope and GC/MS **F**, crystallization **G** | Changes in palm oil crystal structures and appearance of off-flavors. | Patrick et al.(2004) |
| Milk | Volatiles | 24 **A**, 400 **B**, probe **C**, 2.5-20 **D**, 45 **E**, SPME, GC/MS, GC/O **F**, microbial inactivation **G** | Appearance of volatile compounds from lipid degradation: 1-hexene,1-octene, 1-nonene, 5-methyl-1,3-cyclopenatadiene, benzene, toluene, p-xylene, n-hexanal, n-heptanal, 1,3 butadiene and 1-buten-3-yne. | Riener et al.(2009) |
| Tobaco seed oil | Fatty acids | 1865 **A**, 50 **B**, bath **C**, 20 **D**, 25 **E**, GC **F**, extraction **G.** | Drecrease of linoleic acid by 12% and general decrease in polyunsaturated fatty acids with increase of saturated fatty acids. | Stanisavljevic et al. (2009) |
| Sunflower oil | Fatty acids | 40 **A**, - **B**, cutting assembly consisting of a titanium sonotrode **C**, 0.17 **D**, - **E**, DX-100 ion chromatography, conductivity and sensory analysis **F**, cutting **G** | Observation of off-flavors. | Schneider et al.(2006) |
| Olive oil | Fats | 20 **A**, 400 **B**, probe **C**, 5 **D**, - **E**, spectrophotometer **F**, oxidative stability **G** | Oxidation due to ultrasound treatment was used for oxidative stability measurements. | Tabaraki et al. (2011) |

**A**: Frequency (kHz), **B**: Power (W), **C**: Type of ultrasound apparatus, **D**: Exposure time (min), **E**: Temperature (°C), **F**: Detection and analysis method, **G:** Process
**DSC:** Differential scanning calorimetry
**GC/MS:** Gas chromatography mass spectrometry
**GC/O:** Gas chromatography-olfactometry
**IR:** Infra-Red
**SPME:** Solid phase micro-extraction

### 1.3.3. Cutting

Cutting in the food industry is used mainly to separate semi-solid or soft solid materials, by producing two cut faces of the material thanks to an instrument with a predefined velocity, with or without removing material (Feng et al., 2010). Ultrasonic food cutting presents innovations and advantages in cutting and slicing of food products, resulting in less waste and lower maintenance costs, being used specially in multiple layers and highly resistant products such as cheese, fish, candy bars and bakery (Schneider et al., 2008; Chemat et al., 2011). However, studies using sunflower oil as model showed only 10 seconds of exposure to a 40 kHz ultrasound cutting assembly were sufficient to produce the volatiles responsible for typical off-flavors characteristic of lipid oxidation (Schneider et al., 2006).

### 1.3.4. Extraction

Plant extraction applied to food domain is usually done by solvents, representing high cost, pollutant and fastidious techniques. In the case of vegetable oils extraction, most of matrixes are seeds and kernels, which constitute barriers for the penetration of the solvent with consequent low yield. Also, the most common oil extraction is done by normalized Soxhlet extraction. The advantages of the use of low frequency ultrasound have been extensively reported, revealing the increase in substances recovery with milder extraction conditions in a shorter time, either using organic solvents like hexane or more environmental friendly ones such as ethanol.

Oilseed rape have been extracted and even optimized for Ultrasonic Assisted-Extraction (UAE), resulting in a yield almost 23% higher when compared to Soxhlet conventional technique (Wei et al., 2008). This matrix has also been extracted by an ultrasound-assisted Soxhlet extraction, a proposed improvement of the conventional method, with 99% of efficiency (Luque-Garcia and Luque de Castro, 2004). This method was shown to be effective for sunflower and soybean seeds as well. Flaxseed has also been proved to give great recovery results under ultrasound-assisted extraction when compared to conventional maceration in hexane (Zhang et al., 2008). The Aqueous Enzymatic Oil Extraction (AEOE) assisted by ultrasound have also shown great results in seeds oil extraction, such as *Jatropha curcas* L. (Shah et al., 2005) and soybean oil (Li et al., 2004b).

However, high fat containing food products subjected to ultrasound with extraction purposes have also showed changes in organoleptic and/or chemical characteristics, although extraction yields are greater when compared to conventional extraction methods. Kiwi seed oil ultrasound-assisted extraction revealed an oxidation of the samples with differences in the fatty acids composition and detection of limonene (Z)-hept-2-enal and (2E,4E)-deca-2,4-dienal, compounds which are characteristic of lipid degradation. This oil is rich in polyunsaturated fatty acids (PUFAs) (57% in linolenic acid – C18:3) and poor in tocols (tocopherol/tocotrienol 35 $mg.kg^{-1}$), which might influence its oxidative stability (Cravotto et al., 2011). Soybean germ and seaweed submitted to UAE performed in different apparatuses (cup horn, immersion horn and cavitating tube) working at different frequencies presented an oxidation with decrease in the relative percentage of unsaturated fatty acids, irrespective of the degree of unsaturation (Cravotto et al., 2008). Lipid extraction of flaxseed assisted by ultrasound also showed minor losses of fatty acids, although peroxides value increased, bringing the authors to suggest free radicals might also have been generated (Metherel et al., 2009). A general decrease in unsaturated fatty acids and an increase in saturated ones, together with a 3.4% oxidation rate despite the increased oil yield (2.4 to 11.2%) was also observed in UAE of two soybeans varieties (Li et al., 2004a). These observations have also been verified in tobacco seed oil UAE, in this case with a 12% decrease in linoleic acid (the major fatty acid in tobacco seed oil) content (Stanisavljević et al., 2009).

Studies using ultrasound as pre-treatment of Aqueous Enzymatic Oil Extraction of thyme leaves (*Thymus vulgaris* L.) showed an increase of thymol, carvacrol and *p*-cymene, while a significant decrease was observed for γ-terpinene (Kowalski and Wawrzykowski, 2009). The investigation of volatiles from grapes (hybrid cultivar Othello *Vitis sp.*) extracted using ultrasound revealed the appearance of compounds such as E(Z)hexenal and nonanal, which was attributed to an enzymatic degradation of unsaturated fatty acids during the injure-induced stress response to sample preparation (Radulovic et al., 2010).

### 1.3.5. Microbial Inactivation

Considering that food products are generally rich in nutrients and water, creating an appropriate environment for microbial growth, thermal processes were developed to inactivate those microorganisms and preserve food. However, the extreme conditions of temperature in

which those techniques are carried out might result in deterioration of functional properties of those products. Ultrasound have been successfully used for microbial inactivation in high lipid containing food samples as a replacement technology for those conventional methods with the advantages of using milder temperatures that prevent degradation of thermo sensible compounds in a reduced treatment time (Piyasena, 2003; Chemat et al., 2011). Since ultrasound achieves homogenization, reduction of fat globules size and microbial inactivation at the same time, it has been reported that continuous-flow ultrasonic treatment could be a promising technique for milk processing (Villamiel and de Jong, 2000; D'Amico et al., 2006; Cameron et al., 2009).

Ultrasound treatment of milk samples (24 kHz, 400 W, 30 minutes) appears to slightly increase fat content (from 4.04% to 4.25%) when compared to pasteurization, which can be attributed to the breakage of fat globules and release of triacylglycerols in the medium by cavitation bubbles implosion (Bermudez-Aguirre et al., 2009). Nevertheless, other studies suggested that besides homogenization of fat globules, volatile compounds were generated in sonicated milk samples as an indication of degradation of fats present in the sample (Riener et al., 2009a, 2009b; Chouliara et al., 2010). The use of an ultrasonic probe (24 kHz, 400 W) was responsible for the appearance of degradation compounds, namely 1-hexene, 1-octene, 1-nonene, 5-methyl-1,3-cyclopenatadiene, benzene, toluene, p-xylene, n-hexanal, n-heptanal, 1,3 butadiene and 1-buten-3-yne, from which the two last ones are known to be secondary degradation products of free radical-induced lipid oxidation (Riener et al., 2009a). Some of these oxidation compounds and also octanal, 2-butanol and 2,2,4 trimethyl pentane were observed after ultrasound treatment (24 kHz, 200 W) of milk, although a poor relationship was found between lipid oxidation and microbial inactivation and also sensory evaluation was unable to identify those volatiles (Chouliara et al., 2010). Similarly, it has been reported that the application of high-intensity ultrasound in the extraction of a variety of biologically active compounds reduced the antimicrobial activity and degraded some colorless compounds in a time and power dependent manner (Soria and Villamiel, 2010).

### 1.4.Mechanisms and factors of lipid oxidation

Generally, lipid degradation can occur by hydrolysis or oxidation, which can occur by auto-oxidation, photo-oxidation or enzymatic oxidation. The factors affecting lipid

degradation include energy input (light or heat), fatty acids composition, types of oxygen, and minor compounds such as metals, pigments, phospholipids, free fatty acids, mono- and diacylglycerols, thermally oxidized compounds and antioxidants (Choe and Min, 2006).

Triacylglycerols may be hydrolyzed into free fatty acids (hydrolytic rancidity) or oxidized, together with free fatty acids, into volatile compounds influencing flavors in food. It has been suggested that according to its abundance in food products, linoleic acid and its glycerides, which are highly susceptible to oxidation, are the main precursors of aldehyde degradation compounds (Saxby, 1996).

Volatile and semi-volatile materials already present in the crude oil might be responsible for either natural flavors or for the appearance of off-flavors, in the case of volatiles formed from lipid degradation pathways. Degradation products such as alcohols, aldehydes, ketones, alkanes, esters, and short chain acids are secondary metabolites from the degradation of hydroperoxides of unsaturated fatty acids, caused by high temperatures, oxygen presence, metals or other pro-oxidants effect (Jahouach-Rabai et al., 2008). Despite the lack of taste and odor of the primary degradation products from lipid oxidation, secondary products give rise to off-flavors characterized as “green”, “metallic”, “fishy”, “bitter”, “grassy”, “tallow”, “rancid”, etc. which are used to identify degradation of lipid in food products (Saxby, 1996).

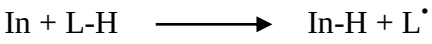
Oxidation of lipids follows a chain reaction that has three different phases: initiation, propagation and termination (Scheme II.1). In the presence of initiators, unsaturated lipids (LH) form carbon-centered alkyl radicals ($L^{\bullet}$) which will then form lipid alkoxyl radicals ($LO^{\bullet}$) and peroxyl radicals ($LOO^{\bullet}$) that propagate by a free radical chain mechanism to form hydroperoxides (LOOH) as the primary products of autoxidation. Those last will be readily decomposed into a wide range of carbonyl compounds, hydrocarbons, ketones and other materials as secondary oxidation products in the termination phase, and those last compounds will contribute to flavor deterioration of foods (Frankel, 1991; Schaich, 2005).

Since lipid oxidation is autocatalytic and once started, the formed lipid hydroperoxides will trigger the chain reaction that will self-propagate and self-accelerate producing even more large amounts of oxidation products. The reaction can produce numerous intermediate products that can influence the course and rapidity of the reaction, although the presence of pro- or antioxidants can have the same effect (Schaich, 2005).

