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Ph.D. course in:

Food and Human Health

Cycle XXXI

Technological Strategies for the Sustainable Valorisation of Fruit and Vegetable Waste

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2019

TABLE OF CONTENTS

SUMMARY

Fruit and vegetable processing generates huge amounts of waste, which represents a wastage of valuable biomass and is characterised by high management cost and environmental impact. For these reasons, great attention has been dedicated in the last years to fruit and vegetable waste (FVW) valorisation, by its exploitation to produce value-added derivatives. Although it is generally assumed that FVW valorisation would deliver economic and environmental advantages, really few data on cost and energetic requirements of valorisation processes as well as on consumer response towards valorisation products are currently available.

Based on these considerations, the aim of the Ph.D. research project "*Technological Strategies for the Sustainable Valorisation of Fruit and Vegetable Waste*" was to develop a rational approach to FVW valorisation, useful to guarantee the production of value-added derivatives, but also to assess their technical feasibility, consumer acceptance, economic and environmental impact.

The developed rational approach to FVW valorisation consists in four steps: waste characterisation, output definition, process design and feasibility study. This approach was validated on waste deriving from the production of fresh-cut lettuce (external leaves and cores), peach juice (pomace) and soy milk (okara). In particular, the characterisation step highlighted that waste generated during the processing of these vegetables ranges from 10 to 40%. Although the high water content (>76%), these wastes are particularly rich in fibre (>26% on dry weight basis) and polyphenolic compounds (>2.0 GAE/g of dry weight). Based on waste characteristics, different possible outputs were defined and specific processes exploiting the application of either traditional or novel sustainable technologies were designed: (i) convenient functional smoothies were produced from lettuce waste and okara using high pressure homogenization; (ii) antioxidant extracts were produced from lettuce waste and peach pomace using ultrasounds and microwaves; (iii) functional flour was obtained by air-drying and grinding of lettuce waste; (iv) supercritical-CO_2-drying of lettuce waste allowed obtaining a porous material with high solvent loading capacity.

The obtained valorisation outputs were then evaluated for their sensory acceptability and consumer response, in order to estimate their market potential. To this aim, the lettuce waste study-case, was analysed by the application of consumer-based methodologies. Obtained results highlighted the possibility to exploit nutritional and environmental claims to promote consumption of waste-derived food. Finally, a multi-objective method was applied to estimate the economic and environmental impact of the proposed valorisation strategies on an industrial scale.

The developed approach could be considered a flexible decision support tool to guide stakeholders' aware choice and investment in the most sustainable valorisation strategies.

RIASSUNTO

Il processo produttivo di derivati di frutta e verdura genera enormi quantità di scarto, che comporta la perdita di prodotti ad alto valore, elevati costi di gestione ed un notevole impatto ambientale. Per queste ragioni, negli ultimi anni, molta attenzione è stata dedicata alla valorizzazione degli scarti vegetali attraverso la loro trasformazione in derivati ad alto valore aggiunto. Sebbene la valorizzazione sia comunemente ritenuta vantaggiosa da un punto di vista economico ed ambientale, in realtà i dati disponibili su costi e richieste energetiche dei processi di valorizzazione, e sulla reazione dei consumatori nei confronti dei prodotti di valorizzazione sono molto limitati.

Sulla base di queste considerazioni, lo scopo del progetto di Dottorato "*Strategie Tecnologiche per la Valorizzazione Sostenibile di Scarti Vegetali*" è stato quello di sviluppare un approccio razionale alla valorizzazione degli scarti vegetali, in grado di garantire l'ottenimento di prodotti ad alto valore aggiunto, nonché la loro fattibilità tecnica, senza tralasciare la stima del livello di accettazione da parte dei consumatori e dell'impatto economico ed ambientale.

L'approccio sviluppato nel corso del progetto consiste di 4 passaggi: caratterizzazione dello scarto, definizione dei prodotti di valorizzazione, *design* del processo produttivo e studio di fattibilità. Questo approccio è stato validato sugli scarti derivanti dalla produzione di insalata di IV gamma (foglie esterne e torsoli), succo di pesca (bucce e polpa residua) e latte di soia (okara). In particolare, la fase di caratterizzazione ha evidenziato che lo scarto generato durante la trasformazione di questi prodotti varia dal 10 al 40%. Nonostante l'elevato contenuto di acqua (>76%), questi scarti presentano rilevanti quantità di fibre (>26% su base secca) e composti fenolici (>2.0 GAE/g su base secca). Sulla base di queste caratteristiche sono stati identificati diversi possibili prodotti di valorizzazione e i corrispondenti processi produttivi, basati sull'utilizzo di tecnologie sia tradizionali che innovative e sostenibili: (i) dagli scarti di insalata e dall'okara, sfruttando l'omogeneizzazione ad alta pressione, sono stati prodotti *smoothies* pronti all'uso; (ii) dagli scarti di insalata e di pesca, utilizzando ultrasuoni e microonde, sono stati ottenuti estratti antiossidanti; (iii) dagli scarti di insalata, disidratati e macinati, è stata ricavata una farina funzionale; (iv) l'applicazione dell'essiccamento supercritico ha infine consentito di convertire gli scarti di insalata in un materiale poroso con elevata capacità assorbente.

I prodotti di valorizzazione così ottenuti sono stati analizzati in termini di accettabilità sensoriale e attitudine dei consumatori, al fine di evidenziarne le potenzialità di mercato. A questo scopo, è stato preso in considerazione il caso studio degli scarti di insalata, applicando metodi *consumer-based*. I risultati ottenuti hanno dimostrato la possibilità di sfruttare *claims* nutrizionali e ambientali per promuovere il consumo di alimenti derivati da scarti vegetali. Infine, è stato applicato un metodo *multi-objective* per stimare l'impatto economico ed ambientale delle strategie di valorizzazione proposte, se applicate su scala industriale.

L’approccio sviluppato in questo progetto può essere considerato un flessibile strumento di supporto alle decisioni, in grado di guidare gli *stakeholders* verso una scelta consapevole circa gli investimenti nelle strategie di valorizzazione più sostenibili.

Chapter 1
Fruit and vegetable waste

1.1 Introduction and main definitions

Around 89 million tons of food are wasted annually in the European Union (Stenmarck, Jensen, Quested, & Moates, 2016) and this value is expected to further increase by 40% in the next years. Moreover, the World and Agriculture Organization calculated that one-third of the edible parts of food intended for human consumption gets lost or wasted (FAO, 2011). The term "food loss" identifies the decrease in edible food mass throughout the part of the supply chain that specifically leads from raw material to food for human consumption. Food losses, thus, take place at production, post-harvest and processing stages in the food supply chain. Food losses occurring at the end of the food supply chain (retail and final consumption) are rather called "food waste", which relates to retailers' and consumers' behaviour (Manzocco, Alongi, Sillani, & Nicoli, 2016; Parfitt, Barthel, & Macnaughton, 2010). Moreover, the term "food by-products" has been increasingly used in the literature relevant to food waste. This term notifies that biomass and waste can be properly treated and converted into valuable marketable products (Galanakis, 2012).

In the fruit and vegetable sector definitions are more controversial. A widely-used term is "fruit and vegetable waste" (FVW). The latter has been defined as the inedible parts of vegetables that are discarded during collection, handling, transportation and processing (Chang, Tsai, & Wu, 2006). According to this definition, thus FVW refers to "unavoidable waste", i.e. waste arising from fruit and vegetable processing that is not and never has been edible (e.g. peels, stones, cores). However, wider definitions have been proposed in the relevant literature. In this regard, Panda, Mishra, Kayitesi and Ray (2016) and Galanakis (2012) indicate FVW as all discards generated from fruit and vegetables along the entire food supply chain, from farm to fork, including thus both pre- and post-consumer stages. This definition is not specifically referred to inedible fruit and vegetable fractions, but includes also "avoidable" FVW, i.e. fruit and vegetables that have been, at some point, edible but are wasted due to different causes (Papargyropoulou, Lozano, Steinberger, Wright, & Ujang, 2014). In this thesis, the term FVW will be used to generally indicate material from fruit and vegetable processing plants and production sites, which is required or intended to be discarded.

1.2 Main causes of FVW

According to FAO estimation (FAO, 2011) pre-consumer phases are particularly critical in terms of FVW generation. To this regard, Segrè and Falasconi (2011) reported that, in Italy, up to 87% of fruit, vegetable and cereals are discarded before reaching consumer. Causes may be different. In

developing countries, wastes are mainly generated in agricultural production, post-harvest and distribution stages, due to seasonality that leads to unsaleable gluts and to the absence of proper conservation strategies for perishable crops. By contrast, wastes in industrialized countries are mostly due to post-harvest evaluation of crops based on quality standards requested by retailers and to programmed overproduction, that leads to "food surplus" (i.e. food produced beyond nutritional needs) that eventually becomes waste (FAO, 2011; Segrè & Falasconi, 2011).

1.3 Main consequences of FVW

As other food waste materials, FVW must be properly managed to guarantee sustainable development of society. According to the Brundtland Commission, "sustainability is the development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (Brundtland, 1987). Even if this definition poses the accent on the fact that sustainable development should not compromise resources for future generations, it gives no indications about what should be done and what should be avoided for achieving this goal. Such indications can be found in the "Corporate Social Responsibility" (CSR) definition, according to which, organizational sustainability must include not only economic performances (profit) but also environmental and social impact: only by balancing these three components, long-term sustainability can be achieved (Elkington, 1994). If specifically referred to food supply chain, sustainability can be achieved by a proper product stewardship, "minimizing the health, safety, environmental, and social impacts of a product and its packaging throughout all lifecycle stages, while also maximizing economic benefits". The manufacturer, or producer, has the greatest ability to minimize adverse impacts of a food product, but other stakeholders, such as suppliers, retailers, and consumers, also play a role (Product Stewardship Institute, 2011). Based on these definitions, it is evident that FVW represents a critical hurdle to sustainability, since negatively affecting economic, environmental and social development. The economic impact of FVW is related to its disposal cost but also to the purchasing cost of a good that becomes unsaleable or inedible, leading to a useless deployment of resources and to a direct reduction in economic income of both farmers and consumers. The main environmental threat related to FVW disposal is the methane production during its decomposition in landfills, highly contributing to greenhouse effect. FVW disposal also results in biogenic cycle alteration and groundwater pollution. Another environmental impact related to FVW is linked to the footprint of food during its lifecycle before becoming a waste, including use of land and resources (water, energy) during harvesting, processing, distribution and retail. FVW has also social and ethical implications, related to an inadequate and uneven distribution of resources in a world increasingly affected by food insecurity and poverty (Papargyropoulou et al., 2014).

1.4 FVW management

Due to the environmental, economic and social issues related to FVW, in the last years, great attention has been focused on the development of policies and methods for its prevention and management (Laufenberg, Kunz, & Nystroem, 2003). In general, waste prevention refers to measures taken before a substance, material or product has become waste (2008/98/EC); waste management "is the collection, transport, recovery and disposal of waste, including the supervision of such operations" (2006/12/EC) and the waste management system consists of "the whole set of activities related to handling, disposing or recycling waste materials". Strategies to tackle FVW have been ordered according to their priority in the so called "waste hierarchy": prevention of waste generation, re-use, recycling, energy recovery and disposal (2008/98/EC; Demirbas, 2011). Table 1 reports the main strategies for FVW prevention and management, that are following presented.

1.4.1 Prevention of FVW

Decrease overproduction is one of the main targets of FVW prevention strategies. Agricultural production has necessarily to be higher than sales forecast, in order to face eventual harvest losses due to natural phenomena (Segrè & Falasconi, 2011). Agronomists suggest an overproduction of 30% to guarantee food security. Despite this indication, the current food overproduction has been quantified around 50, up to 90% in some developed countries (Papargyropoulou et al., 2014). Thus, a decrease in overproduction could be applied to reduce fruit and vegetable surplus and thus FVW, while guaranteeing food security against unpredictable weather patterns affecting crops.

In addition, it has been estimated that huge amounts of small-sized or misshaped fruit and vegetables are wasted because not fulfilling quality standards set by retailers and consumers (Mena, Adenso-Diaz, & Yurt, 2011). Different strategies have been proposed and implemented to tackle waste of these "substandard" fruit and vegetables. The latter have been traditionally downgraded to the production of alternative fruit and vegetable derivatives (e.g. juices, vinegar) (Grewal, Tewari, & Kalra, 1988). Moreover, interesting initiatives to tackle wastage of substandard products are being carried out by the campaigns "Inglorious Fruit and Vegetables" and the line "No Name® Naturally Imperfect™", launched in 2015 by the French retailer Intermarchè and the Canadian one Loblaw, respectively (Intermarchè, 2015; Loblaws, 2015). Such campaigns address the FVW issue by selling substandard fruit and vegetables, while reducing costs for consumers and increasing consumer awareness of the FVW issue (Table 1).

1.4.2 Re-use of FVW

Re-use, or more precisely "preparing for re-use", means "checking, cleaning or repairing recovery operations, by which products or components of products that have become waste are prepared so that they can be re-used without any other pre-processing" (2008/98/EC). When talking about FVW re-use, the difference between food waste and food surplus becomes crucial. As anticipated, food surplus can be defined as food produced beyond nutritional needs. By contrast, food waste refers to substances that can no longer be used for human consumption since they have been never edible (unavoidable food waste) or have been allowed to pass their best (avoidable food waste) (Papargyropoulou et al., 2014). In the case of food waste, re-use is more often referred to the management of food surplus for feeding hungry people. Such management is carried out by the so defined "food rescue programs", which collect perishable food, including fruit and vegetable surplus, and donate it to people affected by food poverty (Schneider, 2013). Since in this case food items are used for their original scope, they must meet safety and quality criteria that are no more satisfied when food has become waste.

1.4.3 Recycle of FVW

According to the above reported discussion, both prevention and re-use cannot be properly defined FVW management strategies, since they are applied at product and food surplus level, respectively and, thus, before waste generation.

Once waste is generated and it is not possible applying prevention and re-use options, "recovery" should be applied. According to the definition given by the Waste Framework Directive (2008/98/EC), recovery refers to "any operation whose result is waste serving a useful purpose by replacing other materials, which would otherwise have been used to fulfil a specific function in the plant or in the wider economy". In this case, waste becomes thus a valuable secondary raw material, giving the basis of a circular economy system in which waste can be converted into resources and materials, returned back to the economy and used again. In particular, recycling and energy recovery strategies can be applied to FVW (Pfaltzgraff, De Bruyn, Cooper, Budarin, & Clark, 2013).

Recycling means "any recovery operation by which waste materials are reprocessed into products, materials or substances whether for the original or other purposes" (2008/98/EC). Current FVW recycling strategies are mainly based on its use for animal feeding and fertilizer production. As other food waste materials, the high fibre content of FVW can be exploited to formulate animal feeds with increased nutritional value (San Martin, Ramos, & Zufía, 2016). However, this re-use strategy is limited by some drawbacks. In particular, low protein content and high presence of indigestible compounds are not always suitable for animal feed (Clemente, Pardo, Madejón,

Madejón, & Bernal, 2015). Moreover, composition of vegetable products varies according to season, forcing manufacturers to often change feed formulations (San Martin et al., 2016).

Aerobic composting is an ancient eco-friendly method to convert organic waste into fertilizer (Chang et al., 2006). This practice is based on the ability of organic waste to increase properties of polluted soil by immobilizing trace metals and metalloids, preventing their transfer to groundwater and living organisms, and promoting the establishment of plants (Clemente et al., 2015). However, this recycling strategy is often difficult to put into practice due to the high biological instability of FVW, responsible for pathogen growth risk and off-odours generation (Ajila, Brar, Verma, & Prasada Rao, 2012). In addition, it is well established that anaerobic digestion (§ 1.4.4) is a more attractive strategy to produce fertilizers from FVW, due to the fact that, beside fertilizing digestate, energy is also recovered (Sharma, Testa, Lastella, Cornacchia, & Comparato, 2000).

FVW has been also largely studied for its conversion into derivatives for human consumption. Recycle strategies applied to this aim can be divided into strategies in which the whole waste mass is recycled (processing into flour, conversion into water) and strategies in which specific compounds are extracted.

Processing into flour of FVW has been exploited with different purposes. The fibrous structure and the high contact surface of FVW flour has been used to adsorb pollutants such as dyes and heavy metals from water and ground (Annadurai, Juang, & Lee, 2002). To this regard, adsorption is due to both physical entrapment into the porous structure of the vegetable and to specific interaction with the functional groups of cellulose, hemicellulose and lignin (Azouaou et al., 2008). FVW flour has also been used as an ingredient for the formulation of food products rich in functional compounds such as polyphenols and fibres (Ferreira et al., 2015).

Water can also be considered a possible output of FVW recycle. To this regard, patented or patent-pending systems able to convert organic material into water are already applied in companies, supermarkets and restaurants (Waste to Water Pdy Ltd, 2017). They are based on the hyper-acceleration of aerobic decomposition through the activity of naturally-occurring microorganisms with enhanced degradation capabilities under tightly controlled environmental conditions (Table 1). Obtained water could be of great interest within the fruit and vegetable derivative production processes, which are particularly water-intensive (Manzocco, Ignat, Anese, et al., 2015). Both processing into flour and conversion into water offer the advantage that no residual waste must be disposed of.

The extraction of specific functional compounds from FVW has been largely studied (Table 1). Bioactive compounds (e.g. carotenoids, polyphenols, anthocyanins) (Ayala-Zavala, Rosas-Domínguez, Vega-Vega, & González-Aguilar, 2010; Choi, Cho, Moon, & Bae, 2015) as well as essential oils (Bustamante et al., 2016; Górnaś, Soliven, & Segliņa, 2015), fibres (Elain et al., 2016; Nawirska & Kwaśniewska, 2005; Piccinno, Hischier, Seeger, & Som, 2015; Zini & Scandola, 2011) and natural dyes (Bechtold, Mussak, Mahmud-Ali, Ganglberger, & Geissler, 2006) are the main

targets of this recycle strategy. Structuring agents, mainly referring to colloidal polymers with interesting gelling or viscosant properties, can also be selectively extracted from FVW (McCann, Fabre, & Day, 2011; Roversi, Radaelli, & Piazza, 2015). Although the added-value of these FVW extracts, after the extraction process, relatively high amounts of residual waste must be still disposed of.

1.4.4 Energy recovery from FVW

Energy recovery, also called waste-to-energy, is performed in order to recover the energy contained in the waste material (Kothari, Tyagi, & Pathak, 2010). In this regard, food waste can be exploited for biorefinery, as renewable biomass source for the production of bioenergy/biofuels, with the final aim of substituting fossil fuels and reducing the depletion of other natural sources (Cherubini & Ulgiati, 2010). Energy can be recovered from waste by the application of several strategies, including thermochemical conversions, such as carbonisation, pyrolysis and gasification or biochemical strategies, such as anaerobic digestion and fermentation. In the case of FVW, only some of these strategies can be applied (Table 1). In fact, thermochemical conversion strategies are not suitable for waste with high moisture, which is responsible for a really low calorific value (Lin et al., 2011). On the contrary, biochemical conversion strategies are quite efficacious. Anaerobic digestion (AD) has been widely used for organic waste disposal. AD is a method to decompose organic matter using anaerobic microorganisms under oxygen-free conditions. After the treatment, the end-product is represented by biogas (60% methane, 40% carbon dioxide) and digestate (or AD effluent) (Sheets, Yang, Ge, Wang, & Li, 2015). Biogas can be used for different purposes, including production of heat, electricity and compressed natural gas, while the AD digestate, rich in nitrogen, can be used as a fertilizer (Yang, Ge, Wan, Yu, & Li, 2014). However, AD of FVW presents some issues. In fact, FVW is generally characterized by a low potential for biogas production, due to low total solid and high volatile fraction that is rapidly hydrolysed during digestion, leading to acidification and inhibition of digestion process. As a consequence, co-digestion of FVW with other organic wastes is increasingly studied and applied (Jiang, Heaven, & Banks, 2012). As a result, companies usually confer their organic waste to centralized biogas production plants (Kothari et al., 2010), dealing with relatively high collection and transport costs (Dereli, Yangin-Gomec, Ozabali, & Ozturk, 2012; Stürmer, Schmid, & Eder, 2011). Moreover, the improper application of AD digestate can lead to serious environmental problems such as over-fertilization and soil pathogen contamination (Nkoa, 2014).

Other energy-recovery strategies have been studied as alternatives to AD. In particular, microbial fuel cells have been recently applied to FVW. This strategy refers to biologically catalysed electrochemical systems in which the chemical energy of an organic substrate is converted into electrical energy through redox reactions (Pant, Van Bogaert, Diels, & Vanbroekhoven, 2010). However, this strategy is limited to carbohydrate-rich wastes (Elmekawy, Diels, De Wever, & Pant, 2013).

1.4.5 FVW landfilling

In the past, FVW was mixed into municipal waste streams and sent to landfills or incinerators (without energy recovery) for final disposal (Nawirska & Kwaśniewska, 2005). However, this is not a good option for FVW, due to its high water content which is, in turn, responsible for microbiological instability, formation of off-odours and leachate (Lin et al., 2011). Beside these disposal issues, landfilling of FVW poses wider sustainability issues, that cover economic, environmental and social aspects (§ 1.3).

Table 1. Main strategies of FVW prevention and management according to food waste hierarchy options.

Food waste hierarchy option	Strategy	Output	Waste origin	References
Reduction	Reduction of overproduction	Reduction of cultivated crops	Fruit and vegetable surplus	Papargyropoulou et al. (2014)
	Market of substandard items	Low cost fruit and vegetables	Substandard fruit and vegetables	Intermarchè (2015); Loblaws (2015)
	Alternative processing of substandard items	Fruit and vegetable derivatives	Substandard apple and grapes	Grewal et al. (1988)
Re-use	Food rescue programs	Fruit and vegetables distributed to hungry people	Fruit and vegetable surplus	Schneider (2013)
Recycle	Dehydration, trimming, pelleting	Fibre-enriched animal feed	Mixed fruit and vegetables	San Martin et al. (2016)
	Composting	Fertilizers	Mixed fruit and vegetables	Chang et al. (2006) ; Clemente et al. (2015)
	Processing into flour	Green and low-cost adsorbents for pollutants in wastewaters	Orange, citrus, banana, olive, apricot	Annadurai et al. (2002); Azouaou et al. (2008)
		Flour rich in antioxidants, phenols, minerals and fibre	Tropical fruit, orange	de Oliveira et al. (2009); Ferreira et al. (2015)
	Conversion into water	Water for industrial facilities	Mixed fruit and vegetables	Waste to Water Pty Ltd (2017)
	Extraction of specific compounds	Bioactive extracts		
		Flavonoids and bio-sugars	Onions	Choi et al. (2015)
		Antioxidants and antimicrobials	Fresh-cut fruit	Ayala-Zavala et al. (2010)
		Oils		
		Essential oils	Citrus fruit	Bustamante et al. (2016)
		Oils for food, biodiesel, pharmaceutical and cosmetic sectors	Watermelon, melon, red currant, pomegranate, grape, apple	Górnaś et al. (2015)
		Fibres		
		Reinforced biopolymers	Banana	Zini & Scandola (2011)
		Bioplastics	Mixed fruit and vegetables	Elain et al. (2016)
		Cellulose nanofibers	Carrot	Piccinno et al. (2015)
		Dietary fibre	Apple, cherry, chokeberry, black currant, pear, carrot	Nawirska & Kwaśniewska (2005)
		Natural dyes	Raspberries, black carrots, currants, onions	Bechtold et al. (2006)
		Structuring agents	Apple, carrot	McCann et al. (2011); Roversi et al. (2015)
Energy recovery	Anaerobic digestion	Biogas Fertilizers	Mixed fruit and vegetables	Sheets et al. (2015)
	Bio-electrochemical systems	Electrical energy	Carbohydrate-rich vegetables	Elmekawy et al. (2013)

1.5 FVW valorisation

The aim of the present paragraph is to define FVW valorisation, trying to find its position within the options presented in the waste hierarchy. In fact, although being increasingly used in the recent literature, there is not an official definition of food waste valorisation. Nevertheless, literature quite fully agrees in defining valorisation strategies as interventions applied in order to produce commercial products from food supply chain waste (Galanakis, 2012; Luque & Clark, 2013; Ravindran & Jaiswal, 2016). Valorisation has thus a strong economic implication. By contrast, although the European Waste Framework Directive (2008/98/EC) advises the Member States to consider also the social and economic impacts of food waste, the waste hierarchy primarily focuses on delivering the best environmental option, which has been the basis of criticism from a number of economists (Rasmussen et al., 2005).

To better define the borders of FVW valorisation, a first step is distinguishing between interventions that can be applied at product (not-waste), food surplus and food waste level. As already anticipated, prevention and re-use are not properly applied to FVW since they avoid waste generation by changing production practices and management of products and food surplus. By contrast, when FVW has been already generated, it should be managed through recycling and energy recovery strategies. The latter can be further prioritized according to the developed added-value. In this regard, when FVW is used as a feedstock to produce energy and fuels (e.g. through anaerobic digestion), animal feeding and fertilizers (e.g. composting), its interesting functional molecules are lost or, at best, underutilised (Pfaltzgraff et al., 2013). The latter are instead maximally exploited when FVW serves as a source of bioactive compounds, functional ingredients and biocompatible materials to be exploited for human consumption. For example, it has been recently proven that the conversion of food waste to bulk chemicals is about 3.5 and 7.5 times more profitable than its conversion to animal feed or transportation fuel respectively, suggesting a marginal economic value of these first-generation valorisation strategies (Papargyropoulou et al., 2014). Thus, valorisation strategies can be defined as those strategies aiming at the maximal exploitation of food waste potentialities, through its recycling into value-added derivatives for human consumption. Based on these considerations, the framework of food waste valorisation in the light of the food waste hierarchy is proposed in Figure 1.

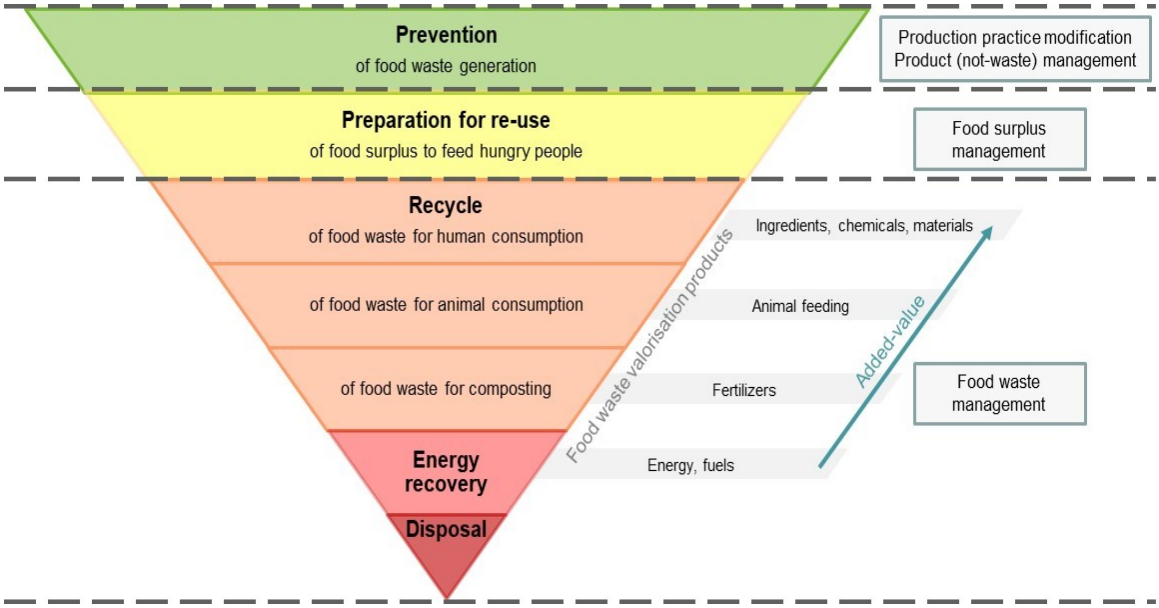


Figure 1. Food waste valorisation framework.

In the field of FVW valorisation, the term "by-product" has been increasingly used instead of waste to indicate discards possibly exploitable for the production of value-added derivatives (Galanakis, 2012). However, it must be underlined that the legal definition of by-product, given by the European Commission (2008/98/EC), often does not apply to FVW exploited for valorisation. According to this definition, in fact, a by-product never becomes a waste since, even if it is not the primary product of a process, it already has a certain use, and thus a market, and its characteristics and specifications meet specific technical, legal, safety, environmental and quality requirements. Since most literature studies aim at valorising substances that are currently a waste and do not meet by-product criteria, the "end-of-waste status" should be most properly considered. The end-of-waste status applies when a waste ceases to be a waste and obtains a status of product or secondary raw material. The reclassification of a substance by the application of the end-of-waste status does not require to meet all the by-product criteria. In particular, to apply this status, the producer should be aware of the specific purpose of the considered substance (intended use) and of its potential for consumer acceptance, that would guarantee a market demand for such waste-derived product. The latter should also meet specific requirements, including technically feasibility, legal, safety, quality and environmental criteria. The end-of-waste status does not require that the substance is produced as integral part of the production processes, opening the possibility, for example, to mix waste streams deriving from different processes. Differently form by-products, in addition, a substance can be assigned with the end-of-waste status also upon the application of industrial practices other than those commonly applied, including, for example, innovative technologies. Table 2 summarises criteria that should be met by a substance to be classified as a by-product or to be assigned with the end-of-waste status.

Table 2. Criteria that should be met by a substance to be classified as a by-product or to be assigned with the end-of-waste status.

Criteria	By-product	End-of waste status
Certain use	v	
Direct use/Use after normal industrial process	v	
Production as integral part of the production process	v	
Specific purpose	v	v
Fulfilment of technical, legal, safety, quality requirements	v	v
Market demand	v	v
No adverse impact on sustainability	v	v

It must be underlined that an incorrect classification of a substance deriving from a production process can cause unnecessary environmental damage or cost for business. In fact, the possibility of classifying a substance as a by-product or of applying the end-of-waste status, will lead to the development of marketable products instead of waste biomass.

The specific by-product and end-of-waste criteria indicated in the Waste Framework Directive offer a useful guideline to analyse FVW characteristics and understanding its potentialities for valorisation.