**Scheme II.1.** Classical free radical chain reaction of lipid oxidation.

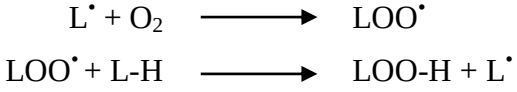
*Initiation*

*Formation of ab initio lipid free radical*

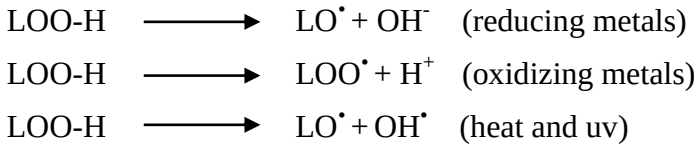


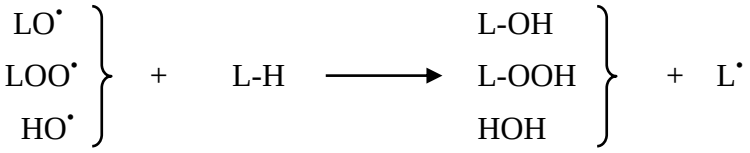

*Propagation*

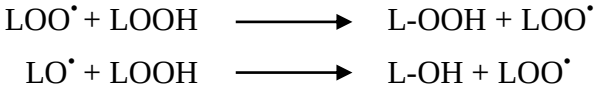
*Establishment of free radical chain reaction*




*Free radical chain branching – Initiation of new chains*

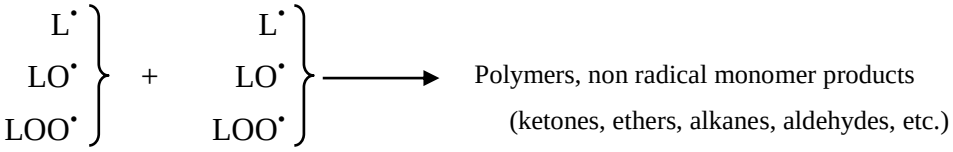


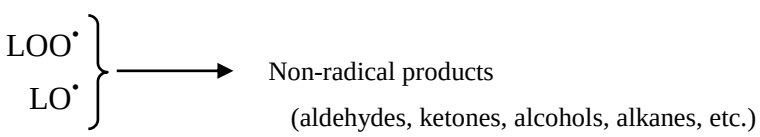




*Termination*

*Formation of non-radical products*



### 1.4.1. Initiation

In the presence of initiators (In), unsaturated lipids (LH) lose a hydrogen radical ($H^{\bullet}$) to form lipid radicals ($L^{\bullet}$) as shown in Scheme II.1. Only traces of the initiators (or catalysts) are needed; therefore, conditions such as the presence of contaminants or exterior factors should not be neglected (Schaich, 2005). Thus, to control or avoid lipid oxidation, it is imperative to control the action of the catalysts, even though at this stage of lipid oxidation the rancid odor and flavor modification are not yet perceptible (Judde, 2004).

The susceptibility to oxidation of a lipid depends on their relative ability to donate hydrogen and, in the case of unsaturated lipids, it depends on the availability of allylic hydrogens to react with peroxyl radicals (Frankel, 1991). Once the peroxyl radical is formed, it can attack another molecular lipid or the starting molecule by removing a hydrogen and forming a hydroperoxide, which is an intermediate product. The peroxide products from the reaction can subsequently participate as initiators of the process at certain circumstances, increasing oxygen consumption over time as hydroperoxides are formed (Yin et al., 2011).

The free radicals production which will trigger the chain reaction can be initiated by a number of factors that group energy input such as light, heat, composition of fatty acids, types of oxygen and minor compounds (metals, pigments, phospholipids, free fatty acids, mono-, di- and triacylglycerols, thermally oxidized compounds and antioxidants (Frankel, 1991; Choe and Min, 2006). The resistance to oxidation can be expressed as the period of time necessary to obtain the critical point of oxidation, whether it is a sensorial change or a sudden acceleration of the oxidative process (Silva et al., 2001). The main initiators of lipid oxidation (metals, light, high temperatures, radicals and minor compounds present in oils) are described below.

#### 1.4.1.1. Metals

Metals increase the rate of oil oxidation due to the reduction of activation energy of the initiation step in the autoxidation and react directly with lipids to produce lipid alkyl radicals and reactive oxygen species, which accelerate oil oxidation (Benjellourr et al., 1991; Choe

and Min, 2006). Metals can also accelerate oxidation by decomposing hydroperoxides or phenolic compounds, reducing oil stability (Keceli and Gordon, 2002).

Only metals undergoing one-electron transfers appear to be active catalysts; these include cobalt, iron, copper, manganese, magnesium, and vanadium, while metals that oxidize by two-electron transfers, e.g., $Sn^{2+}$ and $Tl^{+}$, are not active (Lundberg, 1962). Metals that form complexes with oxygen also form intermediate complexes with hydroperoxides during oxidation and reduction, particularly at low hydroperoxides concentrations and in nonpolar solvents. The mechanisms and rates of metal-catalyzed initiation are determined by a complex mixture of factors: the metal and type of complexes it forms (inner sphere or outer sphere), the chelator or complexing agent, redox potential of the metal and its complexes, solvents, phase localization of the metal, and availability of oxygen or preformed hydroperoxides (Schaich, 2005).

#### 1.4.1.2. Light

Oil oxidation is accelerated by light, especially in the presence of sensitizers such as chlorophylls. Sensitizers in singlet state absorb light energy very rapidly and become excited. Excited singlet sensitizers can return to their ground state via emission of light, internal conversion, or intersystem crossing. Intersystem crossing results in excited triplet state of sensitizers, which may accept hydrogen or an electron from the substrate and produce prooxidative radicals (Choe and Min, 2006). Light plays an important role in $^{1}O_2$ oxidation. Light of shorter wavelengths appears to have more detrimental effects on oils than longer wavelengths (Sattar et al., 1976).

The principal light-absorbing groups of lipids are double bonds, peroxide O-O bonds, and carbonyls; while the last two are the most important. The primary mechanism by which light (principally ultraviolet radiation) initiates lipid oxidation is actually indirect, mediated by homolytic scission of any preformed hydroperoxides to generate the true initiators ($LO^{\bullet}$, $HO^{\bullet}$, and $RO^{\bullet}$) that abstract hydrogens from lipid molecules and form the *ab initio* $L^{\bullet}$ (Schaich, 2005).

#### 1.4.1.3. Temperature

High temperatures produce sufficient energy to break covalent C-C or C-H bonds in the acyl backbone to form a variety of lipid alkyl radicals (Nawar, 1969), which then start the radical chain reactions of oxidation. Moderate temperatures have lower energy, thus the main effect is the breakage of O-O bonds in traces of ROOH or LOOH produced by other reactions. The $RO^{\bullet}$, $LO^{\bullet}$, and $^{\bullet}OH$ generated by this breakage will then abstract hydrogens from neighboring lipids to form $L^{\bullet}$, which will initiate radical chains by propagation. As shown by the activation energies for the individual stages of lipid oxidation, LOOH decomposition and its subsequent contribution to propagation is the major catalytic effect of high temperatures (Marcuse and Fredriksson, 1968; Labuza and Dugan, 1971) and the effects of increased LOOH decomposition are amplified by increased rates of subsequent H abstractions by $LO^{\bullet}$ and $LOO^{\bullet}$, which is reflected in the doubling of oxidation rate for every 10 °C rise in temperature (Lundberg and Chipault, 1947). Although the propagation reaction is the rate-limiting step at ambient temperature, it is not necessarily so at high temperatures where the decomposition of hydroperoxyl radicals is enhanced and the availability of oxygen might be critical (Ragnarsson and Labuza, 1977).

#### 1.4.1.4. Enzymes

The enzymatic initiation of lipid degradation is performed by lipoxygenases, which will catalyze the aerobic oxidation of fatty acids with cis-nonconjugated pentadiene structures to generate optically active conjugated LOOH without releasing a lipid free radical (Schaich, 2005). Even very low levels of lipoxygenases produced by the plant may provide invisible initiators that catalyze oxidation in lipids. The mechanism by which the enzyme catalyzes oxidation is the reaction with hydroperoxides. Glycerides, glycolipids and phospholipids are hydrolysed by lipases into free fatty acids and then lipoxygenase reacts with free acids more readily than with bound lipid to produce hydroperoxides (Hamilton et al., 1997). Hydroperoxides are produced by an electron transfer to the lipid from the ferrous iron atom in the enzyme's active site, while oxygen will bound to a separate site (Aoshima et al., 1977). This activation of the enzyme will allow it to react with the free radical, and then $H^{+}$ donation

from the enzyme completes the LOOH before it is released. The resulting conjugated dienes are always trans-, cis-relative to the hydroperoxide (Egmond et al., 1972).

#### 1.4.1.5. Radicals

As shown above, all the initiating processes generate some form of radical that ultimately reacts with lipids to produce the *ab initio* lipid radical that starts the autoxidation chain reaction. The kinetics of the initiation, however, is determined by the speed of individual radical reactions with lipids, which can vary greatly. Hydroxyl radicals have the fastest reaction rates with lipids. However, $^{\bullet}OH$ are so strongly oxidizing that their reactions are also very nonspecific, and they attack lipids indiscriminately at all sites along acyl chains (Heijman et al., 1985). These radicals then ‘‘migrate’’ (by intramolecular abstraction) to the doubly allylic H’s in dilute monomer solutions, or abstract H’s from doubly allylic sites of neighboring lipids in concentrated solutions, yielding the dienyl radicals that, when oxygenated to $LOO^{\bullet}$ become the main chain carriers (Schaich, 2005). Whether the primary initiator is heat, radiation, or metals, many of the initial oxygen radicals produced react more rapidly with solvent components than with lipids.

#### 1.4.1.6. Effect of minor components present in oils

Although oils are mainly composed of triacylglycerols, some minor compounds present in the oil might accelerate or slow down the autoxidation, influencing the reaction rate. Those components are free fatty acids, mono- and diacylglycerols, metals, phospholipids, peroxides, chlorophylls, carotenoids, phenolic compounds, and tocopherols.

The fatty acids composition might also affect oxidation, especially concerning the unsaturation degree. As unsaturation degree increases, both the formation and the amount of primary oxidation compounds increase (Martín-Polvillo et al., 2004). The autoxidation rate depends greatly on the rate of fatty acid or acylglycerol alkyl radical formation, and the radical formation rate depends mainly on the types of fatty acid or acylglycerol. The relative autoxidation rate of oleic, linoleic, and linolenic acids was reported as 1:40 to 50:100 on the basis of oxygen uptake (Choe and Min, 2006).

Since free fatty acids (FFA) are more susceptible to autoxidation than esterified fatty acids, they can accelerate autoxidation. Because of both hydrophilic and hydrophobic groups of FFA, a decrease of the surface tension of edible oil is observed together with and increase of the diffusion rate of oxygen from the headspace into the oil to accelerate oil oxidation and this same effect on surface tension is observed for mono- and diacylglycerol (Miyashita and Takagi, 1986; Mistry and Min, 1987). Chlorophylls and their degradation products, pheophytins and pheophorbides, act as sensitizers to produce singlet oxygen in the presence of light and atmospheric $^3O_2$, and accelerate the oxidation of oil, although in the absence of those, chlorophylls can act as antioxidants possibly by donating hydrogens to free radicals (Endo et al., 1985; Fakourelis et al., 1987; Gutiérrez-Rosales et al., 1992). Some thermally oxidized compounds produced by high processing temperatures can accelerate autoxidation in a concentration dependent manner (Yoon et al., 1988). Oxidized compounds formed by hydroperoxide decomposition can act as an emulsifier, increasing the introduction of oxygen into the oil to accelerate oil lowering surface tension in the oil (Jung et al., 1989).

Although many minor compounds present in oil can act as prooxidants, some can present antioxidant properties. Such is the case of tocopherols, tocotrienols, carotenoids, phenolic compounds and sterols. Phospholipids present in the oil can also act as antioxidants under certain conditions, and although this protective mechanism has not yet been elucidated, it seems that they sequester prooxidative metals (Choe and Min, 2006).

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### 1.4.2. Propagation

The lipid peroxy radical abstracts hydrogen from other lipid molecules and reacts with hydrogen to form hydroperoxides and another lipid alkyl radical. Hydroperoxides formed can break down to form free radical products (alkoxyde or hydroxyl) or even a peroxy free radical, hydroxyl free radical and water. These steps lead to a proliferation that can catalyze oxidation reaction by propagation steps and thus, the reaction becomes autocatalytic (Hamilton et al., 1997). The hydroperoxides decomposition to form secondary oxidation products is presented in Figure II.2.

The abstraction of a hydrogen atom from an unsaturated fatty acid (LH) by peroxyl radical ($ROO^{\bullet}$) to generate hydroperoxides (LOOH) and another radical ($L^{\bullet}$) is the slowest step in chain propagation and the susceptibility of different fatty acids to this hydrogen

abstraction is dependent on the dissociation energies of C-H bonds present in the fatty acid (Kamal-Eldin, 2003). The presence of a double bond in the fatty acid weakens the C-H bonds on the carbon atom adjacent to the double bond and makes the hydrogen removal easier.

Hydroperoxide decomposition provides one of the most important catalysts for lipid oxidation and once the hydroperoxide content in a system containing polyunsaturated fatty acids reaches a certain critical value, their decomposition becomes significant and the rate of lipid oxidation increases (Schieberle et al., 1979). During the propagation phase, the rate of hydroperoxide formation is greater than the rate of their decomposition and this situation is reversed in the decomposition stage (Kamal-Eldin, 2003).