1.5.1 Sources and targets of food waste valorisation

The availability of substrates presenting a homogeneous composition and a segregated localisation is crucial for implementing FVW valorisation. The absence of these features would require additional collection and separation processes, increasing costs and time needed between FVW collection and valorisation processing. Time aspect is of critical importance, since FVW is extremely perishable (Galanakis, 2012). Currently, the collection of homogeneous wastes, concentrated in rather few locations, is possible only in the initial steps of the food supply chain (harvesting, post-harvest and industrial processing) while, in most cases, food waste generated during distribution, retail and consumer phases is a mixture of heterogeneous and not-segregated materials (Ravindran & Jaiswal, 2016). For these reasons, FVW intended for valorisation is commonly collected in the industrial processing steps.

1.5.2 Open questions related to food waste valorisation

It is generally assumed that FVW valorisation would contribute to sustainable development by delivering environmental, economic and social advantages. FVW valorisation is expected to produce new products from non-fossil sources and contribute to the reduction of greenhouse gas emissions by diverting waste from landfills. Moreover, the production of innovative waste derivatives could bring economic and social advantages, creating marketable value-added products and work positions (Galanakis, 2012).

Nevertheless, most of the valorisation strategies applied to FVW are based on the pioneering exploitation of waste to produce outputs whose impact on the food supply chain sustainability is unknown. In particular, innovative processes based on novel technologies (e.g. ultrasounds, high-pressure processing, pulsed electric field) are often applied to valorise FVW. Despite the encouraging results obtained in the last years on a laboratory scale application of these technologies, evidences of their industrial scalability and impact on the sustainability of the whole production system are very limited. Also, in most cases, there is no evidence of the fact that the products obtained by valorisation possess all the characteristics required for reclassification into new marketable products (e.g. market demand, technical feasibility) (Table 2). Finally, it should be underlined that each one of the recovery strategies shown in Figure 1 may contribute to the increase of food supply chain sustainability. For example, although adding the lowest value to FVW, biogas produced by energy recovery strategies represents an important fossil fuel alternative. In this regard, as clearly stated in the "Rethinking the Waste Hierarchy" report of the Environmental Assessment Institute (Rasmussen et al., 2005), the relevant question in relation to food waste management is not whether to choose re-use, recycling, energy recovery or disposal in landfills, but how much waste should be re-used, recycled, digested or landfilled, respectively. Data about the best proportion of waste to be managed with each strategy are thus required.

1.6 Aim and outline of this Ph.D. thesis

The aim of this Ph.D. thesis was to develop a rational approach to FVW valorisation, able to guarantee the production of value-added derivatives from FVW, but also to assess their technical feasibility and market potential as well as their scalability and impact on the food supply chain sustainability from an environmental and economic point of view.

To this aim, the Ph.D. project was articulated as described in the Thesis outline reported Table 3. The first part of the project was dedicated to the development of a rational approach to FVW valorisation (Chapter 2). The latter was then validated on different vegetable discards (wastes from fresh-cut lettuce, peach juice and soy-milk production), whose management currently represent a critical issue for companies. In particular, after waste characterisation in terms of amount and composition (Chapter 3), outputs that could be obtained from the valorisation of the considered waste materials were identified. In this regard, the selected wastes were turned into fresh homogenates, bioactive extracts and dried derivatives (flour, biodegradable materials) (Chapter 4). This was carried out by the application of specific processes based either on traditional (air-drying, freeze-drying, grinding, solid-liquid extraction) and innovative techniques (supercritical-CO_2-drying, high pressure homogenisation, ultrasounds, microwaves). In this regard, the design of valorisation processes is reported in Chapter 5. Finally, feasibility studies were carried out, aiming at identifying the intended use of obtained valorisation outputs and testing their technical feasibility (Chapter 6). To this aim, the lettuce waste study-case, which provided four different valorisation outputs (fresh homogenate, antioxidant extract, functional flour and biodegradable material) was considered. The possibility of lettuce waste valorisation outputs to have a market demand and the overall impact of proposed valorisation strategies on sustainability were tested using consumer-based methodology and a multi-objective study, respectively.

Table 3. Ph.D. Thesis outline.

Product	**Waste**	**Rational approach to FVW valorisation**								
		Waste characterization	Output definition	Process design			Feasibility study			
				Drying	Extraction	Homogenisation	Specific purpose	Technical validation	Market demand	Sustainability
Fresh-cut lettuce	External leaves	Amount and composition	Fresh homogenate			High pressure homogenization	Healthy ingredient/food			Multi-objective study
			Bioactive extract		Solid-liquid extraction		Food supplement			
					Ultrasound assisted extraction					
			Flour	Air-drying		Grinding	Flour for bakery application	Bread production	Consumer acceptance	
									Consumer response	
				Freeze-drying						
				Supercritical-CO_2-drying						
			Biodegradable material	Supercritical-CO_2-drying			Expanded packaging material			
							Solvent absorber/packaging			
Soy milk	Okara	Amount and composition	Fresh homogenate			High pressure homogenization	Healthy ingredient/food			
Peach juice	Peach pomace	Amount and composition	Bioactive extract		Ultrasound assisted extraction		Food supplement			
					Microwave assisted extraction					

Chapter 2

A rational approach to fruit and vegetable waste valorisation

FVW waste can be considered a cheap source of valuable ingredients. To maximally exploit these potentialities, proper FVW management strategies should be developed, allowing not only the production of value-added derivatives, but also the assessment of their technical feasibility, consumer acceptance, scalability on industrial level and impact on system sustainability. To reach this goal, a rational approach to the development of FVW valorisation strategies should be applied. Such approach consists in a 4 step-procedure, including waste characterization, output definition, process design and feasibility study.

2.1 Waste characterization

The first step for developing a rational valorisation strategy involves an accurate characterization of the waste material, in terms of amount and composition. Amount data are often already available to companies, based on the flow of sources into the company, and during waste transport and disposal.

Secondarily, processing steps mainly involved in waste generation should be identified. It is likely that in these steps, in fact, waste materials presenting a homogeneous composition could be available. This would favour and simplify the implementation of a valorisation strategy, since avoiding or shortening collection and separation processes. Wastes should be then accurately characterized not only in terms of actual homogeneity and composition but also in terms of long-term variability and perishability. This would allow the identification of waste features possibly exploitable in a valorisation strategy (e.g. high fibre or polyphenol content), but also critical ones. For example, a high compositional variability would hinder the standardisation of a valorisation strategy and a high perishability would pose the need for a quick transformation of the waste.

2.2 Output definition

Based on the key properties identified in waste characterisation step, possible final products of waste valorisation can be hypothesized. Beside traditional valorisation outputs (e.g. fertilisers, animal feeding, biogas), in this step, Research and Development expertise should be exploited to hypothesize innovative solutions, leading to the maximal exploitation of waste characteristics. In this regard, it should be underlined that the same waste could offer a wide range of valorisation possibilities. During output definition, thus all the possible valorisation outputs should be identified, with the final aim to reduce to zero the waste. This can be attained by applying a multiple-step

methodology. For example, after extracting bioactive molecules from a vegetable waste, the extraction residue could be further exploited to produce fibre-rich flour, composted or serve as water source for company facilities. Output definition step should result in a clear definition of the possible amounts and compositional features of the waste derivatives, as well as in their classification in terms of possible use (ingredients, products, adjuvants, additives, materials) purpose (increase food functionality, material biodegradability or bio-compatibility) and sector (e.g. food, engineering, bio-medical, packaging).

2.3 Process design

In this step, production processes required to obtain the identified outputs are designed. In this regard, Galanakis (2012) developed the 5-Stages Universal Recovery Process, potentially applicable to every kind of food waste. According to this universal approach, food waste recovery could be accomplished in five distinct stages: macroscopic pre-treatment (adjustment of the water, solids and lipid content, activation or deactivation of enzymes, reduction of the microbial load, increase in the permeability of the matrix); macro- and micro-molecules separation (separation of antioxidants, acids, or ions from biopolymers); extraction (solubilisation of free molecules and dissociation of bound ones); purification (clarification of the target compounds from co-extracted impurities) and product formation (encapsulation or drying to obtain a stable product). This recovery process has the advantage of being potentially applicable to all kinds of food waste materials, since it can be tailored by omitting some stages. In the simplest case, only pre-treatment operations such as selection, cleaning, washing, mincing, partial dehydration can be applied. For example, this applies to traditional valorisation strategies such as animal feeding and composting, but also to innovative valorisation strategies exploiting waste as fresh ingredient. By contrast, when a deeper modification of FVW is required for its valorisation, all steps of the recovery process should be applied and can be accomplished with different conventional or emerging technologies, both of which present specific advantages and pitfalls. Equipment and know-how for the application of traditional technologies are already available and thus they are easy to use and characterized by low or null investment costs. Such technologies include, for example, air-drying and traditional solvent-assisted extraction. However, they are often energy intensive and can damage the treated matrix producing overheating, structural and functionality modifications. In addition, they usually require huge amounts of solvents, which represent an economic and environmental burden for companies. Novel technologies, such as microwaves, ultrasounds, high pressure homogenisation and supercritical drying represent a suitable alternative, since generally reducing thermal effect, matrix damaging and solvent amount while maintaining or increasing process yields. By contrast, since their industrial application is still limited, high investment costs for equipment and dedicated expertise are required for process scaling-up on an industrial level.

2.4 Feasibility study

The final step for the development and implementation of a rational food waste valorisation strategy is the assessment of its feasibility. Based on the criteria indicated in the Waste Framework Directive for the reclassification of FVW into by-products or the application of the "end-of-waste status" (Table 2), the main conditions that should be evaluated to assess the feasibility of a valorisation strategy include:

(i) Specific purpose definition: the final application of valorisation output must be defined.

(ii) Technical validation of the intended use: the valorisation output must be tested for the fulfilment of specific requirements, including its suitably for the proposed final application.

(iii) Evaluation of market demand: the possibility of valorisation output to have a market must be evaluated, by assessing consumer acceptance and response.

(iv) Estimation of the impact of FVW valorisation on food supply chain sustainability: the economic and environmental impact of FVW valorisation must be understood, with reference to industrial scale. This can be achieved by estimating:

- the economic impact of developed strategy: most of food waste valorisation strategies would require high investment cost for scaling up and validation on industrial scale;
- the environmental impact of developed strategy: the implementation of FVW management strategy reduces the amount of waste to be landfilled but is carried out by processes that require energy and resources. Thus, the counterbalancing contribution of these two aspects on the environmental impact should be evaluated;
- the best proportion of FVW to be managed with each strategy: given a specific waste, different strategies can be applied for its valorisation. In this regard, the relevant question is not which the best valorisation option is, but how much waste should be managed in the different ways (Rasmussen et al., 2005). Thus, the best compromise among economic and environmental outcomes of a complex system integrating different valorisation strategies, should be identified.

Feasibility assessment necessarily requires data deriving from the previous steps of the rational approach. In fact, it requires data relevant to waste amount available for valorisation, added-value of the possible final output, and process parameters and yields. Reversely, data collected from the feasibility analysis would not only allow to make aware choices about the implementation of FVW valorisation but would also provide valuable information, potentially applicable to the valorisation of other food waste materials. In this regard, really few and partial information is currently available on different crucial aspects of FVW valorisation, including:

- minimum amount and homogeneity of FVW required for a possible industrial valorisation;
- possible marketing strategies to steer consumer preference towards valorisation outputs;

- scaling-up data, allowing the identification of processing conditions in which valorisation process is profitable from an economic and environmental point of view.

In this way, the proposed approach gets cyclical, since the last step both receives data from the previous steps and provides valuable feedback information (Figure 2).

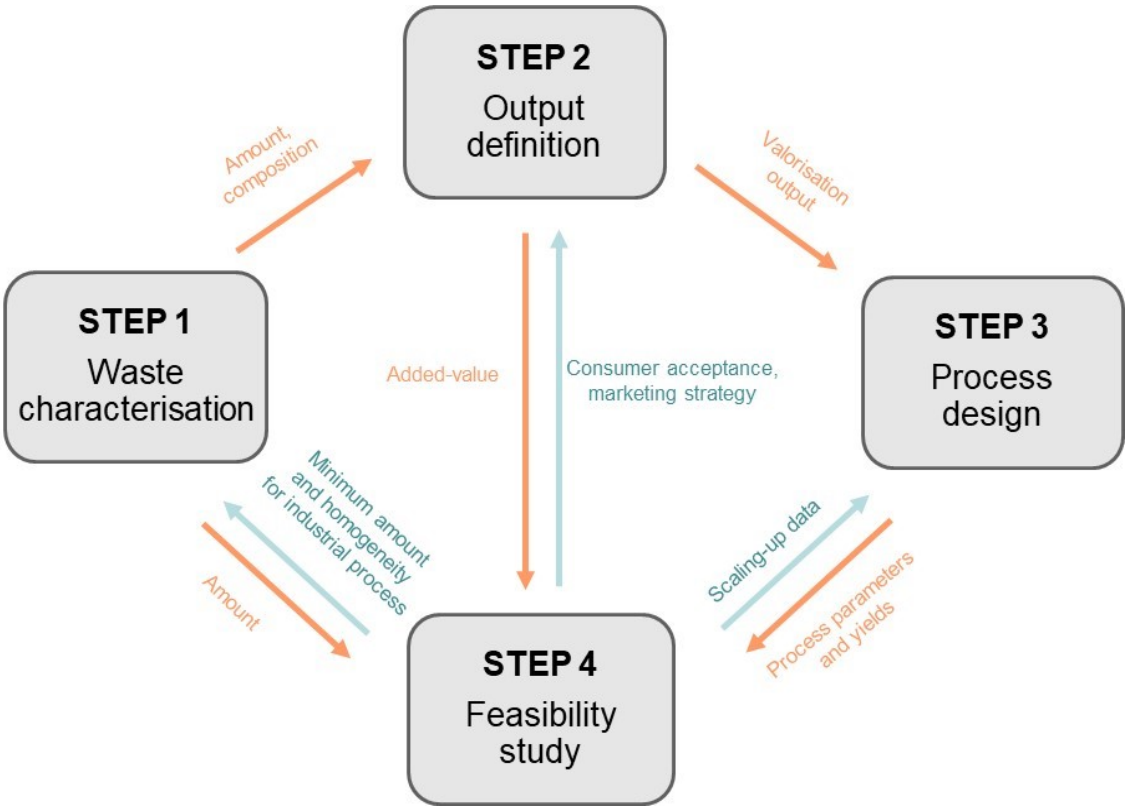


Figure 2. Food waste valorisation approach.

2.5 *Validation of the proposed valorisation approach*

The proposed approach was validated on FVW materials representing a critical issue for companies. In particular, based on company inputs, the following waste materials were selected:

- external leaves generated from fresh-cut processing of *Iceberg* lettuce heads;
- fruit pomace generated from peach juice production;
- okara generated from soy milk and tofu production.

According to the first step of the developed approach, the selected waste materials were characterised in terms of amount and composition (Chapter 3); the second step was the identification of possible valorisation outputs (Chapter 4); following, the design of proper processes was carried out (third step, Chapter 5); finally, the fourth and last step was the feasibility assessment of the proposed valorisation strategies (Chapter 6).

Chapter 3

Step 1 of the valorisation approach: ***Waste characterization***

As indicated in Chapter 2, the first step of the developed rational approach at FVW valorisation is the accurate characterization of the considered food waste material. In this chapter, lettuce waste, peach pomace and okara were characterised in terms of amount and composition. In addition, the current management of such wastes is described, underlining the involved costs for companies.

3.1 Materials and methods

Waste materials

Lettuce waste, peach pomace and okara were provided from companies engaged in the production of fresh-cut lettuce, peach juice and soy milk (Ortoromi s. c. a., Borgoricco, Padova, Italy; Indulleida s. a., Alguaire, Lleida, Spain; Unigrà s.r.l., Conselice, Ravenna, Italy).

Cores, bruised and spoiled parts were removed from lettuce waste and the selected waste (external leaves) was washed with flowing water (18 ± 1 °C) and sanitized 20 min in a chlorinated bath containing 200 mg/L of NaClO with a 100 g/L leaves/water ratio. Leaves were then rinsed with flowing water and centrifuged in a manual kitchen centrifuge (mod. ACX01, Moulinex, France) for 1 min (Manzocco, Ignat, Bartolomeoli, Maifreni, & Nicoli, 2015).

Ground peach pomace was provided either frozen (-18 °C) or dried (140 °C) and was equilibrated at room temperature before use.

Okara was sampled in the company after the soymilk production upon milling and further heating at 80 °C of soybeans. Okara was provided frozen (-18 °C) and equilibrated at room temperature before use.

Quantification

In the case of fresh-cut lettuce, amount data were obtained by analysing lettuce waste generation in a large Italian company. To this aim, a two-step methodology was developed: (i) identification of unit operations in which waste was generated and (ii) waste quantification. Total wasted lettuce (W) was calculated as the sum of wastes generated during preliminary cleaning (W_C), three washing stages (W_{W1}, W_{W2}, W_{W3}) and optical selection (W_{OS}) (eq. 1) (Figure 3). However, direct waste weighting was possible only for wastes generated during washing stages and from the optical selector but not for the preliminary cleaning stage, in which external leaves and core were eliminated. In fact, *Iceberg* lettuce wastes were mixed with those of other vegetables as different raw materials were usually processed in the same day. To quantify waste produced during each production step, an indirect calculation was thus used. In particular, total lettuce waste (W) was

also computed as the difference between the amount of total lettuce accepted after the quality check (L) and the sold one (L_S) (eq. 2).

$$W = W_C + W_{W1} + W_{W2} + W_{W3} + W_{WOS} \quad \text{(eq. 1)}$$

$$W = L - L_S \quad \text{(eq. 2)}$$

W_C was thus calculated by solving the system of eq. 1 and eq. 2.

In the case of okara and peach pomace, waste amount data were obtained based on literature and official databases (Argun & Dao, 2017; Mateos-Aparicio, Redondo-Cuenca, Villanueva-Suárez, Zapata-Revilla, & Tenorio-Sanz, 2010; USDA, 2018).

Composition analysis

Moisture and total dietary fibre content

Moisture content was calculated according to AOAC methods (AOAC, 1997). In the case of lettuce waste and okara total dietary fibre (TDF) was calculated using a total dietary fibre assay kit (TDF-100A, Sigma-Aldrich, St. Louis, Missouri, USA) (AOAC, 1997). In the case of peach pomace, compositional data were obtained from previous studies conducted on this material (Grigelmo-Miguel, Gorinstein, & Martín-Belloso, 1999).

Total polyphenolic content

An amount of 10 g of lettuce waste, peach pomace and okara trimmed with a sharp knife were extracted by reflux with boiling water for 60 min applying a dilution of 1:4 (w/v). Extracts were cooled to room temperature, vacuum filtered thorough Whatman no. 1 filter paper (Maidstone, UK), freeze-dried at -50 °C and stored in a desiccator containing P_2O_5 at room temperature until use. Total polyphenolic content (TPC) was determined using Folin-Ciocalteau reagent (Singleton & Rossi, 1985). The reaction mixture contained 100 µL of waste extract solubilised in water (1:10 w/v), 500 µL of the Folin-Ciocalteau reagent, 4 mL of water and 2 mL of a sodium carbonate aqueous solution (0.15 g/mL). After 2 h reaction at ambient temperature, mixture absorbance was read at 750 nm using UV-Vis spectrophotometer (Shimadzu UV-2501PC, UV-Vis recording spectrophotometer, Shimadzu Corporation, Kyoto, Japan). A calibration curve was made with standard solutions of gallic acid in the range 0.1–1000 mg/L (R^2=0.99). Results were expressed as mg of gallic acid equivalents per g of dry weight.

Current use and cost evaluation

A company survey was performed in order to identify strategies most commonly applied for the management of lettuce, peach and soy wastes. In addition, the mean disposal cost, due to waste transport and disposal, was estimated.

3.2 Results and discussion

For implementing a valorisation strategy, the availability of substrates presenting a homogeneous composition and a segregated localization is crucial. For these reasons, FVW intended for valorisation is commonly collected in the industrial processing steps. The attention was thus focused on industrial discards obtained during processing of fresh-cut lettuce, peach juice and soy milk. In this regard, lettuce waste consisted of outer leaves and cores, while peach pomace and okara appeared as homogeneous matrices. This is due to the specific waste generation process, which is described in Figure 3. In the case of lettuce, after manual removal of external leaves and cores, the latter are simply collected and disposed of. By contrast, peach mincing and soy bean grinding account for the higher homogeneity of peach pomace and okara, respectively.

Quantification

Lettuce is the most important fresh-cut vegetable, representing 50% of the entire fresh-cut market in Europe and US (Cook, 2015; Rabobank International, 2010), up to 70% in Italy (Casati & Baldi, 2012).

Raw material for the production of fresh-cut lettuce can be divided in two main categories: whole-head lettuce (e.g. *Iceberg* lettuce), representing, in Italy, 60% of the total fresh-cut lettuce market and baby lettuce (e.g. rocket lettuce), that accounts for the remaining 40% (Casati & Baldi, 2012). While in baby lettuce processing the whole leaf is harvested and processed, in the case of whole-head lettuce, the percentage of usable product is significantly lower due to preliminary removal of external leaves and core (Martínez-Sánchez et al., 2012) (Figure 3). The typical flow sheet of fresh-cut *Iceberg* lettuce production is reported in Figure 3. The quantification method described in § 3.1 was applied to the production of fresh-cut *Iceberg* lettuce in a large Italian company during 3 production months, in which approximately 800 kg of *Iceberg* lettuce were daily processed. Data indicate that up to 41% of lettuce was wasted during a typical fresh-cut *Iceberg* lettuce process, with removal of external leaves and core stage accounting for nearly the total waste production. Waste production in the following unit operations of washing and optical selection resulted, in fact, negligible. As already reported by Llorach, Tomás-Barberán and Ferreres (2004), external leaves and core differently contributed to total waste, with the former representing more than 78% (w/w) of the overall waste. Based on these results and in order to reduce sample variability, only external leaves were taken into considerations in the present PhD Thesis (§ 2.5).

More than 15 million metric tons of peaches are annually processed into juices worldwide (FAOSTAT, 2010). Peach juice industry produces a huge amount of waste, mainly represented by skin, seeds and some pieces of fruit. As described in Figure 3, upon filtration and centrifugation of minced peach pulp, peach juice is separated from the remaining solid residue, commonly called peach pomace. Depending on the ripeness of the peaches, approximately a 10-15% peach pomace is discarded during processing (Argun & Dao, 2017).

Okara is a Japanese term defining the waste obtained after milling and extraction of the aqueous fraction of soybeans (Redondo-Cuenca, Villanueva-Suárez, & Mateos-Aparicio, 2008). Looking at the soymilk production process (Figure 3), okara is generated upon filtration of ground and soaked soybeans. Due to soybean soaking in water, every kilogram of processed soybeans intended for the production of soy milk and tofu generates about 1.1-1.2 kg of wet okara, leading to about 4 million tons of waste generated every year (O'Toole, 1999; Radočaj et al., 2013).

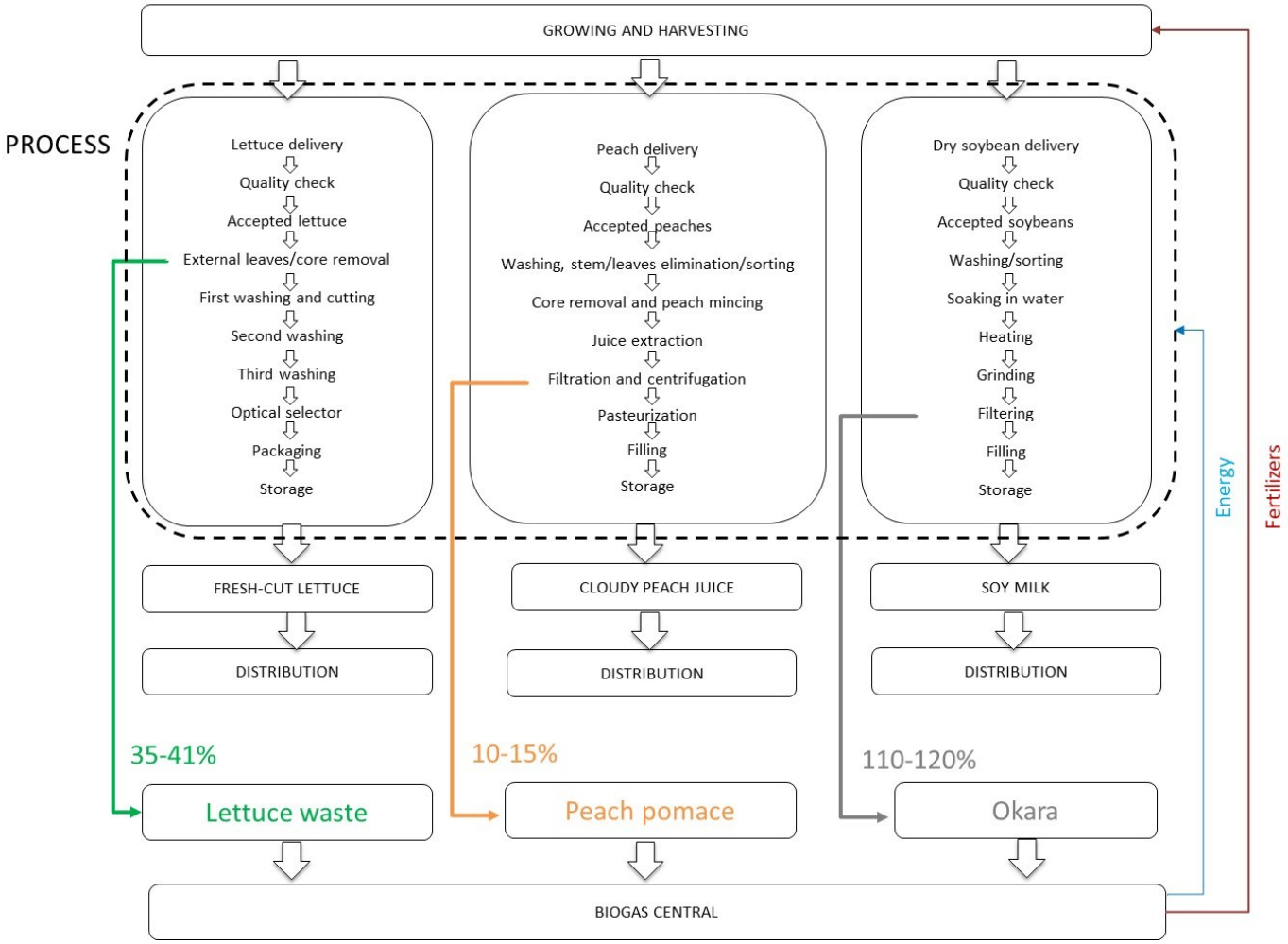


Figure 3. Fresh-cut *Iceberg* lettuce waste, peach pomace and okara flow in a typical company. Operations responsible for the generation of the higher amount of waste are identified. Waste amounts are expressed as percentage ratio to total vegetable accepted after the quality check.

Composition analysis

Composition analysis of lettuce waste, peach pomace and okara was carried out in order to identify exploitable characteristics and support the choice of proper valorisation options (Laufenberg et al., 2003). In this characterisation phase, attention was focused on the determination of water content of waste materials and on the concentration of fibres and polyphenols, as typical healthy compounds found in vegetable products (Table 4).

Table 4. Main characteristics of waste generated from fresh-cut lettuce, peach juice and soymilk production.

Product	**Waste**	**Amount (g/100 g of raw processed vegetable)**	**Composition**		
			Humidity (g/100 g fresh waste weight)	Fibre (g/100 g fresh waste weight)	Polyphenols (mg GAE/100 g dry waste weight)
Fresh-cut *Iceberg* lettuce	External leaves	27.4 ± 2.3	94.5 ± 0.6	1.5 ± 0.1	144 ± 11
Peach juice	Peach pomace	10.5 ± 0.6	84.5 ± 0.3	5.0 ± 0.6	229 ± 10
Soymilk and soy curd	Okara	115 ± 7	76.2 ± 0.4	12.5 ± 0.1	192 ± 4

Although the intrinsic variability due to vegetable variety and cultural practices, obtained compositional data resulted in the ranges reported in the literature relevant to lettuce waste (Llorach, Martínez-Sánchez, Tomás-Barberán, Gil, & Ferreres, 2008; USDA, 2018), peach pomace (Adil, Çetin, Yener, & Bayindirli, 2007; Maurya, Pandey, Dipti, Paras, & Rai, 2017; Pagán, Ibarz, Llorca, Pagán, & Barbosa-Cánovas, 2001; Redondo, Arias, Oria, & Venturini, 2017; Wadhwa, 2015) and okara (Alaiz et al., 2010; Vong & Liu, 2016). As expected, water was found to represent more than 94, 84 and 76% of *Iceberg* lettuce waste, peach pomace and okara fresh weight. In the remaining weight fraction, a significant amount was represented by fibres (Table 4). An interesting polyphenol concentration was also found in all waste materials. Based on these characteristics, lettuce waste, peach pomace and okara can be considered valuable sources of nutrients and functional ingredients.

Current use and cost evaluation

Based on information collected from the companies furnishing lettuce waste, peach pomace and okara, these wastes are currently mainly exploited to produce biogas in centralized plants through anaerobic digestion (Figure 3). Although this strategy produces energy and fertilizing digestate, it is not able to properly valorise the interesting composition of these vegetable wastes (Table 4). In addition, it also implies high costs for its implementation. In fact, in a typical Italian company that delivers FVW at a centralized biogas plant located within a 20-km distance, management of 1 ton

of waste is associated to an average cost of 0.80 € and 60 € for transport and disposal, respectively. Based on the amount of waste generated by *Iceberg* lettuce fresh-cut processing, peach juice extraction and soy milk production (Table 4), it can be estimated a medium waste management cost of about 22.00, 6.00 and 67.00 € per ton of processed raw material. This cost is expected to increase with the distance between production site and disposal centre. In addition, it must be underlined that such waste management often represents a net cost for companies, due to the risible or null return in terms of biogas or digestate to be used as fertilizer upon anaerobic digestion of waste.