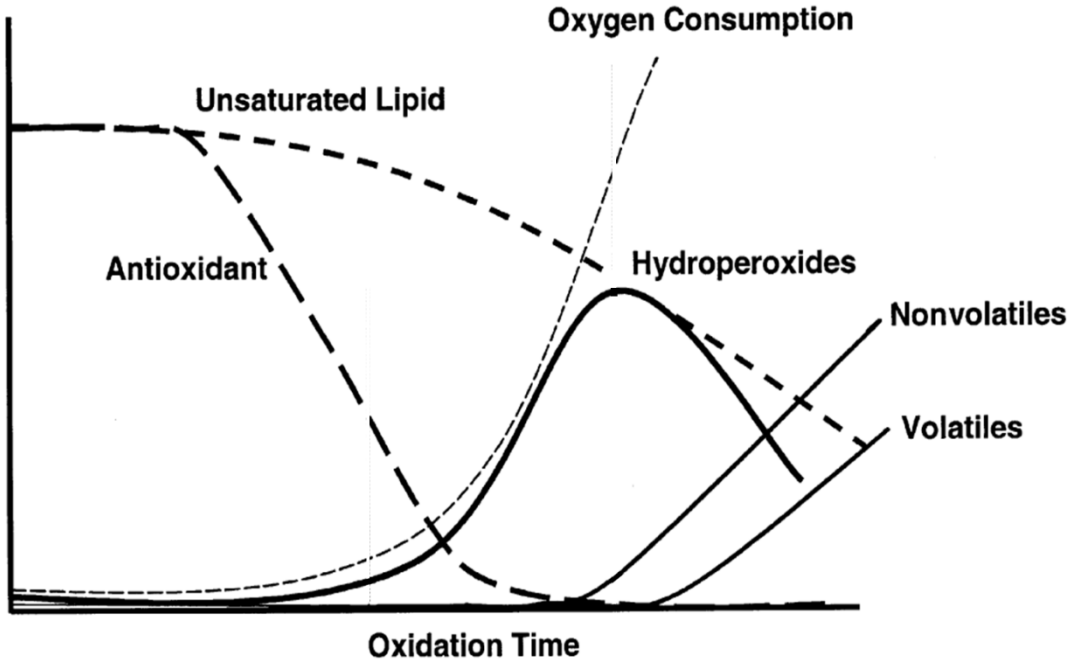


**Figure II.2.** Kinetic curve of autoxidation of polyunsaturated fatty acids.

Multiple mechanisms are well established in radical chemistry and have been applied to peroxyl and alkoxyl radical reactions in lipid oxidation, although not all rate constants and reaction details are available. All the pathways of the propagation step lead to H abstraction to form intermediate products that will then breakdown to form secondary products. One example of mechanisms of hydroperoxides decomposition to form secondary oxidation products is represented in Figure II.3.

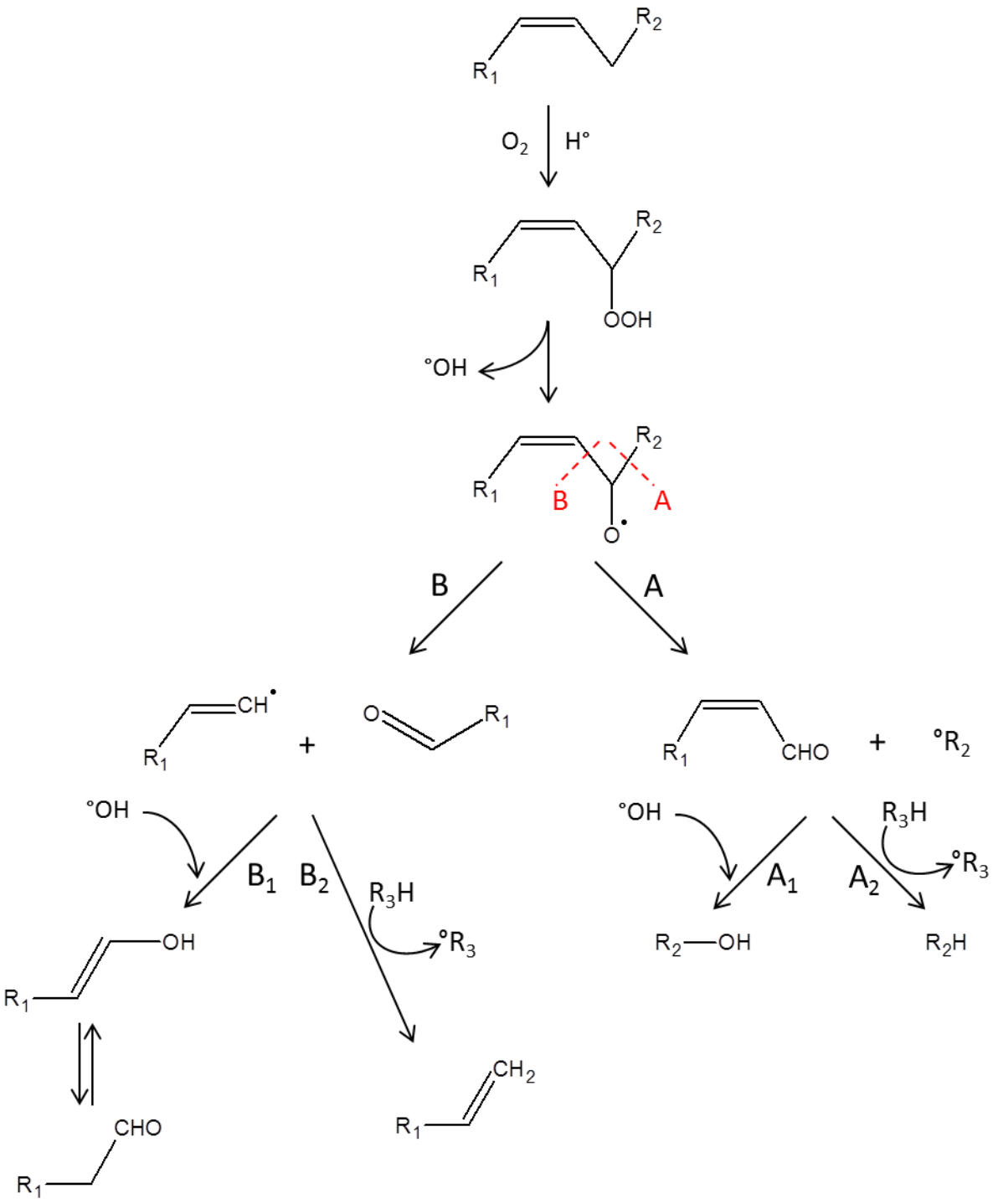


**Figure II.3.** Mechanism of hydroperoxide decomposition to form secondary oxidation products.

### 1.4.3. Termination

A complex mixture of volatile, nonvolatile, and polymeric secondary oxidation products is formed through the decomposition reactions of hydroperoxides (Figure II.4) and the structures of some of these decomposition products are known relatively well on the basis of the studies done in various model systems (Kamal-Eldin, 2003). However, the exact mechanisms for their formation and the kinetic and thermodynamic factors governing their quantitative and qualitative distribution are not yet completely understood. Variables that may have an effect on the relative reaction rates and product distribution include temperature, reaction media, and antioxidative and prooxidative compounds. Most likely the relative distribution of the decomposition products is determined by several competitive reaction pathways whose relative importance depends on the reaction conditions.

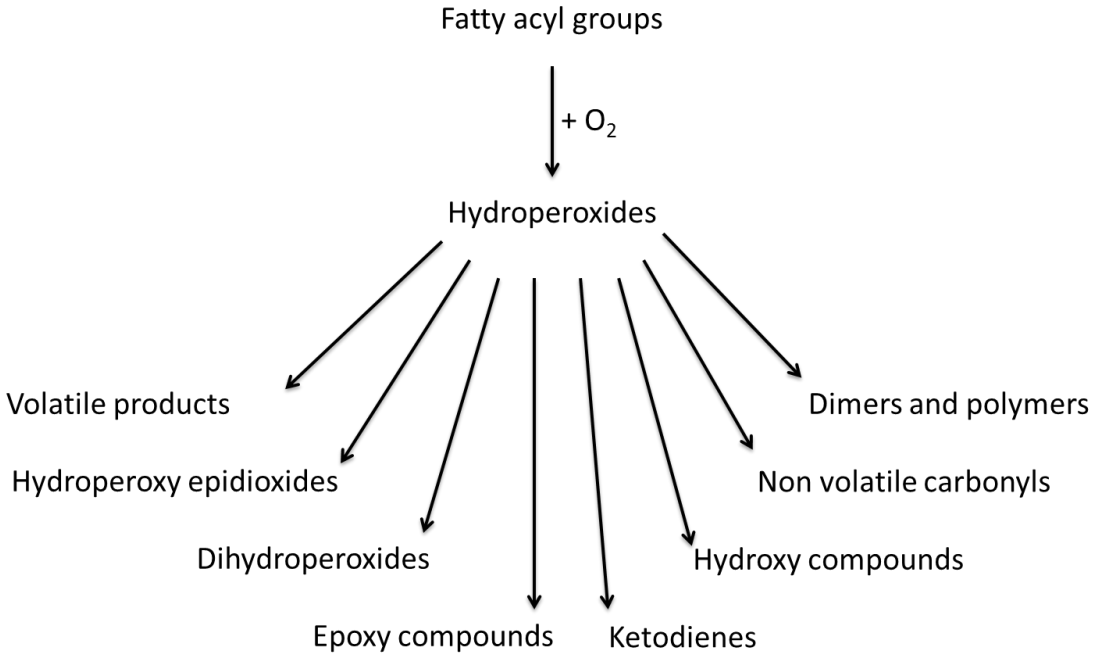


**Figure II.4.** Types of compounds formed from hydroperoxides decomposition.

## 1.5. Lipid degradation assessment methods

The autoxidation process in lipids lead to rancidity, which represents the off-flavors observed in lipid containing food products. Although the objective of analytical methods is to determine the oxidation in products, the evaluation of the degradation is often done by monitoring the intermediate compounds (Hamilton et al., 1997). The assessment of multiple oxidation products and stages of the oxidative pathway enables a better follow-up with more details for better understanding of the lipid degradation process (Devasagayam et al., 2003).

In order to evaluate lipid decomposition, some tests are capable of evaluating the extent of degradation, such as conjugated dienes, peroxides value, acid value and the analysis of volatile compounds. Conjugated dienes may not be as sensitive as hydroperoxides and their decomposition products including aldehydes, ketones and low molecular weight acids for determining lipid peroxidation induced by ultrasound (Chemat et al., 2004a).

Oxidative stability is not considered a standard parameter of oil quality, although it might be useful for shelf-life estimation. A reduction of unsaturated fatty acids can be used for quantification of oxidation, since saturated fatty acids are practically unaltered by auto oxidation (Krichene et al., 2010).

In order to monitor oxidation process in oils, peroxides value and K270 are useful for quantification of hydroperoxides content and the formation of secondary oxidation products (Frankel 2005) and it has been suggested that volatile molecules degrade first under ultrasound (Tiwari et al., 2008). Peroxides value increase indicate primary oxidation and it is verified in oils after ultrasonic treatment, which seems to be time dependent (Jahouach-Rabai et al., 2008).

High temperatures (below 100°C) can provoke irreversible degradation of triacylglycerols in edible oils that react with the atmospheric oxygen, producing hydroperoxides, which trigger a radical-chain reaction that produces volatile and non-volatile compounds, while water present in oil samples might increase free fatty acids, mono and diacylglycerols as also glycerols (Achir et al., 2006).

Vegetable oils can contain a variety of minor components, such as hydrocarbons, sterols, tocopherols, ascorbic acid, polyphenols, color compounds and trace metals, which might be subjected to degradation. Tocopherols and tocotrienols, are supposed to protect fats and oils from thermal oxidation, which provokes their oxidation into quinines and dimmers,

although tocotrienols appear to be less stable than tocopherols (Rossi et al., 2007; Krichene et al., 2010). It has been frequently reported that the oxidative stability of oil is influenced by its fatty acid composition, the oil quality parameters and tocopherol content (Van Hoed et al., 2009). Antioxidants that are supposed to protect the fats from oxidation also present considerable degradation under ultrasound treatment, for instance, a decrease of 11% was observed in ascorbic acid content and seemed to be dependent upon amplitude level and treatment time (Tiwari et al., 2008).