Chapter 4
Step 2 of the valorisation approach: ***Output definition***

As discussed in Chapter 3, the management of waste deriving from fresh-cut lettuce, peach juice and soymilk production represents not only a cost for companies but, based on their rich composition, also a loss of valuable biomass (Table 4). The second step of the proposed FVW valorisation approach (Chapter 2), is the definition of waste valorisation outputs able to exploit this rich composition. In this Chapter, some of the most recent studies aiming at valorising lettuce waste, peach pomace and okara to produce value-added derivatives are reviewed. This information was used to define possible valorisation outputs.

4.1 Literature review

Table 5 reports recent studies about the valorisation of lettuce waste, peach pomace and okara.

Lettuce waste

Really few studies are available on lettuce waste valorisation options other than its use for anaerobic co-digestion (Lin et al., 2011). Nevertheless, different studies conducted on FVW other than lettuce and on edible lettuce (not waste) highlight several alternative outputs that can be obtained from lettuce waste. In particular, lettuce has been used as an ingredient for the formulation of mixed fruit and vegetable fresh juices (Pop, Muste, Mureșan, & Jula, 2014). Alternatively, lettuce has been exploited for the extraction of bitter and gelling compounds, as well as polyphenols for the production of functional ingredients (Llorach et al., 2004; Mai & Glomb, 2013; Roversi, Ferrante, & Piazza, 2016). Finally, lettuce waste drying has been implemented to produce functional flour or natural adsorbents (Ferreira et al., 2015; Pavlović et al., 2015).

Peach pomace

Based on its composition, peach waste offers different possibilities for valorisation. Most of literature studies relevant to peach pomace valorisation deal with the extraction of bioactive compounds from this waste material, including carotenoids, polyphenols and structuring agents (Adil et al., 2007; Pagán et al., 2001; Vargas, Jablonski, Flôres, & Rios, 2017) (Table 5). Peach pomace has been also integrally used as fresh ingredient in the production of extruded snacks with enhanced fibre content (Sarkar & Choudhury, 2014). However, as reported for other FVW, the main issue related to fresh peach waste management is its high humidity content, making it quickly prone to microbial spoilage (Ajila et al., 2012). For this reason, peach waste drying has been extensively applied to produce fibre concentrates and flour, which have been exploited in the formulation of

different goods with increased nutritional value (Grigelmo-Miguel, Carreras-Boladeras, & Martín-Belloso, 2001; Grigelmo-Miguel et al., 1999; Yangilar, 2016) (Table 5).

Okara

Besides being commonly used to produce traditional food in East Countries with huge soy consumption, okara has been exploited as fat replacer and fibre supplement in different bakery products, including snacks, cookies and breakfast cereals (Osho, 2003; Palermo, Fiore, & Fogliano, 2012; Park, Choi, & Kim, 2015; Radočaj et al., 2013; Santos, Dedani, & Rossi, 2004). Moreover, fibres, gelling agents, antioxidant molecules, proteins, and lipids extracted from okara have been tested on the laboratory scale as ingredients of foods, cosmetic and pharmaceutical products (Fung, Yuen, & Liong, 2010; Li, Lu, Nan, & Liu, 2012; Ma, Liu, Kwok, & Kwok, 1996; Mateos-Aparicio, Mateos-Peinado, Jiménez-Escrig, & Rupérez, 2010; Quitain, Oro, Katoh, & Moriyoshi, 2006). Finally, dried okara flour has been exploited for the production of functional foods, enriched in fibres and proteins (Grizotto, Andrade, Miyagusku, & Yamada, 2012; Grizotto, Rufi, Yamada, & Vicente, 2010; Katayama, Wilson, & Quality, 2008; Ostermann-Porcel, Rinaldoni, Rodriguez-Furlán, & Campderrós, 2016) (Table 5).

4.2 Conclusions

Although the huge number of strategies proposed in the literature for valorisation of lettuce waste, peach pomace and okara, they can be classified in three main categories, based on the obtained outputs:

- fresh food ingredient;
- antioxidant extract;
- dried material or flour.

All these outputs present advantages and pitfalls, which should be carefully evaluated. The direct use of waste materials in food formulation seems the easiest way to valorise their rich composition. However, the high humidity content would require a quick transformation of vegetable waste, limiting this application to local foods and processing plants. Alternately, waste should be stored in refrigerated or freezing conditions, which would imply huge storage volumes, energy consumption and logistical costs. The extraction of bioactive and functional ingredients would lead to high value-added derivatives. However, upon extraction, a huge amount of waste should be still disposed-off. Finally, drying would allow to turn waste into microbiologically stable derivatives, presenting a lower storage volume but would also require high energy consumption, due to the high water amount that must be removed from vegetable matrix (Table 4).

Starting from these available data, it was decided to turn selected waste materials into juices, antioxidant extracts, and dried materials/flour. To this aim, proper production processes were designed (Chapter 5).

Table 5. Valorisation outputs obtained in recent literature studies from lettuce waste, peach pomace, and okara.

Valorisation output	Function	Proposed use	References
Lettuce waste			
Fresh ingredient	Juice	Blended juices	Pop et al. (2014)
Functional extract			
Sesquiterpene lactones	Bitter compounds	Bitter foods and beverages	Mai & Glomb (2013)
Cell wall materials (pectins)	Structuring and gelling agent	Functional foods	Roversi et al. (2016)
Polyphenols	Antioxidant	Functional foods	Llorach et al. (2004)
Dried material or flour			
Vegetable flour	Nutritional value improvement	Cookies and cereal bars	Ferreira et al. (2015)
Dried material	Green adsorbents for dyes	Wastewater treatment	Pavlović et al. (2015)
Peach pomace			
Fresh ingredient	Fibre-content increase	Extruded foods	Sarkar & Choudhury (2014)
Functional extract			
Carotenoids	Antioxidant	Functional foods	Vargas et al. (2017)
Polyphenols	Antioxidant	Functional foods	Adil et al. (2007)
Cell wall materials (pectins)	Structuring and gelling agent	Functional foods	Pagán et al. (2001)
Fibre	Fat replacement	Muffins, ice cream	Grigelmo-Miguel et al. (2001); Yangilar (2016)
Dried material or flour			
Vegetable flour	Nutritional value improvement	Fibre-enriched foods	Grigelmo-Miguel et al. (1999)
Okara			
Fresh ingredient	Fat replacement	Coconut-based snack	Radočaj et al. (2013)
	Nutritional value improvement	Cookies	Park et al. (2015)
		Cassava-based product (Gari)	Osho (2003)
		Extruded wheat breakfast cereals	Santos et al. (2004)
Functional extract			
Fibre	Structure and nutritional value improvement	Cosmetic and food formulations	Fung et al. (2010)
Cell wall materials (pectin)	Structuring and gelling agent	Functional foods	Li et al. (2012)
Carbohydrates	Antioxidant	Functional foods	Mateos-Aparicio, Mateos-Peinado, et al. (2010)
Proteins	Ingredient	Functional foods	Ma et al. (1996)
Lipids	Ingredient	Cosmetic and food formulations	Quitain et al. (2006)
Dried material or flour			
Vegetable flour	Meat protein replacement	Frankfurter type sausage	Grizotto et al. (2012)
	Nutritional value improvement	Soy-based snack food	Katayama & Wilson (2008)
		Moulded biscuit	Grizotto et al. (2010)
	Gluten replacement	Cookies	Ostermann-Porcel et al. (2016)

Chapter 5

Step 3 of the valorisation approach: ***Process design***

Given the definition of the main outputs of the waste valorisation strategies (Chapter 4), relevant production process should be designed. In this regard, it must be underlined that FVW valorisation cannot be contemplated without the hand-in-hand development of sustainable processes. In fact, as previously discussed (Table 2), the reclassification of FVW into an useable raw-material or product needs the fulfilment of environmental requirements and must not be related to adverse impacts on sustainability. In this regard, based on the definition reported by Chemat, Rombaut, Meullemiestre, et al. (2017), Green Food Processing "*is based on the discovery and design of technical processes which allow a reduction of energy and water consumption as well as recycle of waste while guaranteeing safety and quality of the final product*". A central objective in valorisation process design, thus, is the development of technical solutions that are economically competitive to traditional processes but also allow optimal use of raw materials, energy and solvents (Chemat, Fabiano-Tixier, Abert, Allaf, & Vorobiev, 2015). This can be attained by both the improvement and optimization of existing processes and their innovation. In this regard, great attention has been lately dedicated to the development of green processes based on the application of innovative techniques. Pressure-based technologies such as high pressure homogenization and high-pressure carbon dioxide have been proposed for fresh juice production and could thus be exploitable to produce novel functional beverages containing vegetable waste (Koutchma, Popović, Ros-Polski, & Popielarz, 2016). Innovative extraction technologies, such as ultrasounds and microwaves could be applied to vegetable waste for the extraction of bioactive compounds at mild temperature conditions, to maximally preserve ingredient properties (Chemat et al., 2015). Similarly, innovative drying technologies such as supercritical fluid drying could offer the possibility of drying vegetable wastes at low temperature, while maintaining tissue structure (Brown, Fryer, Norton, Bakalis, & Bridson, 2008).

Based on these considerations, the present chapter presents the design of processes aiming at obtaining the outputs identified in Chapter 4. In particular, § 5.1 and 5.2 present the valorisation of lettuce waste and okara as fresh ingredients for the production of innovative fresh homogenates, by exploiting high pressure homogenisation; § 5.3 and 5.4 show the application of innovative extraction techniques based on ultrasounds and microwaves on lettuce waste and peach pomace; finally, § 5.5 and 5.6 report the application of either traditional and innovative drying technologies for the production of flour and dried materials from lettuce waste.

5.1 Valorisation of lettuce waste into an ingredient for juice production by means of high-pressure homogenization

The objective of this work was to evaluate if high pressure homogenisation (HPH), combined with blanching, could be used to turn lettuce waste into a value-added ingredient, possibly exploitable in the formulation of blended juices, smoothies and comminuted food. Hereto, the work was divided in three parts. In the first one, lettuce waste was ground, pre-homogenized at 40 MPa and subsequently submitted to HPH treatments at 80 (1 pass) and 150 MPa (1 and 10 passes). In the second part, lettuce waste was submitted to the same grinding, pre-homogenization and homogenization treatments after blanching. Finally, in the last part of the work, blanched lettuce juice was stored up to 15 days in refrigerated conditions. In order to understand the effect of the different processing operations on sample characteristics, lettuce waste samples were analysed for colour, microscopic structure, phase separation, viscosity, phenolic content, polyphenoloxidase and pectin methylesterase activity, and microbial load (total bacterial count, yeasts, lactic acid bacteria) both immediately after the treatments and during refrigerated storage.

5.1.1 Introduction

Increasing consumer demand for low-caloric foods with fresh-like characteristics and high nutritional quality has encouraged the research of alternative vegetable products. In this context, ready to drink juices, smoothies and enriched beverages are experiencing an increasing market demand due to their fresh-likelihood, health benefits, convenience and clean-label (Yi et al., 2018). Among these products, blends of freshly extracted fruit and vegetable juices offer the possibility to develop new products, which present innovative flavours and improved nutritional quality, due to the high concentration of bioactive compounds and low caloric content (De Carvalho, Maia, De Figueiredo, De Brito, & Rordrigues, 2007). Vegetables used to produce commercial blended juices include tomato, carrot but also leaf-vegetables such as spinach, celery, kale and parsley (Hao, Zhou, Koutchma, Wu, & Warriner, 2016).

Being the stability of fresh blended juices limited by microbial, enzymatic and physical alterations, different technologies have been investigated to guarantee an adequate shelf-life. In particular, the application of high hydrostatic pressure allows juice stabilization from a microbial and enzymatic point of view (Deliza, Rosenthal, Abadio, Silva, & Castillo, 2005). However, this technology cannot provide an adequate physical stability of the juices, which undergo rapid phase separation during storage (Laboissière et al., 2007). Although the addition of hydrocolloids (e.g. pectin, carboxymethyl cellulose, and sodium alginate) have been repeatedly suggested to control juice sedimentation (Ibrahim et al., 2011), this strategy hardly fits with consumer expectations for clean label products, leading to the need for alternative solutions. In this regard, high pressure homogenisation (HPH) has been demonstrated to represent a valid alternative to cloudiness

preservatives, due to its ability of modifying the structure-forming properties of plant fibre suspensions in the juice. During HPH, a fluid is forced to pass through a narrow gap, leading to rapid acceleration followed by sudden pressure drop. In this way, the fluid undergoes simultaneous energetic phenomena including elongational stresses, cavitation and turbulent flow. This leads to reduction of suspended particle size, lowering sedimentation rate, but also to a modification in the fibre physicochemical properties such as water holding, swelling and structuring capacity (Van Buggenhout et al., 2015). HPH have been shown to be particularly effective in physical stabilization of different beverages, including tomato and banana juices (Calligaris, Foschia, Bartolomeoli, Maifreni, & Manzocco, 2012; Colle, Van Buggenhout, Van Loey, & Hendrickx, 2010). Pressures between 50 and 150 MPa are generally applied for juice HPH treatment and the juice can also be recirculated in the homogeniser to increase treatment intensity without necessarily increase treatment pressure (Karacam, Sahin, & Oztop, 2015; Yi et al., 2018).

Although the recognised efficacy of HPH in reducing particle size of vegetable suspensions, tissue disruption is often responsible for a rapid juice colour depletion, due to the activity of oxidative enzymes on phenols and natural-occurring pigments (Liu, Liu, Liu, et al., 2009). The inactivation of these enzymes by the application of blanching prior to HPH can allow obtaining a colour-stable product. In this case, the vegetable is submitted to a "heat-shock" treatment, during which a heat treatment is rapidly followed by a quick cooling of the product. Plant tissue enzymes are thus inactivated, leading to a reduced colour change upon further process and storage (Devece et al., 1999).

Lettuce waste has been shown to present a high content in dietary fibres and polyphenols (Table 4) and can be supplied continuously and in large quantity by the fresh-cut industry. For these reasons, lettuce waste could be considered an interesting raw material for producing healthy blended juice.

5.1.2 Materials and methods

Lettuce waste preparation

Lettuce waste was then prepared as described in § 3.1.

Blanching

Lettuce waste was immersed in water (100 g/L lettuce/water ratio) at 90 °C for 30 s and then immediately placed 1 min into an ice bath (100 g/L lettuce/water ratio). After that, lettuce waste was accurately dried using absorbing paper and stored at 20 °C for 10 min before treatment.

Grinding

Lettuce waste was ground using a domestic grinder (MC3001, Moulinex, Milan, Italy) at ambient temperature for 5 min.

High pressure homogenisation (HPH)

A continuous lab-scale high-pressure homogeniser (Panda Plus 2000, GEA Niro Soavi, Parma, Italy) supplied with two Re+type tungsten carbide homogenisation valves with a flow rate of 10 L/h was used. A scheme of the used plant is shown in Figure 12B. Ground lettuce waste (150 g) was pre-homogenised at 40 MPa to reduce valve obstruction risk. High pressure homogenisation treatments were then conducted at 80 and 150 MPa. Moreover, at 150 MPa, 10 subsequent cycles were performed. The different combinations of treatments performed on lettuce waste and the identification of sample names are reported in Table 6.

Table 6. Treatments performed on lettuce waste and obtained samples.

Sample		**Pre-treatment**				**Treatment**	
		Blanching	Grinding	Pressure (MPa)	Passes	Pressure (MPa)	Passes
Not-blanched	Control	no	no	/	/	/	/
	Ground	no	yes	/	/	/	/
	Pre-homogenised	no	yes	40	1	/	1
	HPH 80	no	yes	40	1	80	1
	HPH 150	no	yes	40	1	150	1
	HPH 150x10	no	yes	40	1	150	10
Blanched	Control	yes	no	/	/	/	/
	Ground	yes	yes	/	/	/	/
	Pre-homogenised	yes	yes	40	1	/	1
	HPH 80	yes	yes	40	1	80	1
	HPH 150	yes	yes	40	1	150	1
	HPH 150x10	yes	yes	40	1	150	10

Analytical determinations

Colour

Colour was determined using a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-300 measuring head. The instrument was standardized against a white tile. Colour was expressed in L*, a* and b* CIELAB scale parameters.

Image acquisition

Images were acquired using an image acquisition cabinet (Immagini & Computer, Bareggio, Italy) equipped with a digital camera (EOS 550D, Canon, Milan, Italy) placed on an adjustable stand, positioned 45 cm above a black cardboard base where samples were placed. Light was provided by 4 23 W frosted photographic floodlights, in a position allowing minimum shadow and glare. Images were saved in jpeg format resulting in 3456 x 2304 pixels.

Optical microscopy

Samples were observed at room temperature using a Leica DM 2000 optical microscope (Leica Microsystems, Heerbrugg, Switzerland). The images were taken at 200× magnification using a Leica EC3 digital camera and elaborated with the Leica Suite Las EZ software (Leica Microsystems, Heerbrugg, Switzerland).

Phase separation

Samples were poured in 50 mL-graduated cylinders for 24 h at 4 °C. Phase separation was visually assessed and expressed as % (mL of separated phase per 100 mL of sample).

Viscosity

Rheological analyses were performed using a RS6000 Rheometer (Thermo Scientific RheoStress, Haake, Germany), equipped with a Peltier system for temperature control. Measures were performed using a bob-cup geometry at 20 °C. Flow curves were recorded increasing shear rate from 0.1 to 100 s^{-1}.

Supernatant preparation

Samples were poured in 1.5 mL Eppendorf tubes and centrifuged (Hittich MIKRO 20, Centrifuge, Tuttlingen, Germany) at 13,000 rpm for 15 min.

Polyphenoloxidase activity

The polyphenoloxidase (PPO) activity was assayed spectrophotometrically (Shimadzu UV-2501PC, UV-Vis recording spectrophotometer, Shimadzu Corporation, Kyoto, Japan) at 25 °C according to the methodology of Kahn (1985). The reaction was started by the addition of 200 µL of supernatant to 1.8 mL of 0.1 mol/L potassium phosphate buffer pH 7 and 1.5×10^{-3} mol/L L-Dopa (Carlo Erba, Milan, Italy). The absorbance at 420 nm was monitored each 10 s for 10 min. The changes in absorbance per min were calculated by linear regression, applying the pseudo zero order kinetic model. The eventual final stationary phase was excluded from regression data. The slope of the very first linear part of the reaction curve was used to determine PPO activity (k_{PPO}). PPO activity was expressed as the % activity as compared to that of the Ground sample not submitted to HPH treatments (Table 6). The latter presented a PPO activity of 0.0453 ± 0.0040 ΔAbs/min.

Pectin methylesterase activity

Pectin methylesterase (PME) activity was measured using the method described by Martin-Diana et al. (2005) with some modifications. Briefly, the initial pH of 10 g of sample (3.1 ± 0.3) was adjusted at 7.5 using NaOH 1 M (Carlo Erba, Milan, Italy). After that, 0.2 mL of NaOH 0.05 M were added, and the time required by each sample to reach again a pH value of 7.5 was identified. To this aim, sample pH was continuously monitored using a pHmeter (pH-Meter BASIC 20, Crison,

Barcelona, Spain) equipped with a measuring head for liquids (52 02, Crison, Barcelona, Spain). The changes in pH per min were calculated by linear regression, applying the pseudo zero order kinetic model. The eventual final stationary phase was excluded from regression data. The slope of the very first linear part of the reaction curve was used to determine PME activity (k_{PME}). PME activity was expressed as the % activity as compared to that of the Ground sample not submitted to HPH treatments (Table 6). The latter presented a PME activity of -0.0372 ± 0.0002 ΔpH/min.

Total polyphenolic content

Total polyphenolic content (TPC) was determined using Folin-Ciocalteau reagent as described in § 3.1 (Singleton & Rossi, 1985). The reaction mixture contained 50 µL of supernatant, 2 mL distilled water and 250 µL of the Folin-Ciocalteau reagent.

Microbial analyses

For microbiological analyses, 25 g of Control sample was diluted with 100 mL Maximum Recovery Diluent (Oxoid, Basingstoke, UK) and homogenised for 1 min in a Stomacher (PBI International, Milan, Italy). By contrast, Ground, Pre-homogenized, HPH 80, HPH 150 and HPH 150x10 samples (Table 6) were directly used. Serial dilutions of each suspension were made in Maximum Recovery Diluent (Oxoid) and analysed for microbial counts. Appropriate aliquots (0.1 or 1 g) were spread on agar plates. Plate Count Agar (Oxoid) and Man Ragosa Sharpe (MRS) were used for enumeration of total bacterial count and lactic acid bacteria respectively, and plates were incubated for 48 h at 30 °C. Oxytracycline-Glucose- Yeast Extract (OGY) agar (Oxoid), was used for enumeration of yeasts, and plates were incubated for 72 h at 28 °C.

Sample storage

Aliquots of 50 mL of sample were introduced in sterile falcon tubes and stored for up to 15 days at 4 °C in a refrigerated cell. At increasing time during storage, samples were removed from the refrigerator, equilibrated at 22 °C and submitted to the analyses.

Data analysis

Analyses were carried out at least three times in two replicated experiments. Analysis of variance ($p<0.05$) and linear regression analysis were performed using R (The R foundation for statistical computing, v.3.1.1). Bartlett's test was used to check the homogeneity of variance, one-way ANOVA was carried out and Tukey test was used to determine statistically significant differences among means ($p<0.05$).

5.1.3 Results and discussion

Effect of high pressure homogenisation without blanching pre-treatment

In the first part of the study, the effect of HPH treatments on lettuce waste was investigated. Hereto, lettuce waste was subjected to grinding, equilibrated at room temperature, and pre-homogenised at 40 MPa, to avoid valve blockage. The obtained lettuce dispersion was then homogenised at 80 and 150 MPa, the latter treatment being applied for 1 or 10 cycles. Grinding and pre-homogenisation increased sample temperature by 2 and 5 °C, respectively. By contrast, further HPH application resulted in a progressive temperature increase, so that samples reached 38, 65 and 85 °C after treatments at 80, 150 and 150 MPa applied for 10 passes, respectively. Ground lettuce waste showed a non-homogenous appearance due to the presence of particle aggregates with different size. The visual homogeneity of samples progressively increased with the HPH treatment intensity, as also confirmed by microscopic images (Table 7). Tissue cellular organization was well-evident in both Control and Ground samples. The 40 MPa-pre-homogenised sample (Pre-homogenised) still presented several intact cells, although the broken cell material was the most abundant. No intact cells were observed in samples submitted to higher intensity treatments (HPH 80, HPH 150, HPH 150x10), in which the broken cell material appeared uniformly distributed. HPH treatment is well-known to promote vegetable tissue disruption, due to the highly energetic phenomena taking place during the pass through the homogenising valve, including shear and elongational stress, cavitation and turbulence. In this regard, similar disruptive effects have been reported upon HPH treatment of tomato and algae (Bot et al., 2017; Samarasinghe, Fernando, Lacey, & Faulkner, 2012). Grinding promoted a visible change in lettuce colour, as confirmed by the sensible decrease in luminosity (L*) and yellow point (b*), and the concomitant increase of red point (a*) (Table 7). These results suggest a significant loss of the original lettuce green colour, in favour of a brownish one. The application of 40 MPa pre-homogenisation and HPH treatments up to 150 MPa, inverted this tendency, leading to a bright green colour, as suggested by the increase in L* and the decrease of a*. By contrast, upon 10 passes at 150 MPa, samples tended again to become more brownish (Table 7). Such results can be attributed to the effect of different phenomena taking place simultaneously. The significant browning induced by grinding can be attributed to the decompartmentalization of oxidative enzymes and their phenolic substrates upon tissue disruption, leading to polymerized brown derivatives (Espín, Jolivet, & Wichers, 1998). Although tissue disruption was further promoted by HPH, as well-evidenced by microscopic images (Table 7), a greener colour was obtained upon HPH treatments up to 150 MPa. This result can be attributed to the higher surface area developed by small particles produced by HPH, leading to an increased light scattering and thus in sample luminosity (L*) (Ahmed, Shivhare, & Raghavan, 2000). In addition, HPH has been reported to effectively release chlorophyll from intracellular spaces (Carullo et al., 2018), possibly accounting for the greener colour (lower a* value) of samples subjected to one HPH pass up to 150

MPa as compared to Ground sample. The further loss of green upon 10 passes at 150 MPa can be possibly attributed to the pronounced thermal effect of this treatment, promoting the degradation of both chlorophyll and polyphenols (Espín et al., 1998; Koca, Karadeniz, & Burdurlu, 2007). To further study the role of oxidative phenomena upon HPH treatment of lettuce waste, total phenolic content (TPC) and polyphenoloxidase (PPO) activity were evaluated (Figure 4). Despite the inherent vegetable variability and the application of different extraction parameters and quantification methods, the TPC value of the Ground sample (about 14 mg GAE/100 g of lettuce waste) resulted in the range reported in the literature for green-leaf lettuce. In this regard, a TPC of 18.2 mg and 14 mg/100 g fresh weight were obtained by Llorach et al. (2008) and (2004), respectively. A TPC value similar to that observed in the Ground sample was also found upon the 40 MPa-pre-homogenisation treatment of lettuce waste. By contrast, a further increase in HPH intensity up to 150 MPa led to a progressive reduction in the TPC, while the application of 10 passes at 150 MPa did not promote a further phenol loss (Figure 4A). This can be attributed to both the thermal effect of HPH treatment, leading to phenol degradation and the activation of PPO (Figure 4B). It can be inferred that, upon cell disruption induced by HPH (Table 7), PPO, which has been reported to be highly active in *Iceberg* lettuce, was no longer separated from its phenolic substrates, which were thus easily oxidised (Mai & Glomb, 2013). A 60, 90 and 40% PPO activity increase was actually observed in Pre-homogenised, HPH 80 and HPH 150 samples, respectively. The HPH-induced PPO activation can be due to multiple effects of the treatment. Firstly, lettuce cell disruption has been reported to promote the release of proteases, responsible for the activation of latent PPO which, differently from the free soluble one, is bounded to the cellular membrane (Cantos, Espín, & Tomás-Barberán, 2001). In addition, HPH processing is well-known to affect PPO conformation and activity. In this regard, a progressive PPO activation was also observed in Chinese pear and mushroom submitted to high pressure microfluidisation at pressures in the range from 80 to 200 MPa (Liu, Liu, Liu, et al., 2009; Liu, Liu, Xie, et al., 2009). Figure 4B also shows that only the application of 10 passes at 150 MPa led to an almost complete PPO inactivation, possibly explaining the lack in further TPC reduction (Figure 4A). However, in this case, the intense PPO inactivation should be mainly attributed to the fact that multiple HPH passes made temperature sample exceed that of PPO inactivation (70 °C) by about 15 °C (Terefe, Delon, Buckow, & Versteeg, 2015).

Table 7. Visual appearance, microscopic image, and colour of not-blanched and blanched lettuce waste submitted to grinding, pre-homogenisation and HPH treatments at increasing pressure and number of passes.

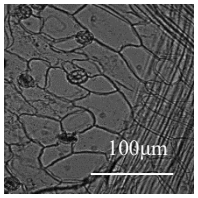
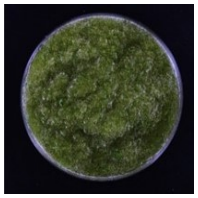
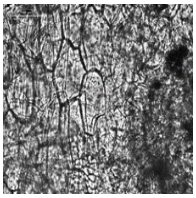
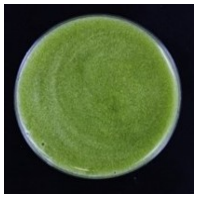
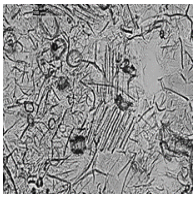
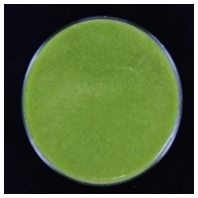
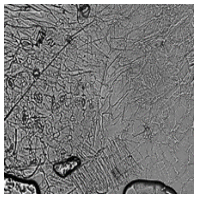
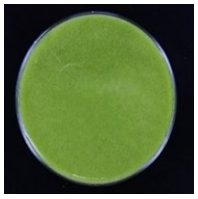
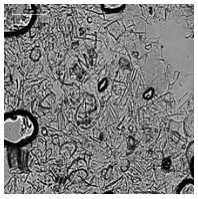
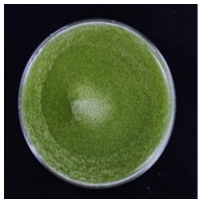
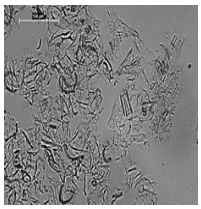
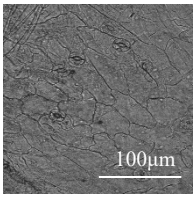
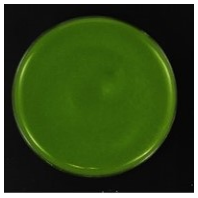
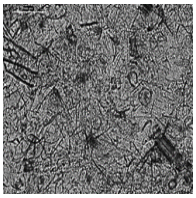
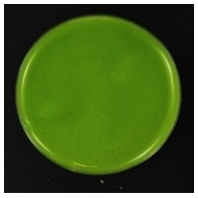
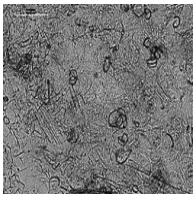
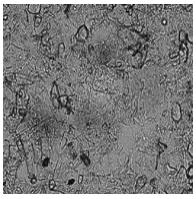
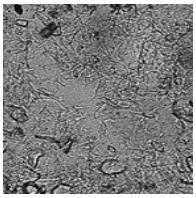
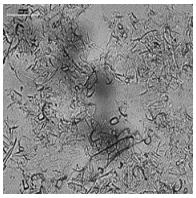
Sample		**Appearance**	**Microscopy**	**Colour**		
				L*	a*	b*
Not-blanched	Control			71.4 ± 1.3 [a]	-16.9 ± 1.2 [g]	31.6 ± 1.4 [a]
	Ground			39.8 ± 0.7 [g]	-3.5 ± 0.3 [a]	17.0 ± 1.0 [g]
	Pre-homogenised			43.6 ± 0.3 [f]	-4.3 ± 0.1 [a]	20.7 ± 1.0 [f]
	HPH 80			48.6 ± 0.1 [c]	-6.2 ± 0.1 [c]	26.1 ± 0.2 [cd]
	HPH 150			49.7 ± 0.1 [c]	-6.3 ± 0.1 [c]	25.4 ± 0.3 [d]
	HPH 150x10			46.6 ± 0.1 [d]	-3.8 ± 0.1 [ab]	21.4 ± 0.2 [ef]

Table 7. (continues).