### 1.5.1. Primary oxidation compounds

As shown in Figure II.2, at the beginning of the lipid oxidation process, the formation rate of hydroperoxides outweighs their rate of decomposition during the initial stage of oxidation, and this becomes reversed at later stages (Hamilton et al., 1997; Shahidi and Zhong, 2005). Many of the primary products formed in the free radical chain oxidation of lipids are unstable and difficult to isolate and identify (Yin et al., 2011).

#### *1.5.1.1. Hydroperoxides*

Hydroperoxides are relatively stable at room temperature, except if they are in presence of heat, UV or metals, which will provoke a rapid decomposition in alkoxy radicals to form aldehydes, esters, ketones, acids, alcohols, and short chain hydrocarbons. For this reason, the measurement of the amount of formed hydroperoxides (Figure II.5) is an indicator of initial stages of lipid degradation. The most probable pathway of hydroperoxides decomposition is homolytic cleavage between oxygen and the oxygen bond, producing alkoxy and hydroxyl radicals (Choe and Min, 2006).

A number of analytical methods are available for determining the amount of hydroperoxides and those can be classified in those determining the total amount of hydroperoxides and those based on chromatographic techniques giving detailed information on the structure and the amount of specific hydroperoxides present in a certain oil sample (Shahidi and Zhong, 2005).

Peroxide (hydroperoxides) value (PV) is the most common measurement of lipid oxidation and represents the total hydroperoxides content in a sample. Hydroperoxides have no flavor or odor of their own, but they are unstable and break down rapidly to other products such as aldehydes that have a strong, unpleasant flavor and odor. Peroxide value measures the miliequivalents of oxygen hydroperoxides per gram of oil. A number of methods have been developed for determination of PV, among which the iodometric titration, ferric ion complex measurement spectrophotometry, and infrared spectroscopy are most frequently used. The iodometric AOCS Method Cd 8-53 (AOCS, 2009) is the most widely used for determination of edible oil quality. The maximum PV of 0.1 and preferably less than 0.05 is expected for freshly refined oils. A peroxide value higher than 10 meq/kg is considered unacceptable (Shahidi, 2005). It is also possible to monitor the amount of formed hydroperoxides over time, which can give details on the stage of lipid oxidation, i.e. whether a lipid is in the growth or decay portion of the hydroperoxide concentration curve – Figure II.2.

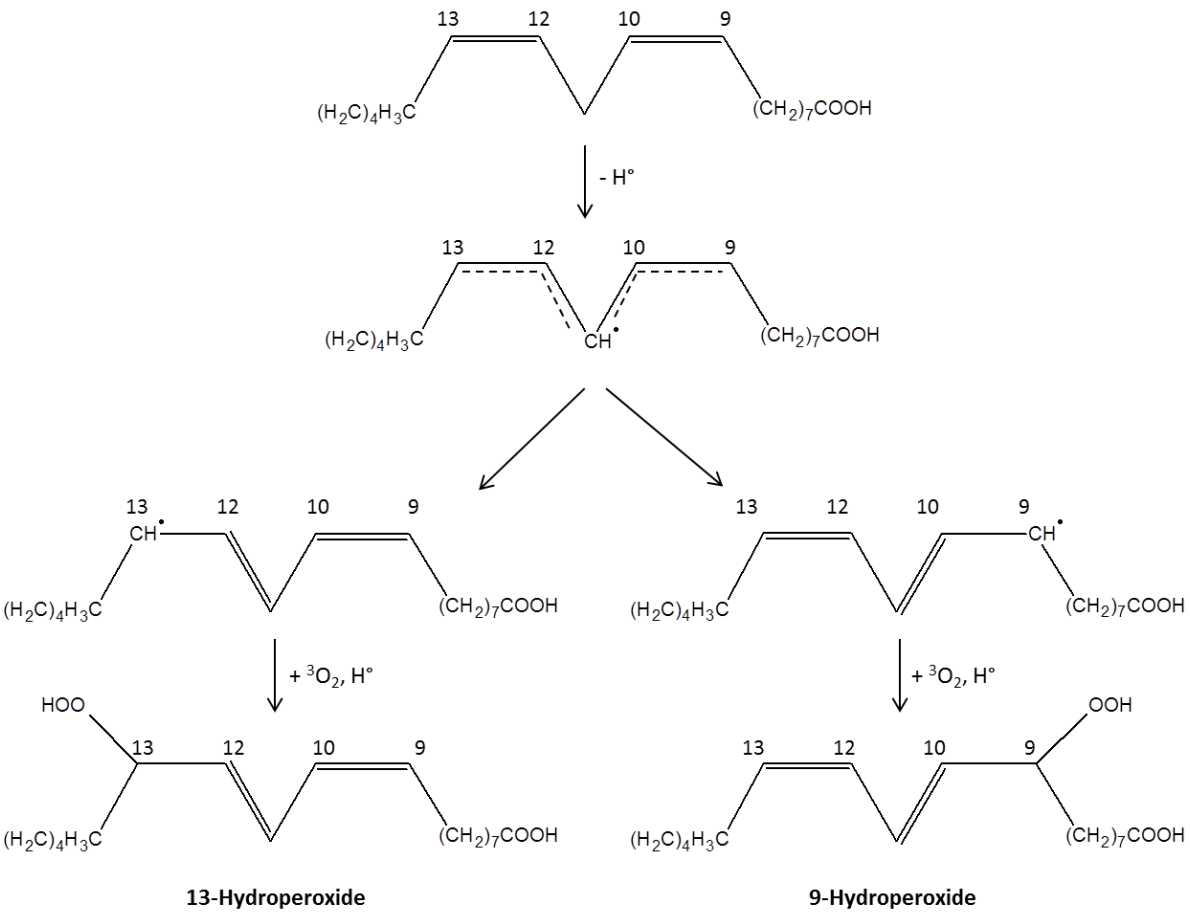


**Figure II.5.** Formation of hydroperoxides from linoleic acid autoxidation.

### *1.5.1.2. Conjugated dienes*

The autoxidation of linoleic and linolenic acids produce only conjugated products (Choe and Min, 2006). A peroxidative sequence is initiated by the attack of an unsaturated lipid (LH) by an initiator that abstracts a hydrogen atom leaving an unpaired electron in the carbon atom. Methylene groups adjacent to double bonds are particularly susceptible to the attack and the resultant carbon radical ($^{\bullet}$CH) is stabilized by molecular rearrangement to form a conjugated diene (Devasagayam et al., 2003) as shown in Figure II.6. The conjugated dienes have a strong absorbance at 234 nm, with allows a rapid assessment and quantification of the oxidation state in lipids.

Conjugated dienes and trienes absorbing at 234 and 268 nm, respectively, are directly related to hydroperoxides and are often used in addition or in place of PV (Shahidi, 2005).

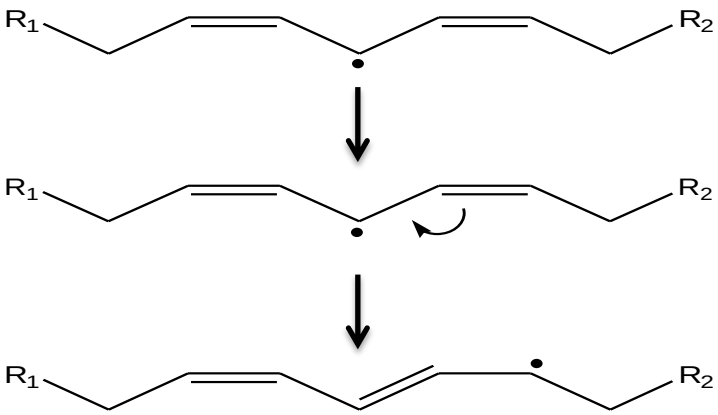


**Figure II.6.** Rearrangement to form conjugated dienes.

## 1.5.2. Secondary oxidation compounds

The primary oxidation products (hydroperoxides) are unstable and susceptible to decomposition. A complex mixture of volatile, nonvolatile, and polymeric secondary oxidation products is formed through decomposition reactions, providing various indices of lipid oxidation (Kamal-Eldin, 2003). Secondary oxidation products include aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, among others

(Shahidi and Zhong, 2005). The period for secondary products formation varies upon the oil type. Those compounds are formed immediately after hydroperoxides formation in rapeseed and olive oils; however, in safflower and sunflower oils secondary compounds are only formed after a certain accumulation of hydroperoxides (Guillén and Cabo, 2002; Choe and Min, 2006).

A certain number of spectrophotometric methods are available for the measurement of secondary oxidation products, all based on the assessment of the color intensity resulting from the combination of a family of secondary degradation products with a specific reagent and the choice of the method must be considered in function of the studied model (Judde, 2004).

#### *1.5.2.1. Anisidine value*

The p-anisidine value (*p*-AnV) method measures the content of aldehydes (principally 2-alkenals and 2,4-alkadienals) generated during the decomposition of hydroperoxides in fats and oils. It is based on the color reaction (Figure II.7) of *p*-methoxyaniline (anisidine) and the aldehydic compounds. The reaction of *p*-anisidine reagent with aldehydes under acidic conditions results in yellowish products that absorb at 350 nm. The color is quantified and converted to *p*-AnV and the color intensity depends on the amount of aldehydes as well as on their structure. The *p*-AnV is defined as the absorbance of a solution resulting from the reaction of 1 g of fat in isooctane solution (100 ml) with *p*-anisidine (0.25% in glacial acetic acid) and the AOCS Method Cd 18-90 (AOCS, 2009) has been standardized for anisidine value analysis.

This test is more sensitive to unsaturated aldehydes than to saturated aldehydes because the colored products from unsaturated aldehydes absorb more strongly at this wavelength (Yanishlieva et al., 2001). However, it correlates well with the amount of total volatile substances and is a reliable indicator of oxidative rancidity in fats, oils and lipid containing foods (Doleschall et al., 2002; Van Der Merwe et al., 2004). A highly significant correlation between *p*-AnV, flavor scores and PV has been described (List et al., 1974). Nevertheless, some authors have indicated that p-AnV is comparable only within the same oil type because initial *p*-AnV varies among oil sources (Guillén and Cabo, 2002). For instance, oils with high levels of polyunsaturated fatty acids might have higher *p*-AnV even when fresh.

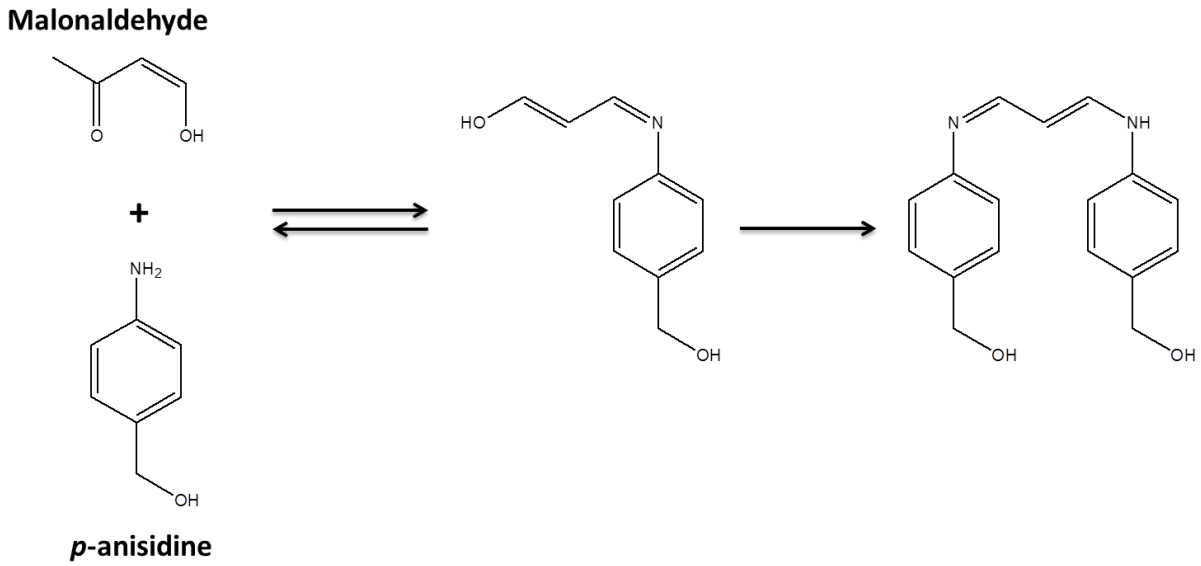


**Figure II.7.** Reaction between *p*-anisidine and malonaldehyde.

### *1.5.2.2. TOTOX value*

The Totox value is a measure of the total oxidation, including primary and secondary oxidation products and is calculated by 2 PV + *p*-AnV, providing information about the current status of oxidation as well as its history and is used by the industry (Shahidi, 2005). During lipid oxidation, it is often observed that PV first rises, and then falls as hydroperoxides decompose (Figure II.2). PV and p-AnV reflect the oxidation level at early and later stages of oxidation reaction, respectively. Totox value measures both hydroperoxides and their breakdown products, and provides a better estimation of the progressive oxidative deterioration of fats and oils (Stauffer, 1996).