Sample		Appearance	Microscopy	Colour		
				L*	a*	b*
Blanched	Control			69.0 ± 3.9 [a]	-15.5 ± 0.7 [f]	30.7 ± 0.9 [a]
	Ground			45.1 ± 0.2 [ef]	-13.5 ± 0.4 [d]	21.6 ± 0.8 [ef]
	Pre-homogenised			46.7 ± 0.1 [d]	-14.3 ± 0.1 [e]	28.3 ± 0.1 [b]
	HPH 80			46.8 ± 0.1 [d]	-14.6 ± 0.1 [e]	26.9 ± 0.1 [c]
	HPH 150			46.0 ± 0.1 [de]	-14.4 ± 0.1 [e]	26.0 ± 0.4 [cd]
	HPH 150x10			51.6 ± 0.4 [b]	-6.7 ± 0.2 [c]	22.3 ± 0.2 [e]

[a-g]: In the same column, means indicated by different letters are significantly different ($p<0.05$)

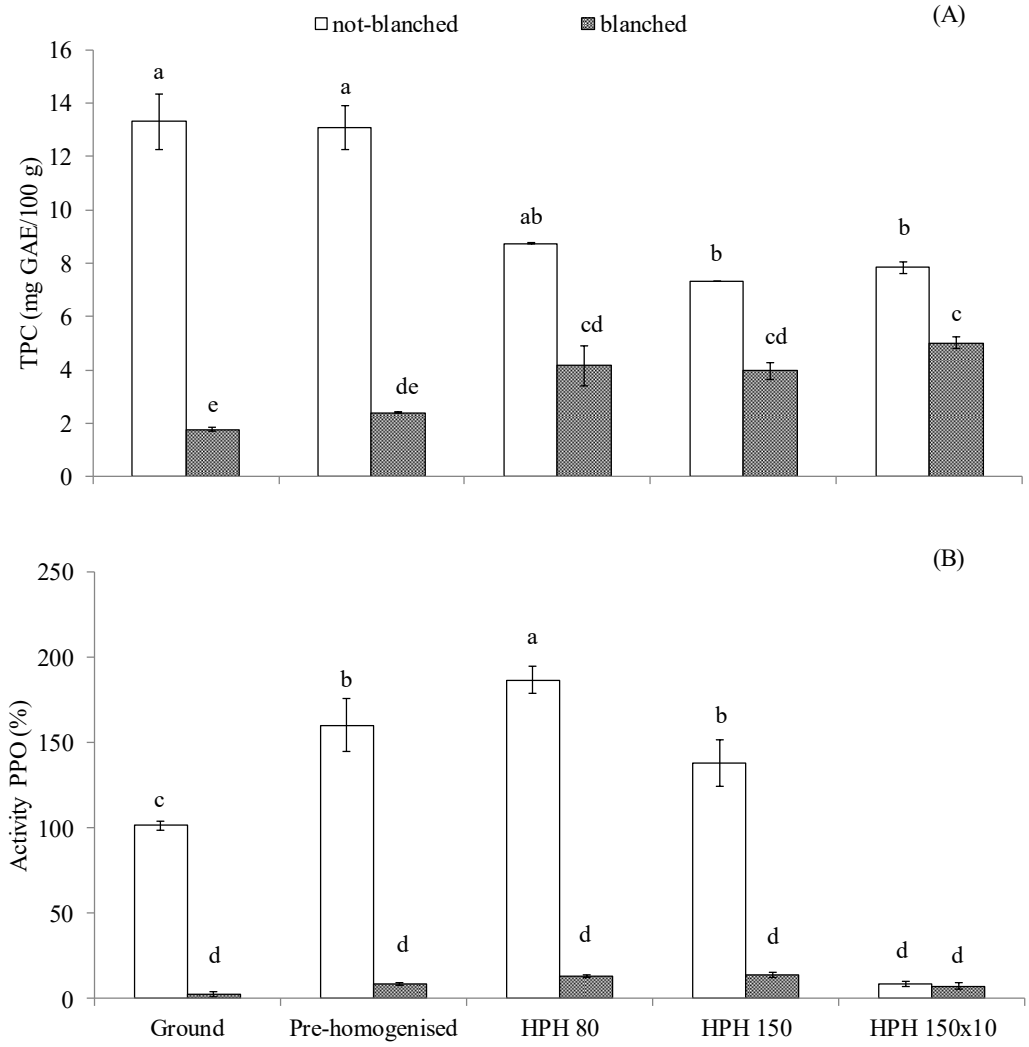


Figure 4. Total phenolic content (TPC) (A) and polyphenoloxidase activity (Activity PPO) (B) of not-blanched and blanched lettuce waste submitted to grinding, pre-homogenisation and HPH treatments at increasing pressure and number of passes; [a-d]: Means indicated by different letters are significantly different ($p<0.05$)

To evaluate the physical stability of samples, phase separation after 24 h of refrigerated storage was assessed (Figure 5A). Interestingly, the Ground sample showed no phase separation, while the latter increased with the applied pressure. These results can be possibly due to the effect of HPH on network-forming ability of vegetable fibres. It is likely that in Ground sample, lettuce fibres tended to intertwine and form a network trapping the free liquid which would otherwise separate from the product. By disrupting the vegetable components (Table 7), HPH reduced the length of lettuce fibres to a point where they were no longer able to form an effective network. In this regard, Colle et al. (2010) highlighted a significant change in water holding capacity of tomato fibres submitted to HPH up to 130 MPa. Although the sample obtained by the 150 MPa treatment for 10 passes

showed the highest tissue disruption (Table 7), it presented a lower phase separation as compared to the 150 MPa single-pass treatment. In addition to the effect of fibre length, an effect of applied treatments on pectolytic enzymes can be accounted for the observed separation data. In fact, as a consequence of the activity of these enzymes, cross-linking between carboxyl groups in pectin molecules are favoured, leading to structure changes in vegetable derivatives. In particular, the activity of pectin methylesterase (PME) has been reported to destroy the cloudy stability in citrus fruit juices (Welti-Chanes, Ochoa-Velasco, & Guerrero-Beltrán, 2009) and to favour crispness loss in *Iceberg* lettuce (Martín-Diana et al., 2005). HPH treatments up to 150 MPa actually reduced PME activity by about 20% (Figure 5B). The application of 10 passes at 150 MPa led to a further inactivation, leading to a residual PME activity around 50%. It is thus likely that only the most intense treatment promoted a sufficient PME inactivation to reduce separation phenomena. This agrees with results obtained by Welti-Chanes et al. (2009) in orange juice, in which the application of 5 passes at pressures up to 250 MPa was shown to enhance PME inactivation.

Finally, samples were analysed for total bacterial (TBC), yeast and lactic acid bacteria (LAB) counts (Table 8). LAB resulted always lower than detection limit (1.7 CFU/g), while TBC and yeasts progressively decreased with the HPH intensity. Only the 150 MPa treatments were able to attain lettuce juices presenting microbial loads below limits usually indicated for vegetable and fruit juice quality (3.7-4.7 Log CFU/g for TBC) (Simforian, Nonga, & Ndabikunze, 2015). Such result should be attributed not only to HPH effect, but also to the intense heating promoted by the treatment (Comuzzo et al., 2017).

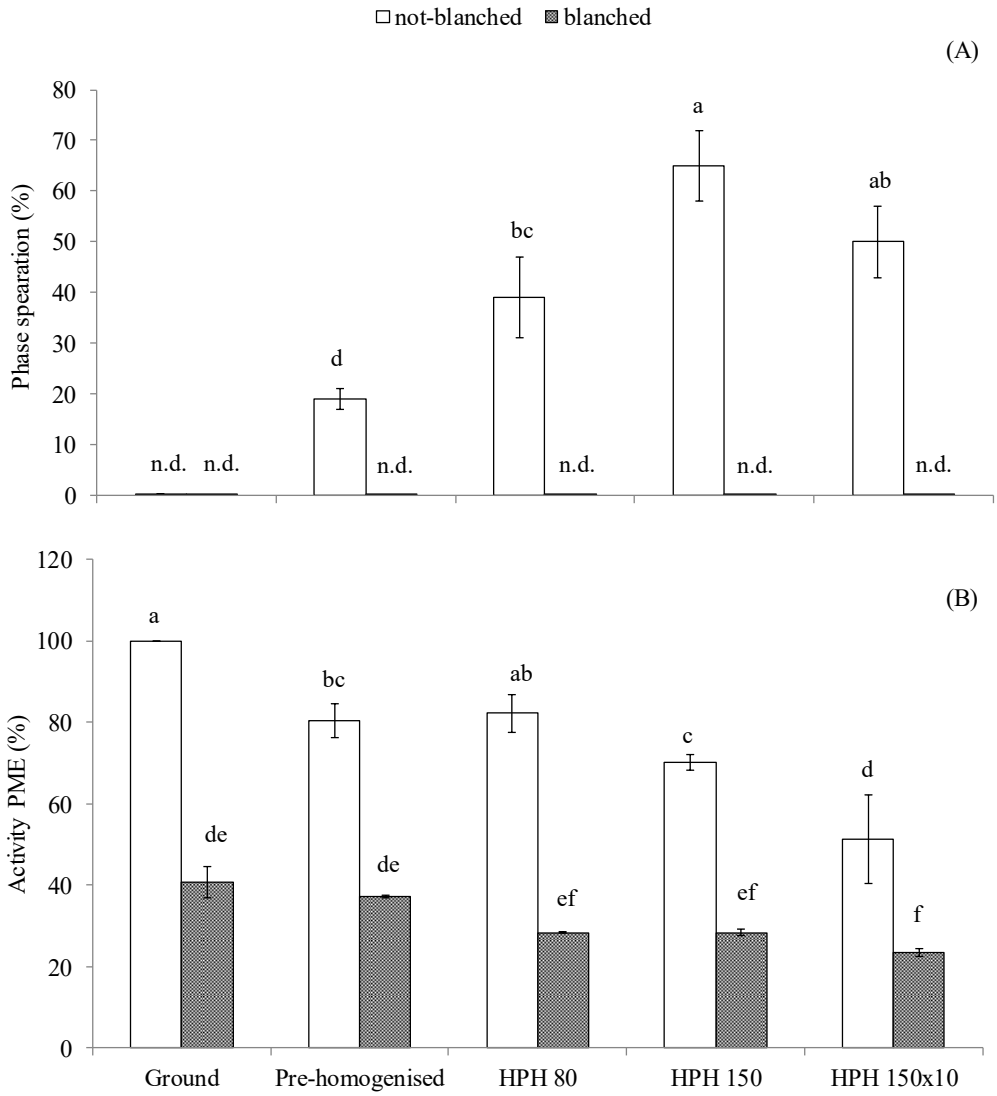


Figure 5. Phase separation (A), and pectin methylesterase activity (Activity PME) (B) of not-blanched and blanched lettuce waste submitted to grinding, pre-homogenisation and HPH treatments at increasing pressure and number of passes; [a-f]: Means indicated by different letters are significantly different ($p<0.05$); n.d. not detected

Table 8. Total bacterial count (TBC), yeast and lactic acid bacteria (LAB) load of not-blanched and blanched lettuce waste submitted to grinding, pre-homogenisation and HPH treatments at increasing pressure and number of passes.

Sample		TBC (Log CFU/g)	Yeasts (Log CFU/g)	LAB (Log CFU/g)
Not-blanched	Ground	5.71 ± 0.31 [a]	5.48 ± 0.14 [a]	<D.L.
	Pre-homogenised	5.57 ± 0.21 [a]	5.44 ± 0.46 [a]	<D.L.
	HPH 80	4.31 ± 0.39 [ab]	4.13 ± 0.55 [b]	<D.L.
	HPH 150	1.85 ± 0.22 [c]	<D.L.	<D.L.
	HPH 150x10	3.20 ± 0.10 [bc]	<D.L.	<D.L.
Blanched	Ground	3.23 ± 0.54 [bc]	<D.L.	<D.L.
	Pre-homogenised	3.16 ± 0.44 [bc]	<D.L.	<D.L.
	HPH 80	2.00 ± 0.79 [c]	<D.L.	<D.L.
	HPH 150	3.20 ± 0.38 [bc]	<D.L.	<D.L.
	HPH 150x10	2.00 ± 0.37 [bc]	<D.L.	<D.L.

D.L. = detection limit = 1.7 Log CFU/g; [a-c]: Means indicated by different letters are significantly different ($p<0.05$)

Effect of high pressure homogenisation with blanching pre-treatment

The application of HPH treatments to lettuce waste resulted in juices showing a critical instability of physical and microbiological parameters. In the light of these findings, in the second part of the study, the possibility to improve the stability of HPH treated lettuce waste by the application of a blanching pre-treatment was evaluated. The visual appearance and the microscopic structure of obtained samples is reported in Table 7. As compared to not-blanched samples, Ground blanched ones presented a lighter colour and an apparently more homogeneous structure. Microscopic images revealed that a good cellular disruption was obtained also by the application of grinding and that HPH treatments further increased the homogeneity of the sample, which showed uniformly distributed cellular content (Table 7). Blanching treatment actually promotes cell turgidity loss and cell wall degradation, leading to a more deformable and softer texture, which is expected to favour grinding and homogenisation (Xu, Yu, & Li, 2015). As compared to not-blanched samples, pre-homogenised and homogenised blanched ones presented significantly lower a* data. As expected, blanching hindered browning phenomena, allowing to better maintain the original lettuce green colour. This can be attributed to the effect of blanching on oxidative enzymes, responsible for polyphenol oxidation and chlorophyll degradation (Devece et al., 1999). To this regard, Figure 4B shows that blanched samples presented PPO activity always lower than 14%. Similar to not-blanched samples (Table 7), also the blanched ones obtained by the application of single-pass HPH treatments presented a greener colour (lower a* value) than the blanched Ground sample, possibly due to HPH-induced chlorophyll extraction (Carullo et al., 2018). Reversely, as already pointed out,

the application of 10 passes at 150 MPa led to colour bleaching, probably due to the intense thermal effect of this treatment, possibly leading to severe thermal degradation of both polyphenols and chlorophyll (Manzocco, Mastrocola, Nicoli, & Marangoni, 2001; Weemaes, Ooms, Van Loey, & Hendrickx, 1999). Beside colour, blanching-induced PPO inactivation also affected the phenolic content of HPH treated lettuce waste. As shown in Figure 4A, TPC of blanched samples as a function of HPH treatment intensity followed an opposite pattern as compared to not-blanched ones, resulting in progressively higher TPC values. However, given the treatment, TPC of blanched samples resulted always lower than that of not-blanched ones. This apparently contrasting result can be explained considering the counterbalancing effect of HPH and blanching on phenol content. HPH-induced tissue disruption (Table 7) promoted the extraction from blanched lettuce cells of phenolic compounds that, in the absence of an intense oxidative PPO activity, were largely maintained. In this regard, HPH has been extensively used as cell-breakage technology favouring extraction of different target molecules from vegetable tissues (Zhu et al., 2016). However, vegetable blanching is known to cause significant depletion in phenolic content, due to both applied temperature and leaching effect in the water used for the treatment (Eyarkai Nambi, Gupta, Kumar, & Sharma, 2016).