### *1.5.2.3. Volatiles*

Flavor deterioration of high lipid containing food products is mainly caused by the production of volatile lipid oxidation products, which can impair organoleptic characteristics even in very low concentrations (Frankel, 1998).

### 1.5.3. Other methods

#### *1.5.3.1. Polar compounds*

The oxidation of fats and oil under high temperatures is characterized by a decrease in the total unsaturation of the fat with increases in viscosity and the content of polar compounds and polymeric material (Shahidi and Zhong, 2005). The determination of polar material in frying fats is a reliable approach for oil quality evaluation and is an official method in Europe. The content of polymers and polar components in oils increases during frying process and size exclusion chromatography and HPLC may be used for the analysis of such components. The content of polar lipids should not exceed about 20% (Shahidi, 2005).

#### *1.5.3.2. Free fatty acids*

Free fatty acids (FFA) are formed due to hydrolysis of triacylglycerides and can get promoted by reaction of oil with moisture (Frega et al., 1999). Hydrolytic processes lead to the formation of free fatty acids by splitting of acylglycerols that can affect flavor. The Standard AOCS Method Ca 5a-40 and Cd 3a-63 (AOCS, 2009) for acid value are commonplace. Free fatty acids are normally calculated as free oleic acids on a percentage bases. Free fatty acids are important quality indicators during processing and storage of fats and oils.

#### *1.5.3.3. RPE*

Since the radicals formed in the initial states of lipid oxidation have a short half-life, the oxidation levels of lipid can also be assessed by the measurement of the amount of radicals present in the medium, providing a good indication of the oxidation state of the sample.

Electron spin resonance (ESR), also referred to as electron paramagnetic resonance (EPR) spectroscopy, rely on the paramagnetic properties of the unpaired electrons in radicals and have been developed for assessing the formation of free radicals originated in the early

stages of oxidation and the onset of primary oxidation (Velasco et al., 2004). The assay measures the absorption of microwave energy when a sample is placed in a varied magnetic field (Andersen and Skibsted, 2002; Shahidi and Zhong, 2005). Quantification of radical concentrations is complicated by the comparison with stable paramagnetic compounds, such as transition metals and nitroxyl radicals; however, the short lifetimes and low steady-state concentrations of the highly reactive lipid-derived radicals make it difficult to detect these radicals at concentrations lower than the minimum detectable concentration of $10^{-9}$ M (Andersen and Skibsted, 2002). This technique is of great value for the study of the early stages of lipid oxidation and prediction of oxidative stability of fats and oils. It has high sensitivity, allows mild conditions by applying significantly low temperatures and requires little sample preparation (Shahidi and Zhong, 2005).

## 1.6. Conclusion

The use of ultrasound on food products has proved to be advantageous in numerous processes. However, some modifications in the physicochemical parameters or structures of components and the degradation of some compounds have been increasingly reported. In the case of food products rich in lipids, the analysis tend to point to lipid degradation induced by cavitation, although very few studies have tried to elucidate the mechanism by which ultrasound energy degrades those compounds. In this chapter a brief theory on lipid degradation and some analytical oxidation assessment methods were presented and in the next chapter, a more detailed study on edible oils treated by ultrasound will be presented, in order to better understand the effects of ultrasound energy on those compounds.

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# Chapter II

# *Degradation of edible oils during ultrasound processing*

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## 2.1. Introduction

The increasing demands of higher quality and quantity of products in the food industry brought the attention to the use of ultrasound (US) as a food processing technique of interest as technological benefit and/or as a technique to alter product functionalities with several advantages over conventional methods in terms of energy consuming, time and higher throughput (Jambrak et al., 2010; Soria & Villamiel, 2010).

Ultrasound are used in the food industry for numerous processes on high lipid containing food products such as milk (Bermudez-Aguirre, Mawson, & Barbosa-Canovas, 2008; Bermudez-Aguirre, Mawson, Versteeg, & Barbosa-Canovas, 2009; Cameron, McMaster, & Britz, 2009; Chouliara, Georgogianni, Kanellopoulou, & Kontominas, 2010; Riener, Noci, Cronin, Morgan, & Lyng, 2009; Riener, Noci, Cronin, Morgan, & Lyng, 2009), yogurt (Riener, Noci, Cronin, Morgan, & Lyng, 2010; Wu, Hulbert, & Mount, 2001), cheese (Arnold, Leiteritz, Zahn, & Rohm, 2009), etc., presenting great results in cooking (Pingret, Fabiano-Tixier, Petitcolas, Canselier, & Chemat, 2011; Pohlman, Dikeman, Zayas, & Unruh, 1997), cutting (Arnold, Zahn, Legler, & Rohm, 2011; Rawson, 1998; Schneider, Zahn, & Rohm, 2008), emulsification/homogenization (Abismaïl, Canselier, Wilhelm, Delmas, & Gourdon, 1999; Ashokkumar et al., 2008; Behrend, Ax, & Schubert, 2000; Bermudez-Aguirre et al., 2008; Gaikwad & Pandit, 2008) and microbial inactivation (Adekunte, Tiwari, Scannell, Cullen, & O'Donnell, 2010; D'Amico, Silk, Wu, & Guo, 2006; Gómez-López, Orsolani, Martínez-Yépez, & Tapia, 2010). The interest of applying ultrasound and sonochemistry in food processing techniques lies in the fact that power ultrasound are able to induce modifications (chemical, functional, physical, and structural, etc.) in some of the food properties (Mason, Paniwnyk, & Lorimer, 1996).

Although power ultrasound present numerous technological benefits (Ashokkumar et al., 2008; Mason et al., 1996), some undesirable changes in food composition or characteristics have been reported after ultrasound treatment in the last few years (Chouliara et al., 2010; Rawson et al., 2011; Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008; Tiwari, O'Donnell, Patras, & Cullen, 2008). Despite the observation of these phenomena, the potential restrictions and/or disadvantages of chemical effects have often been overlooked. Acoustic cavitation might be responsible for initiating formation of degradation products,

which can trigger radical chain reactions and provoke substantial quality impairments in those products.

The particles displacement caused by the application of ultrasound in liquid media induce the formation of cavitation bubbles that provoke extreme conditions of pressure and temperature that can generate violent physical forces by collapse that include microjets, shear forces and shock waves, influencing the bulk liquid surrounding the bubble or inside the bubble itself (Flint & Suslick, 1991; Mason, Riera, Vercet, & Lopez-Buesa, 2005; McNamara, Didenko, & Suslick, 1999).

Within the collapsing cavitation bubble, the extreme temperature and pressure conditions can induce the dissociation of water into hydroxyl radicals and hydrogen atoms, which can trigger chain reactions at the interface of the bubble and/or in the surrounding liquid (Riesz, Berdahl, & Christman, 1985). The formation of free radicals in both aqueous and non-aqueous media has been evidenced by electron spin resonance (Makino, Mossoba, & Riesz, 1983; Riesz et al., 1985).

Some studies have shown the appearance of off-flavors in some food products containing lipids when submitted to ultrasound (Chemat, Grondin, Costes, et al., 2004; Chemat, Grondin, Shum Cheong Sing, & Smadja, 2004; Chouliara et al., 2010; Cravotto et al., 2008; Cravotto et al., 2011; Jahouach-Rabai et al., 2008; Patrick, Blindt, & Janssen, 2004; Pingret et al., 2011; Riener, Noci, et al., 2009; Riener, Noci, Cronin, Morgan, & Lyng, 2010). Besides flavor impairments, degradation of fats in food might decrease the nutritional quality and safety from those products (Frankel, 1980; Kasai et al., 2005). Chemat, Grondin, Shum Cheong Sing, & Smadja (2004) verified an oxidation in oil emulsions after sonication even when in indirect contact to ultrasound source. Although the use of ultrasound is disseminated in the food industry, the potential restrictions induced by effects of sonication on food matrices have not been extensively examined.

To the best of our of knowledge, only four reports on the study of lipid degradation in oil samples using EPR spectroscopy have been published (Ottaviani, Spallaci, Cangiotti, Bacchiocca, & Ninfali, 2001; Papadimitriou et al., 2006; Szterk, Stefaniuk, Waszkiewicz-Robak, & Roszko, 2011; Valavanidis et al., 2004). In this work, sunflower oil was used as a model of high lipid containing food products and thus was submitted to a treatment with two different ultrasonic probes in order to better understand the phenomena of oil degradation by cavitation or the shear forces induced by ultrasound. A spin trapping study coupled to electron

paramagnetic resonance spectroscopy as well as the assessment of the classical physicochemical parameters of oxidation were performed.

## 2.2. Materials and Methods

### 2.2.1. Materials and Reagents

All oil samples were purchased at a local supermarket. α-Phenylbutylnitrone phenyl-N-tert-butylnitrone (PBN) was synthesized according to the procedure by Huie & Cherry (1985) and purified by two successive recrystallizations from ethyl acetate/diethyl ether at the laboratory. 2,2,6,6-tetramethyl-1-piperidine-*N*-oxyl (Tempo) and *p*-anisidine were purchased from Sigma-Aldrich Srl (France) and *p*-anisidine was purified according to the AOCS analytical method. All other reagents were of analytical grade. Ultrasonic devices used were a pyrex horn of 17 mm tip (Milestone, Italy) operating at 24kHz and a titanium alloy microprobe of 6 mm tip (Ultrasonic Processor, Fisher Bioblock Scientific, France) operating at 20kHz.

### 2.2.2. Ultrasound treatment of samples

All ultrasound treatments were performed in 50 mL of sample for 15 minutes in double mantle glass vessels at 25°C. All experiments were carried out in triplicates. Ultrasound treatments (US) were performed with two different ultrasonic probes and in order to obtain the same ultrasonic energy in samples with both probes, their power and amplitude were calculated and adjusted to obtain the same ultrasonic intensity. Considering the actual input power from each device is converted to heat which is dissipated in the medium, the actual ultrasound power was determined by calorimetry, calculated as shown in the equation 1 below (Toma, Fukutomi, Asakura, & Koda, 2011).

$$\mathbf{P} = m.\mathbf{Cp}.\frac{d\mathbf{T}}{d\mathbf{t}} \qquad (1)$$

Where *Cp* is the heat capacity of the solvent at constant pressure ($J.g^{-1}.°C^{-1}$), *m* is the mass of solvent (g) and *dT/dt* is the temperature rise per second. The consequent ultrasonic

intensity (*UI*) was calculated for each ultrasonic probes using the calculated power (equation 1) as shown in the equation 2 (Tiwari, O'Donnell, Patras, & Cullen, 2008).

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

Where *UI* is the ultrasonic intensity ($W.cm^{-2}$), *P* is the ultrasound power (W) as calculated by the equation 1, and *D* is the internal diameter (cm) at the tip of the probe. The UI of 3.49 $W.cm^{-2}$ was used for both ultrasonic treatments. The temperatures in the beginning and at the end of ultrasound treatment for each probe were assessed for further comparison.

### 2.2.3. Physicochemical analysis

The determination of the following physicochemical parameters in samples was carried out according to the analytical methods described by AOCS (AOCS, 2009): FFA or free fatty acids (AOCS official method Ca 5a-40), conjugated dienes level by UV spectrophotometric method (AOCS Cd 7-58), polar compounds (AOCS Cd 20-91), peroxide value (AOCS Cd 8-53), anisidine value (AOCS Cd 18-90) and fatty acid composition by gas chromatography (AOCS Ce 1-62 and Ce 2-66). The TOTOX (total oxidation value) value was calculated by the sum of 2 PV and AV (Wai, Saad, & Lim, 2009). The determination of polymerized triglycerides was performed by High-performance Size-exclusion Chromatography (ISO 16931) and the water content in the sunflower oil samples was determined by the Karl Fisher method.

### 2.2.4. Electron paramagnetic resonance analysis

EPR measurements were carried out on a MS 300 benchtop EPR spectrometer from Magnettech GmbH. The instrument settings were as follows: B0 field, 3347.80 G; sweep width, 198.8 G; modulation amplitude, 2 G; microwave power, 10 dB; receiver gain, 9×102; scan time, 40 s, number of scan 40. After ultrasound treatment, the oil samples were cooled down in a freezer and a solid amount of PBN was immediately dissolved into each sample to obtain a 20 mM solution. The PBN-oil mixture was left to equilibrate under agitation on a RT-10 magnetic stirrer plate (IKAMAG, Germany) over 24 hours in the dark. A quartz flat

cell fixed in the EPR cavity was filled by the oil mixture and immediately tested by EPR. Toluene solutions of PBN were tested as a control and no paramagnetic signal was detected demonstrating the high purity of the PBN used in this study. The absolute concentrations of nitroxides were calculated by comparing the intensities of the EPR spectra of oil solutions with TEMPO solutions in toluene in a concentration range. Essays were performed in triplicates. The EPR spectra were simulated by an automatic fitting program (Rockenbauer and Korecz, 1996). The g-factor, the nitrogen (aN) and proton (aH) couplings and the three-line parameters were adjusted until convergence is achieved. The total radical concentration was calculated from the adjusted spectral amplitude of the adduct spectra compared to the TEMPO spectrum.

### 2.2.5. Fatty acid methyl esters derivatives analysis

Fatty acids contents of samples were determined using a modified fatty acids methyl ester (FAME) method (Morrison & Smith, 1964). 20 mg of samples were weighed to the nearest mg into a pyrex tube fitted with a Teflon-lined cap. Nonadecanoic acid (400 µL of a 1 g/L solution) in dichloromethane was added as internal standard. Methylation was performed using Boron trifluoride (10%) in methanol (1 mL), which was added to the samples followed by dichloromethane (1 mL) and the mixture was vortex-mixed. The tubes were placed in a Stuart SBH200D block heater from Bibby Sterilin LTD (Stone, Staffordshire, UK) at 100 °C for 30 minutes. Then, the tubes were removed and cooled down to room temperature before adding dichloromethane (1 mL) to extract FAMEs. Hydrogenocarbonate 0.5 M (2 mL) was added, and the mixture was shaken to allow phase separation and the supernatant was discarded. This step was realized twice. Anhydrous sodium sulfate was then added to bind any residual water.

Fatty acid methyl esters (FAMEs) were analyzed on a HP 5890 gas chromatograph equipped with a FID detector and auto sampler, attached to a DB-225 capillary column (30 m· 0.25 mm·0.5 µm film thickness). One µL of sample was injected in split mode at 250 °C. The carrier gas was used at the velocity 35 $cm.s^{-1}$. The oven temperature program was as follows: the initial temperature was 50 °C, from 50 to 180 °C at 20 $°C.min^{-1}$, from 180 °C to 220 °C at 3 $°C.min^{-1}$, and then held at 220 °C for 10 min. Identification of fatty acids was performed by comparison with 37 FAME standards (SUPELCO). Fatty acid methyl esters were quantified

as percentages of the total methyl ester peak areas. Analyses were performed at least three times and the mean values were reported.

### 2.2.6. Volatiles and off-flavors analysis

The HS-SPME (Headspace Solid-Phase Microextraction) procedures were performed using a A0C 5000 (Shimadzu, kyoto) equivalent to CTC CombiPAL autosampler (Zwingen, Switzerland). The silica fibers and automatic SPME holder were purchased from Supelco (Bellefonte, PA) and a carboxen-polydimethylsiloxane (CAR/PDMS, 75 µm) fiber was used. For each extraction, 5 g of oil were hermetically sealed in 20 mL screw-top clear vials with aluminum seal and PTFE/silicone septa (Supelco). The samples were equilibrated during the incubation time at 40 °C for 10 min. Subsequently, the SPME device was automatically inserted into the sealed vial through the septum and the fiber was exposed to the sample headspace at 40 °C for 35 min. The agitator tray was turned on during the incubation and extraction procedure. Following the sampling procedure, the SPME fiber was immediately inserted into the GC-MS (Gas Chromatography - Mass Spectrometry) injector and the fiber was thermally desorbed for 3 min at 250 °C.

GC-MS analysis were performed on QP2010 (Shimadzu, Kyoto, Japan) equipped of a UBWAX capillary column (30 m, 0.25 mm i.d., 0.5 µm film thickness). The injection port (250 °C) operated in split mode with the ratio 10. The carrier gas was He at the constant velocity of 35 $cm.s^{-1}$. The initial oven temperature of 35 °C was held for 3 min, ramped at 3 $°C.min^{-1}$ to 150 °C and then ramped at 10 $°C.min^{-1}$ to 230 °C. This final temperature was held for 10 min. The mass spectrometer operated in the electron impact mode at 70 eV with continuous scans (every 0.5 s) from mass to charge ratio (m/z) 29 to 300. Data were collected with GC-MS Solution software.

### 2.2.7. Sensory analysis

The influence of US on the sensory characteristics of the samples was evaluated. Sensory evaluation was conducted by a panel consisting of 18 graduate students and staff members from the University of Avignon, France. The subjects were seated in sensory booths

with appropriate ventilation and lighting. The randomly coded samples were presented to each panelist and the sensory attributes were evaluated. Tap water was supplied to the panelists for rinsing between samples.

### 2.2.8. Statistical analysis

One-way analysis of variance (ANOVA) was conducted to determine the effect of the two ultrasound treatments on sunflower oils physicochemical parameters using Statgraphics V® software (Statistical Graphics Corp., Rockville, MD). Each measurement was replicated three times. In order to determine which means are significantly different from each other, Turkey multiple range test method was used. Trends were considered significant when means of compared parameters differed at $P < 0.05$ significance level.

## 2.3. Results and Discussion

### 2.3.1. Physicochemical analysis

In the case of lipid degradation, the analytical methods for estimating oxidation include the quantification of some primary and secondary oxidation products by direct or indirect methods and the assessment of several oxidation stages provide more detailed information on the oxidative pathway (Hamilton, Kalu, Prisk, Padley, & Pierce, 1997). In the radical oxidation process, the formed peroxides can become initiators at certain settings, increasing the rate of oxygen consumption as they are formed (for a general review see: Yin, Xu, & Porter, 2011). A significant general increase in all oxidation measurement parameters of ultrasound treated oil is observed with a marked difference between treatments. Indeed, a higher oxidation was observed in most cases for the pyrex horn compared to the titanium alloy one (Table II.3).

During the formation of hydroperoxides in polyunsaturated fatty acids oxidation, a rearrangement of the double bond can occur, resulting in the formation of conjugated dienes (CD) with an intense absorption at 234 nm. The formation of conjugated trienes (CT) can also take place, with a typical absorption at 270 nm. Although the increase in CD and CT values

reflect the formation primary oxidation product in oils, these parameters are not easily correlated to the extent of the degradation, since the dienes participate in additional oxidative reactions. Nevertheless, the CD and CT increase is proportional to the oxygen uptake and generation of peroxides, being well correlated to peroxides value (Shahidi & Zhong, 2005; Thompson, 2001). When compared to the pure untreated oil, the measurement of primary oxidation products presented an increase of 23% for conjugated dienes and of 55% for conjugated trienes in the case of treatment by the titanium alloy horn against 23% and 60% for the pyrex horn for the same parameters. A more significant difference was observed in the peroxides value (PV) from the oil treated by the titanium alloy horn, with an increase of 67% compared to the untreated oil, while the pyrex treatment presented an increase of 76% compared to titanium treatment, which represents 3 times the value of the untreated oil.

**Table II.3.** Characterization of sunflower oil for untreated and sonicated samples.

| Sample | Peroxides value (meq $O_2$/kg) | $E_{233}$ | $E_{268}$ | Anisidine Value | TOTOX | Free Fatty Acids (mg KOH/g) | Polar Compounds (%) | Nitroxides (µM) |
|---|---|---|---|---|---|---|---|---|
| SO Untreated | 2.12 ± 0.29 | 0,217 ± 0,001 | 0,106 ± 0,001 | 0,06 ± 0,001 | 4.3 | 0.02 ± 0.01 | 6.5 ± 0.10 | 1.09 ± 0.10 |
| SO Titanium | 3.55 ± 0.01 | 0,273 ± 0,001 | 0,164 ± 0,001 | 1,21 ± 0,010 | 8.31 | 0.04 ± 0.01 | 5.5 ± 0.10 | 0.95 ± 0.18 |
| SO Pyrex | 6.27 ± 0.23 | 0,268 ± 0,001 | 0,170 ± 0,001 | 2,62± 0,001 | 15.16 | 0.12 ± 0.01 | 5.5 ± 0.10 | 2.39 ± 0.18 |

**SO.** Sunflower Oil

In the lipid oxidation, an initial increase in the peroxides value (PV) is observed, with a subsequent decrease as the secondary volatile products are formed (Hamilton et al., 1997). Due to their low sensory threshold value, aldehydes are considered the main responsible for the appearance of off-flavors subsequent from lipid degradation. The anisidine value (AV) is often used for the assessment of secondary oxidation products of unsaturated fatty acids, specially dienals and 2-alkenals and even though it lacks of specificity, associated to peroxides value the AV can be a useful indicator of oil quality, particularly for oils presenting low peroxides value (Labrinea, 2001).

The measurement of secondary oxidation products presented a similar behavior to that of the primary oxidation products. The Anisidine value (AV) presented the most substantial increase, with for the pyrex and the titanium alloy horn an increase of 43 and 20 times, respectively, when compared to the value of the pure untreated oil. It has been found that a high rate of hydroperoxide generation (high PV) does not always involve a high rate of

generation of secondary oxidation products (low AV) (Guillen & Cabo, 2002). Similarly, AV determination is useful for assessing the quality of products such as frying oils that often has low PV values (Labrinea et al., 2001). Actually, some interference might occur in the primary oxidation products measurement in samples containing conjugated trienes, since some secondary products such as ethylenic diketones and conjugated ketodienes and dienals also absorb at 268 nm (Guillén & Cabo, 2002). The sum of 2 PV and AV is known as the total oxidation value (TOTOX), and this parameter provides a better representation of the overall quality status and a better estimation of the progressive deterioration of the oil (Shahidi & Zhong, 2005; Wai et al., 2009). This general increase in oxidation evaluation parameters can be confirmed by the TOTOX value, which increased 2 times when compared to the untreated sample for the titanium alloy horn, against an increase of 6 times for pyrex horn when compared to this same value.

The same behavior was observed for the Free Fatty Acids (FFA), with an increase of 2 times the untreated oil value for the titanium alloy horn and of 6 times for the pyrex horn. Free fatty acids are usually already present in minor quantities in edible oils and are more susceptible to oxidation than esterified fatty acids and can be formed by hydrolysis and pyrolysis from the cleavage of TAG (Triacylglycerol). Since FFA decrease the surface tension, an increase of the diffusion rate of oxygen from the headspace into the oil with subsequent acceleration of oxidation is observed (Mistry & Min, 1987). The augmentation of those compounds suggest a consequent increase in the oil degradation. Therefore, a noticeable oxidation is evidenced for both ultrasound treatments, with a pronounced oxidation issued from the treatment with the pyrex horn, especially for the secondary oxidation products. Formed hydroperoxides present conjugated double bond resulting from 1,4-pentadiene (dienes) or from 1,4,7-octatriene (trienes) units present in linoleic or in linolenic acyl groups, which can be measured by a direct UV-method at 232-234 and 268-270 nm, respectively. However, other secondary oxidation products could interfere with the measurement of trienes such as some ethylenic diketones, conjugated ketodienes and dienals, which also absorb at 268 nm (Guillén & Cabo, 2002). Water content quantification by the Karl Fischer method showed samples present 0.13% of water, which can also play a role in the oxidation process.

During prolonged heating of edible oils, a polymerization of triglycerides and an increase of the polar compounds can take place and results in quality impairments of the food product (Ferrari, Schulte, Esteves, Brühl, & Mukherjee, 1996). The polymerized triglycerides were quantified to less than 3% in both treated and untreated samples. Those compounds are

formed exclusively by frying conditions; therefore a time factor is intrinsically related to this type of degradation product. Despite the extreme temperature micro-conditions in the cavitation bubbles, the temperature conditions during the short time of ultrasonic treatment did not cause polymerization of triglycerides or changes in the polar compounds.

The fatty acids composition (Table II.4) of the sunflower oil before and after both ultrasound treatments was verified and results show no significant difference between those three samples. From these results, it is possible to observe the sample is rich in MUFA, having the oleic acid as major fatty acid, accounting for 60%, followed by linolenic acid (30%), while the total SFA account for 11%. Sunflower oil is a significant source of long-chain unsaturated fatty acids, especially linoleic and linolenic acids (Gallegos Infante et al., 2007). However, for some food applications, high oleic sunflower oil is preferable to high linoleic sunflower oil, since it is supposed to be more resistant to oxidative degradation under frying and storage conditions (Smith, King, & Min, 2007). Also, sunflower oil rich in MUFA might help decrease the risk of coronary heart diseases (Ashton, Best, & Ball, 2001), emphasizing the interest for the food applications of this type of oil.

**Table II.4.** Fatty acids composition of both untreated and sonicated sunflower oil.

| | SO Pure (%) | SO Pyrex (%) | SO Titane (%) |
|---|---|---|---|
| ***C16:0*** | 4.5 | 4.4 | 4.4 |
| **C16:1** | 0.1 | 0.1 | 0.1 |
| ***C18:0*** | 5.8 | 5.4 | 6.1 |
| ***C18:1*** | 59.0 | 59.2 | 59.3 |
| ***C18:2*** | 29.6 | 30.4 | 29.3 |
| **C18:3** | 0.1 | 0.1 | 0.1 |
| **C20:0** | 0.2 | 0.2 | 0.2 |
| **C20:1** | 0.1 | 0.2 | 0.1 |
| **C22:0** | 0.6 | 0.5 | 0.6 |
| ***∑SFAs*** | *11.0* | *10.5* | *11.3* |
| ***∑MUFAs*** | *59.3* | *59.5* | *59.5* |
| ***∑PUFAs*** | *29.7* | *30.5* | *29.4* |
| **C18:1/ C18:2** | *2.0* | *1.9* | *2.0* |
| **MUFA/SFA** | *5.4* | *5.6* | *5.3* |
| **MUFA/PUFA** | *2.0* | *2.0* | *2.0* |

**SO.** Sunflower Oil

### 2.3.2. Electron paramagnetic resonance analysis

The spin trapping technique is based on the reaction of a transient radical with a spin trap leading to a stable and persistent spin adduct that is detectable by electron paramagnetic resonance (Figure II.8). Over the past four decades, the spin trapping technique has been widely employed for the detection and characterization of transient radicals that are undetectable under normal conditions (For general reviews on the technique see: Janzen & Haire, 1990 and Villamena & Zweier, 2004). More recently, it has found interest in food processing and has been successfully used to investigate the oxidative stability of oil (Ottaviani et al., 2001; Papadimitriou et al., 2006; Szterk et al., 2011; Valavanidis et al., 2004; Velasco, Andersen, & Skibsted, 2004). Among several classes of spin traps available, nitrone compounds are the molecules of choice due to their specificity and ability to quantify radicals.

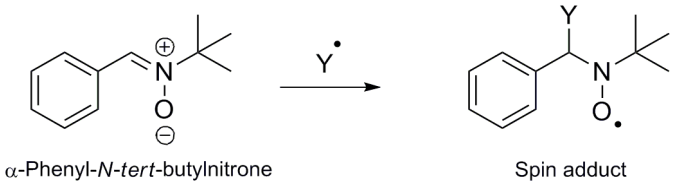


**Figure II.8.** The spin trapping mechanism of a free radical (Y□) by the α-phenyl-N-tert-butylnitrone.

The addition of α-phenyl-*N*-*tert*-butylnitrone (20 mM) to non-treated sunflower oil led, after 24 hours, to an EPR signal resulting from the trapping of transient radicals by the nitrone function. The same spectrum was observed with the ultrasound treated oil with a higher spin-adduct concentration (Figure II.9). In both cases, i.e. non-treated and treated oil, the spectra were characterized by three hyperfine lines from the coupling between the electron spin and the nitrogen atom ($a_N$ = 15.0 G). Another splitting from the β-hydrogen was also observed with a coupling constant $a_H$ = 1.85 G. This in agreement with values of the literature for oxygen-centered adduct in apolar media. Indeed, Ottaviani et al. (2001) observed a nitrogen hyperfine splitting constant of 15.3 G in olive oil that was attributed to a hydroxyl spin adduct.

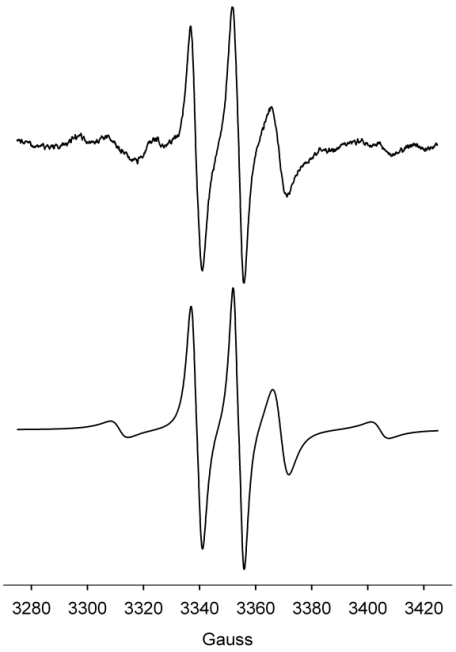


**Figure II.9.** Experimental (top) and simulated (bottom) EPR spectra of ultrasound treated sunflower oil.

Similarly, Szterk et al. (2011) reported that POBN, a PBN derivative, was able to form two spin-adducts with vegetables oil whose splitting constants were respectively $a_N$ = 13.78 G and $a_H$ = 1.80 G for the first spin-adduct and $a_N$ = 15.54 G and $a_H$ = 2.00 G for the second one. Based on these hyperfine splitting constant values, they concluded that the former species came from the trapping of the superoxide anion radical spin whereas the latter one originated from the trapping of the hydroxyl radical. The large beta and gamma relaxation parameters obtained using the program ROCKY (Rockenbauer & Korecz, 1996) indicates a slow rotation of the nitroxide in a medium of high viscosity (Table II.5). The good quality of spectral fit indicates the presence of a single radical adduct.

**Table II.5.** EPR parameters of the PBN-spin adduct observed in sunflower oil.

| **Parameters of the spin adduct (Gauss)** | |
|---|---|
| $a_N$ | 15 |
| $a_H$ | 1.85 |
| α | 2.2 |
| β | -1 |
| γ | 1.2 |

In the absence of ultrasonic treatment, the concentration of radicals after 24 hours was found to be equal 1.09 ± 0.10 µM (Table II.3). This confirms that even in the absence of ultrasound treatment, residual free radicals are present in sunflower oil as it was also demonstrated by indirect techniques (this work). When using the titanium horn, no significant change in the free radical concentration was observed whereas a significant increase by ~ 2.2 times was measured with the pyrex horn. Such an increase is in full agreement with the data obtained by the other techniques. Indeed, except for polar compounds and polymeric triglycerides, all the other physicochemical parameters in the oil samples treated by the pyrex horn presented a more prominent increase, denouncing that oxidation is more accentuated by the sonication with this type horn when compared to the titanium one. The results from spin trapping analysis suggest the increased degradation observed for the pyrex horn is due to the formation of radicals in the treated oil, in opposition to the consequent degradation observed in the oils treated by the titanium horn, which shows no evidence of radicals formation. Therefore, in the case the pyrex horn, data suggest the consequent degradation verified by the other physicochemical parameters (peroxides value, free fatty acids, anisidine value, etc.) might be due to the radicals formed during sonication using this type of ultrasonic horn, which might not be the case for the titanium horn.

### 2.3.3. Volatiles, sensory and off-flavors analysis

The off-flavors observed in the samples treated by ultrasound corresponding to the formation of volatile secondary compounds characteristic of oil oxidation such as pentanal, hexanal, heptanal, 2-heptenal and 1-octen-3-ol were confirmed by SPME analysis. Since these compounds present a very low odor threshold, their presence even at low concentrations prejudice the sensory quality of the oil (Jeleń, Obuchowska, Zawirska-Wojtasiak, & Wąsowicz, 2000). The results show an increase of 2 times the concentration of pentanal and hexanal in the sonicated oil for the titanium horn compared to untreated oil, against 1.5 times for the pyrex horn. The heptanal concentration increased 3 times for the titanium and 2 times for the pyrex horns. The 2-heptenal increased 10 times for titanium and 4 times for pyrex horn. The concentration of 1-octen-3-ol increased 20 times for titanium horn against 8 times for the pyrex one.

From the volatiles analysis we noticed the appearance of degradation compounds on both ultrasound treatments. While, the treatment with the titanium alloy horn induced the production of a greater amount of degradation compounds, the pyrex horn treatment induced a significantly lower amount of those compounds.

### 2.3.4. Generalization

A general increase of peroxides value is also observed for all typer of oils treated by ultrasounds (Table II.6). Between the other oil samples, an increase of 3 (rapeseed oil) to 32 times (palm oil) was observed, evidencing the fatty acids composition is a main factor in lipid degradation induced by ultrasound. It is possible to observe differences even between different sunflower samples, which presented increase in the PV from 5 to 13 times the untreated sample value, evidencing different oil qualities can interfere in the degradation rate of samples. In all samples, a certain degradation was already present in the untreated oil, as evidenced by an initial value of PV, which also varies upon the oil type and nature, as well as its quality.

**Table II.6.** Peroxides value for different oil samples treated by ultrasounds.

| Samples | Peroxides value (meq $O_2$/kg) | |
|---|---|---|
| | Untreated sample | US treated sample |
| Sunflower oil 1 | 9.52 ± 0.27 | 45.26 ± 0.03 |
| Sunflower oil 2 | 6.30 ± 0.31 | 39.94 ± 0.05 |
| Sunflower oil 3 | 4.50 ± 0.11 | 33.72 ± 0.10 |
| Sunflower oil 4 | 6.09 ± 0.45 | 77.91 ± 0.19 |
| Rapeseed oil | 10.92 ± 0.01 | 30.17 ± 0.26 |
| Peanut oil | 2.77 ± 0.15 | 9.75 ± 0.04 |
| Olive oil | 14.03 ± 0.10 | 81.68 ± 0.34 |
| Grapeseed oil | 6.33 ± 0.05 | 52.50 ± 0.05 |
| Flaxseed oil | 5.01 ± 0.19 | 58.22 ± 0.10 |
| Palm oil | 0.39 ± 0.30 | 12.31 ± 0.07 |

### 2.3.5. Comprehension of the mechanism of ultrasound lipid degradation

Generally, lipid degradation arise from hydrolysis or oxidation, which can occur by auto-oxidation, photo-oxidation or enzymatic oxidation and depends on multiple factors that include photosensibilization, fatty acids composition, types of oxygen, as well as the presence of minor compounds such as metals, pigments, phospholipids, free fatty acids, mono- and diacylglycerols, thermally oxidized compounds and antioxidants (Choe & Min, 2006). Degradation of fats in food products can not only prejudice acceptance by consumers but also result in a decrease in safety by the formation of molecules which are able to couple to intracellular nucleophiles. The binding to proteins and DNA result in their denaturation or impairment of normal physiological function of induction of mutation (; Kasai et al., 2005).

Lipid oxidation follows a radical chain reaction mechanism through initiation, propagation and termination stages. However, multiple factors can be involved in the initiation step, resulting in a faster or slower degradation of the lipid sample. This autocatalytic reaction generally starts with the formation of $L^{\bullet}$ (lipid alkyl radical) in the presence of an initiator. Since the spin angular momentum needs imperatively to be conserved during reactions and C=C is usually in the singlet state and O-O are in the triplet state, to overcome the spin barrier, initiators or catalyst are required either to remove an electron from the lipid or oxygen or change the electron spin of the oxygen to start the oxidation process (Choe & Min, 2006; Frankel, 1998; Schaich, 2005). The known catalysts (or initiators) of lipid oxidation are metals, light, heat, enzymes, ozone and free radicals (Choe & Min, 2006; Frankel, 1980, 1998; Hamilton et al., 1997; Kamal-Eldin, 2003; Lundberg & Chipault, 1947; Schaich, 2005; Yin et al., 2011). Due to exposure to one or more initiators, the processing method has great influence in the oxidative stability of the oil. In our studies, the initiation pathways of light, enzymes and ozone were disconsidered, since no addition of compounds or light was made in the short time of treatment. Therefore, only intrinsic minor constituents were considered to be susceptible to co-initiate the oxidation process.

In the case of exposure to high temperatures, the breakage of the covalent bond C-C or C-H is observed as well as the formation of a variety of lipid alkyl radicals that initiate the radical chain reactions of lipid oxidation. However, in moderate temperatures, preferably the covalent bonds of O-O are broken in existing traces of ROOH or LOOH that generates, by the means of intermediate reactions, the L° that initiates the chain reaction (Schaich, 2005).

Therefore, after the sonication treatment, the temperatures of both horns were measured by an infrared thermometer (Testo845 - Testo, France) and it was observed an increase of 21 degrees Celsius for the pyrex horn while the titanium one presented an increase of 60°C, although the temperature of the oil remained the same (60°C) in both conditions (Figure II.10).

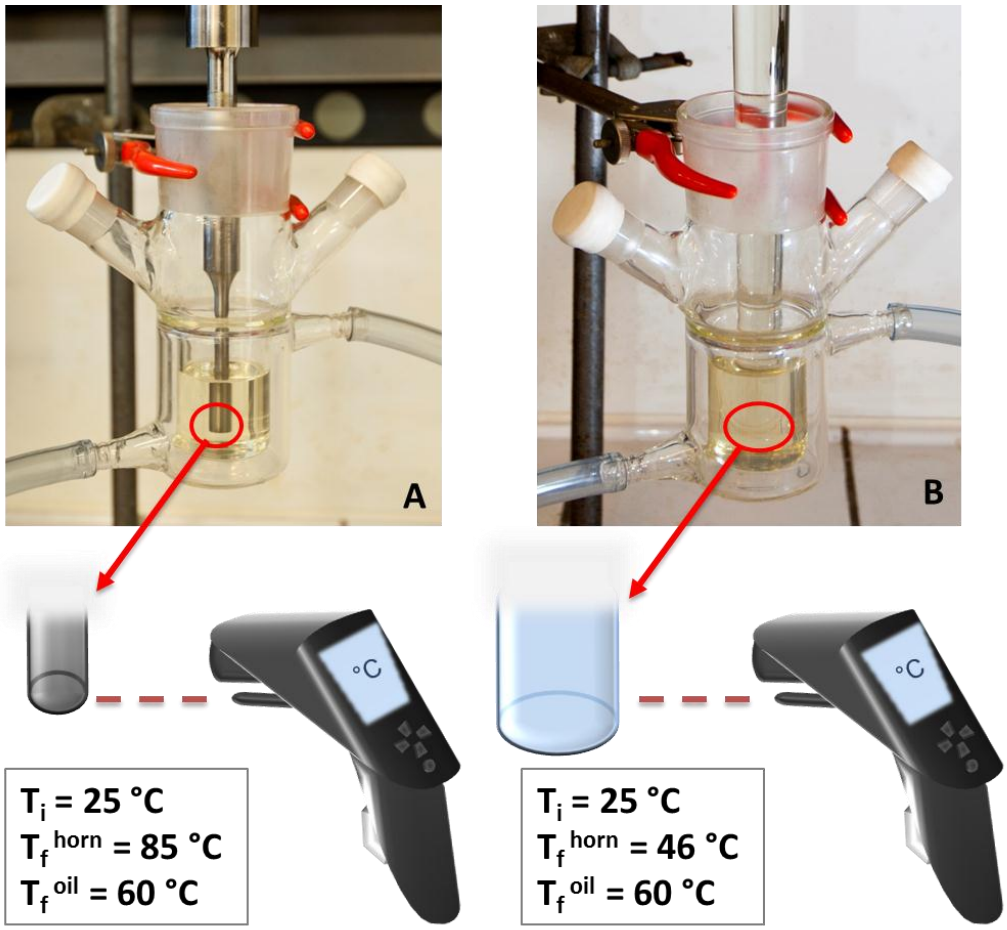


**Figure II.10.** Temperature assessment of both ultrasonic horns after sonication (Ti. initial temperature, Tf. final temperature).

For that reason, it is possible that the moderate temperatures generated by the pyrex horn induce the formation of numerous sub-products that, with the addition of ultrasonic energy, might then follow the free-radical initiation reaction. Some studies were carried out to determine the temperature attained in cavitation bubbles using molecular emission of diatomics ($C_2$) (Flint & Suslick, 1991), or emission from metal atoms (Fe, Cr, Mo) originating from volatile organometallics (McNamara et al., 1999) and the order of magnitude of the

temperatures attained reaches 5,000 K in some cases. On the other hand, studies using radioactive collision processes in a weakly ionized gas, temperatures near 20,000 K have been proposed (Brenner, Hilgenfeldt, & Lohse, 2002; Hammer & Frommhold, 2000) experiments in sulfuric acid yielded a 2,700-fold increase in light intensity, and according to the authors, the bubble would contain a hot plasma core at 30,000 K (Flannigan & Suslick, 2005).

Metals can also initiate the oxidation process and sunflower oil might contain from 2.2 to 8.5 ppb of copper and from 0.22 to 0.31 ppm of iron (Choe & Min, 2006), knowing the Codex Alimentarius (Codex Alimentarius Commission, 1999) preconizes the maximum level for those metals not to exceed 0.1 ppm and 1.5 ppm, respectively. Therefore, this small amount of metal, associated with other initiators such as high temperature or radicals could help co-initiate the oil oxidation process, producing lipid alkyl radicals. It is possible that water contained in those samples have also a role in the oxidation pathway. The initial concerns at the time of first appearances of degradation in samples treated by a metallic ultrasonic horn were that metal particles of the probe would co-initiate the oxidation reaction. However, Chemat et al. (2004) demonstrated that oxidation also occur in a glass vessel immersed in an ultrasonic bath, discarding the hypothesis of the sole oxidation by metals from ultrasonic apparatus. Our study corroborates those findings by evidencing degradation by a pyrex horn in oils contained in glass vessels; however, both laboratory glasses and the horn are made of pyrex, which contains 14% boron, 51% oxygen, .3% sodium, 1% aluminum, 38% silicon, and less than 1% potassium according to the National Institute of Standards (USA). Therefore, the treatment by ultrasound is not performed in metal-free conditions and those metals compounds contained in the glass structures might co-initiate lipid oxidation during sonication. However, the type of metal compound has a major importance in the oxidation induction rate, since different metals catalyze different reactions; while some accelerate hydrogen peroxide decomposition, others will accelerate hydroperoxides decomposition (Choe & Min, 2006). In our study, the metals present in the two sonication settings are different for the titanium and the pyrex horn, which might explain the differences in the extent of oxidation produced by each horn.

According to the overall results, it is possible to conclude that there is an increase in the formation of degradation products due to the treatment performed with the titanium horn. This behavior is also verified for the pyrex horn, but with a more marked formation of those compounds, suggesting therefore a more pronounced degradation. Indeed, the EPR analysis showed a significant increase of radicals in the oil after only 15 minutes of treatment for the

pyrex horn, which was not the case for the titanium one. The temperature analysis of both horns allowed the observation that titanium horn presents a higher increase of temperature when compared to the pyrex horn.

For those reasons, it is possible to infer that the oil degradation mechanism for the titanium horn might be mainly thermal, while the one for the pyrex horn might be mainly radical. Since the treatment with the pyrex horn stimulates the production of radicals, when the temperature parameter is included, the degradation occurs more rapidly than for the treatment by the titanium horn, which does not stimulate the formation of radicals. And this is the probable reason why the increase in not that remarkable for the other physicochemical parameters.

## 2.4. Conclusion

Ultrasound is used in emerging technologies that have been applied to numerous food industry domains alone or coupled to other techniques with advantages over other conventional techniques. Nevertheless, the effects of sonicated foods are often overlooked and, in high fat content foods, the appearance of off-flavors is observed, suggesting lipid degradation. This work demonstrates the role of ultrasound as an initiator in the oxidation of lipids with the increase of free radicals and oxidative products in sonicated oils when compared to untreated samples. Further studies should be carried out to better elucidate the degradation mechanism in sonicated edible oils.

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# *General Conclusion*

# Conclusion Générale

L'objectif de ce travail a consisté en l'étude et surtout en la compréhension du mécanisme d'action des ultrasons dans les procédés agroalimentaires.

Dans une première partie, après une synthèse bibliographique, nous avons observés l'impact positif de l'utilisation des ultrasons avec l'augmentation des rendements d'extraction des polyphénols des drèches de pomme aussi bien à l'échelle laboratoire qu'à l'échelle pilote après avoir réalisé cependant au préalable, une étape d'optimisation des différents paramètres susceptibles d'influencer l'extraction à savoir la puissance des ultrasons, la température et la durée d'extraction. Aucune dégradation des principaux polyphénols n'a été observée à l'issue de cette étude.

Par contre nous avons observés aussi bien expérimentalement que dans la littérature une altération des caractéristiques organoleptique, des paramètres physico-chimiques et/ou structuraux des produits alimentaires lors de l'exposition de ces derniers aux ultrasons. Les produits alimentaires les plus altérés semblent être les produits riches en lipides, avec l'apparition d'une odeur de rance, métallique caractéristiques de l'oxydation des ces derniers.

Dans une seconde partie, à l'issue de cette étude et au vu des résultats que nous avons obtenus, nous nous sommes donc interrogés sur l'innocuité ou non des ultrasons dans les procédés agroalimentaires et pour cela nous avons réalisé une étude plus fondamentale concernant la compréhension des mécanismes de dégradation engendrés lors de l'utilisation des ultrasons dans les procédés alimentaires.

Après avoir fait un état des lieux des problèmes rencontrés lors de l'utilisation des ultrasons en agroalimentaire, ce dernier nous a permis de mettre en avant que c'était bien les produits riches en corps gras qui étaient les plus sensibles aux traitements ultrasonores. Nous nous sommes donc intéressés à l'étude de ce phénomène de dégradation en travaillant sur les huiles végétales comme modèle. Nous avons pu mettre en évidence à l'issu d'étude physico-chimique réalisés sur nos échantillons avant et après traitement ultrasonore, que les ultrasons induisent une dégradation à la fois thermique et radicalaire. Cette étude nous a donc permis de comprendre les phénomènes ainsi que les mécanismes d'action des ultrasons dans les procédés agroalimentaires, ce qui entrainera à plus long terme le contrôle voir l'élimination de ces phénomènes en maitrisant aussi bien la température que la production de radicaux au sein de nos préparations.

Ces deux premières phases ont permis de mettre en avant les avantages et inconvénients concernant l'utilisation des ultrasons dans ce domaine.

En perspective, il est essentiel d'englober ces conclusions aux travers de deux approches qui permettront de prendre en compte la sécurité du produit alimentaire et d'assurer des conditions de travail sures en vue d'un transfert vers l'industrie. En effet, ces démarches prennent de plus en plus d'importance afin de pouvoir réaliser une production dans les meilleures conditions possibles. De plus, dans l'industrie chimique, il est commun d'adapter le processus de fabrication afin d'y introduire une technologie nouvelle telle que les ultrasons. Alors qu'à l'inverse dans l'industrie agroalimentaire, l'image du produit, l'étendu du marché et les risques alimentaires sont tels que c'est la technologie innovante qui doit s'adapter au processus de fabrication et au delà à toute la chaîne de l'usine.

Pour répondre à ces précédents constats, diverses méthodes existent pour identifier l'apparition des risques qui peuvent se produire. Les concepts d'**analyse des risques et l'étude des points critiques** (**HACCP**) et de l'**analyse des risques et d'opérabilité** (**HAZOP**) sont employés pour garantir la sécurité alimentaire et pour optimiser l'efficacité et la gestion des risques liés à la fabrication, aux personnes et à l'environnement.

Il faut noter que ce sont des étapes importantes dans l'approbation des produits et des procédés alimentaires par les organismes de normalisation et de certification. Une des exigences majeures pour un programme de sécurité alimentaire est qu'il doit être basé sur les principes de l'analyse du risque et l'étude des points critiques, comme mentionné dans l'édition courante du *Codex Alimentarius*.

Nos résultats vont donc fournir des éléments essentiels à prendre en compte dans le cahier des charges lors de la conception, de la réalisation et de la mise en route de procédés de transformation ou de préservation assistés par ultrasons en agroalimentaire.