Blanched samples resulted physically stable after 24 h refrigerated storage, showing no visible phase separation (Figure 5A). Such result can be partially attributed to the effect of blanching on pectolytic enzymes. As shown in Figure 5B, in fact, blanched samples presented a PME activity always lower than 40% and progressively decreasing with the increase of HPH intensity. Blanching at temperatures higher than 80 °C has been actually reported to inactivate PME in different vegetables (Ni, Lin, & Barrett, 2005). Nevertheless, microscopic images evidenced a considerable HPH effect on blanched lettuce structure (Table 7), that was evaluated by means of rheological measurements (Figure 6). Sample viscosity decreased with the increase in HPH treatment intensity. Similar results were also reported for apple and banana juices and can be possibly attributed to the HPH-induced reduction of fibre dimension, favouring fibre-fibre interaction rather than fibre-water ones (Colle et al., 2010). Finally, the microbial quality of blanched samples was determined (Table 8). LAB and yeasts resulted always lower than detection limit. The TBC of Ground sample resulted lower than 3.5 Log CFU/g and progressively decreased with the HPH intensity. These values resulted not only lower than those of not-blanched samples, but also below limits usually indicated for vegetable and fruit juice quality (3.7-4.7 Log CFU/g for TBC). Blanching, in fact, can reduce the microbial load of vegetable surface, due to the applied temperature and microorganism leaching into treatment water (Xiao et al., 2017).

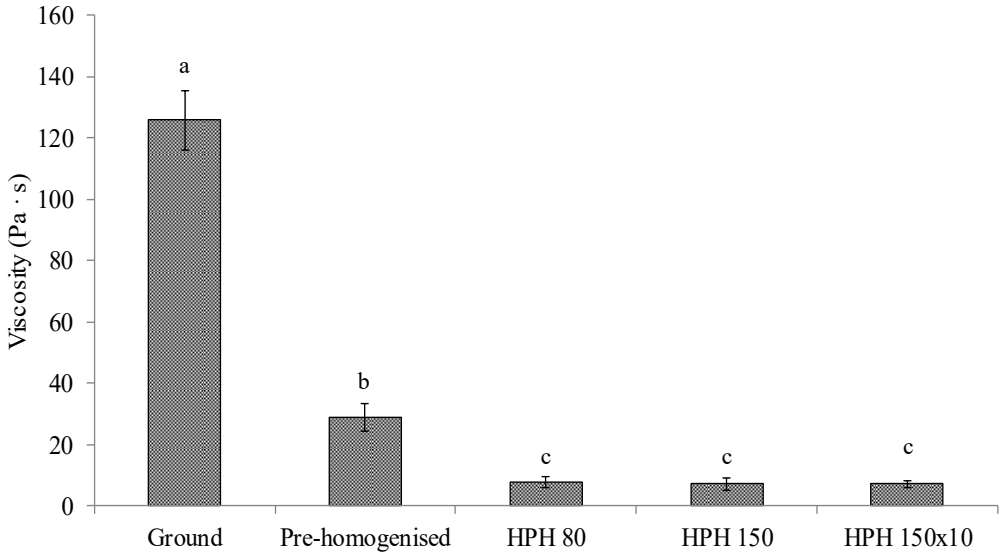


Figure 6. Viscosity of blanched lettuce waste submitted to grinding, pre-homogenisation and HPH treatments at increasing pressure and number of passes. [a-c]: Means indicated by different letters are significantly different ($p<0.05$)

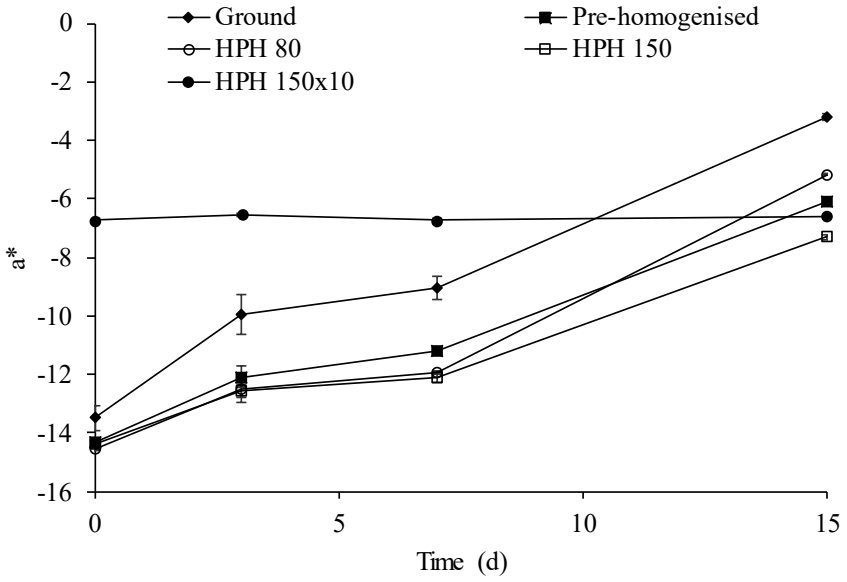


Figure 7. Red-point (a*) of blanched lettuce waste submitted to grinding, pre-homogenisation and HPH treatments at increasing pressure and number of passes, during 15-day refrigerated storage.

Storage test

Based on the obtained results, HPH treatments associated to a blanching pre-treatment would allow obtaining a lettuce juice presenting good physical stability and acceptable microbial load, potentially exploitable for the formulation of blended juices, smoothies and comminuted foods. These samples were thus selected for a storage test that was conducted in refrigerated conditions up to 15 days. During this period, no significant changes in L* and b* parameters of the samples were observed (data not shown). However, in the Ground sample and in the samples subjected to a single HPH pass a progressive increase in a* was observed, indicating sample browning (Figure 7). This effect should be probably attributed to the progressive degradation of chlorophyll during storage (Perucka, Olszówka, & Chilczuk, 2014) and to the formation of chemically oxidised polyphenols upon contact of samples with oxygen (Le Bourvellec, Le Quéré, Sanoner, Drilleau, & Guyot, 2004) rather than to PPO activity. The latter was actually very low in the just prepared samples (Figure 4B) and remained below 8% (data not shown) during the entire storage test, indicating that applied treatments were able to irreversibly inactivate this enzyme. At the end of storage, the a* value of samples subjected to one HPH pass resulted lower than that of Ground sample. This higher greenness was already observed immediately after the treatment (Table 7) and possibly attributed to the ability of HPH to release intracellular compounds, such as chlorophyll. Only the sample obtained by 10 passes at 150 MPa showed no changes in a* during storage. As already pointed out, this sample showed, immediately after the treatment, an a* value significantly higher than that of other blanched samples (Table 7). Thus, it is likely that the high temperature reached on multiple HPH passes promoted intense degradation of polyphenols and pigments during the treatment. This, in turn, led to negligible changes in juice colour on further storage, probably due to a low concentration of degradable pigments remaining in the sample.

No phase separation neither viscosity changes were observed during the 15 day-storage (data not shown) in agreement with PME irreversible inactivation by applied treatments. In fact, PME showed no changes in activity during storage time (data now shown). Figure 8 shows the evolution of microbial load during time. Yeasts resulted always lower than detection limit (1.7 UFC/g). Except for HPH 150x10 sample, in which LAB growth resulted inhibited, TBC and LAB progressively increased in all samples (Figure 8A and B), exceeding values commonly indicated for fruit and vegetable juice quality (3.7-4.7 Log CFU/g for TBC) after only 3 days. As widely reported in the literature, HPH presents a reduced antimicrobial efficacy and is thus usually combined with other treatments able to attain a microbiologically stable product. The latter include thermal treatments, acidification of the product or high hydrostatic pressure processing (Georget, Miller, Callanan, Heinz, & Mathys, 2014; Patrignani, Tabanelli, Siroli, Gardini, & Lanciotti, 2013).

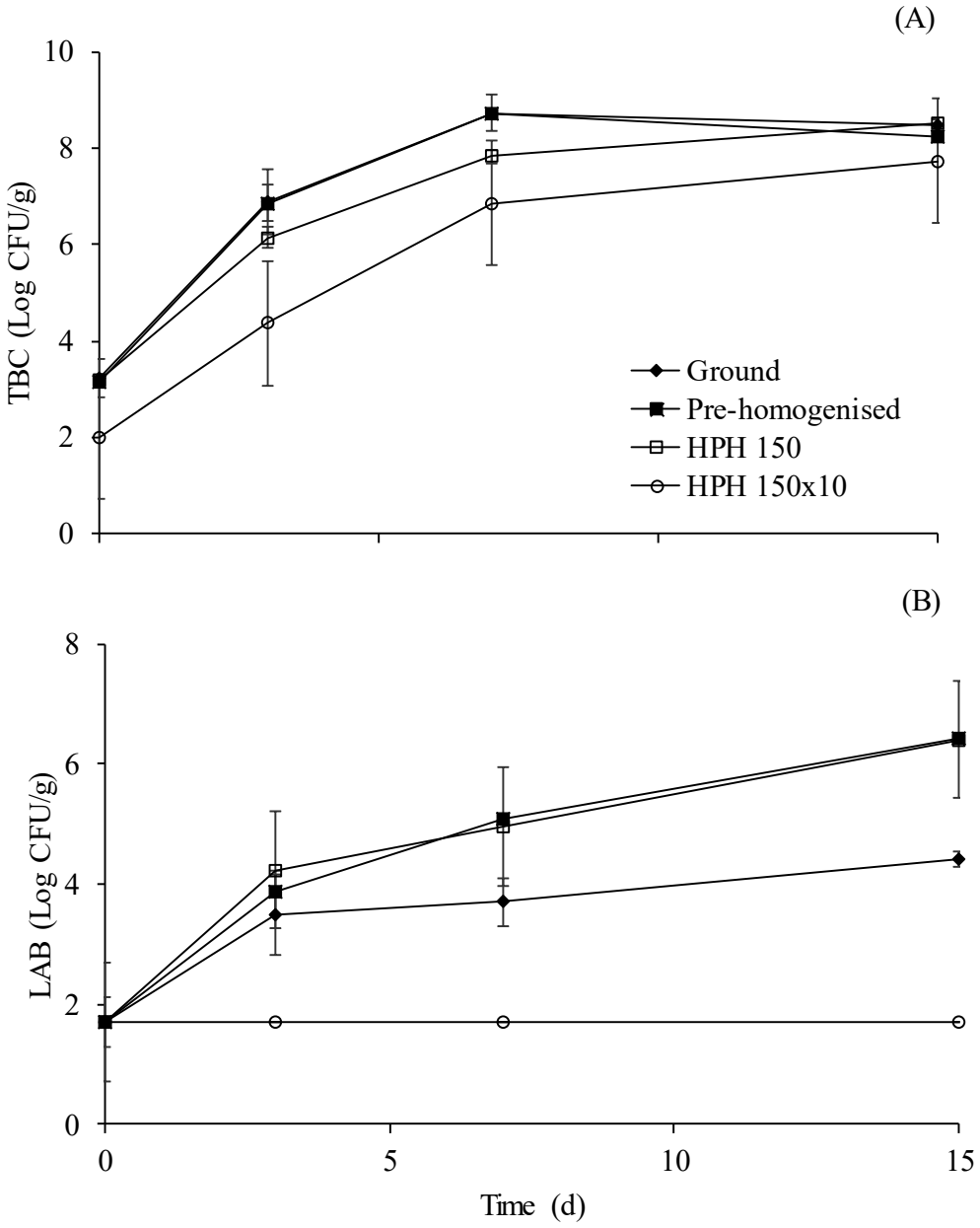


Figure 8. Total bacterial count (TBC) and lactic acid bacteria (LAB) load of blanched lettuce waste submitted to grinding, pre-homogenisation and HPH treatments at increasing pressure and number of passes, during 15-day refrigerated storage. Detection limit = 1.7 Log CFU/g

5.1.4 Conclusions

Results obtained in this study suggest that high pressure homogenisation might be an interesting technology to fully exploit lettuce waste and obtain innovative healthy ingredients to be further used in food production. The combination of this technology with a blanching pre-treatment resulted in a homogeneous lettuce juice, presenting a partially maintained phenol content, a bright green colour, and high physical stability during storage. Although promoting an irreversible inactivation of alterative enzymes, the proposed treatment was not adequate for guaranteeing juice microbial stability. HPH of blanched lettuce should be thus associated to a further stabilization step (e.g. acidification or high hydrostatic pressure), reasonably applied at the level of the final formulation, being a blended juice, a smoothie or a comminuted food. Although the case here presented was relevant to lettuce waste, obtained results could be easily extended to other vegetables discards, largely broaden their applicability and impact. This effort is worth making considering that HPH is being increasingly introduced as processing operation in different industrial contexts, showing good feasibility and cost effectiveness. For these reasons, the efficacy of HPH in turning waste into functional homogenates was further tested taking in consideration the okara study-case (§ 5.2).

5.2 Valorisation of soy okara into a functional homogenate by means of high pressure homogenization

The aim of this study was to investigate the impact of HPH treatments, carried out at increasing intensity, on soy okara. To this purpose, okara dispersions (10% w/w) were submitted to 1 pass at 50, 100 and 150 MPa and to 5 passes at 150 MPa. Homogenised samples were then analysed for physical properties, including stability, particle size, microstructure, viscosity, and solubility. To study the effects of HPH on soy okara biopolymers, samples were characterised in terms of fibre and protein content. Protein conformational and dimensional changes were also evaluated by assessing -SH groups and absorbance at 280 nm as well as performing gel permeation HPLC. Results were discussed to provide a structural interpretation of the physical effects of HPH on soy okara and infer indications about their possible exploitation as functional homogenates.

5.2.1 Introduction

Okara generated from soy milk production has been extensively exploited for the extraction of functional compounds or producing functional flour (Table 5). However, these strategies present some issues. In particular, upon extraction a significant amount of remaining waste should be still disposed of, along with extraction solvents. Similarly, okara drying involves huge costs due to the high water content of this waste material (Table 4). Moreover, important alterations in flavour, aroma and colour can occur during thermal treatment (Li et al., 2013). The possibility to directly exploit fresh okara, by turning it into a functional ingredient or semi-finished product could represent a valuable alternative strategy for okara valorisation. In particular, as also described in § 5.1, the use of HPH on different vegetable matrices has been widely reported to promote cell disruption, bioactive extraction and modification of biopolymer physical properties (Augusto, Ibarz, & Cristianini, 2013; Bot et al., 2017).

Based on these considerations, HPH treatment of okara dispersions might have different advantages comprising: (i) extraction of proteinaceous and fibrous materials as a consequence of cell disruption and (ii) increase of functionality resulting from biopolymer structure modification and development of novel inter particle interactions and networking. Preece, Hooshyar, Krijgsman, Fryer, and Zuidam (2017a) recently studied the effect of HPH on soybean processing materials. It was observed an improvement of the extraction yield of proteins from soy okara dispersions after homogenisation at 100 MPa for 1 pass. Beside these data, to our knowledge no information is available on the effect of HPH on okara biopolymer structure and interactions.

5.2.2 Materials and methods

Preparation of okara dispersion

Soy okara was prepared as described in § 3.1. Okara was dispersed in deionized water at 10% (w/w) concentration under magnetic stirring for 1 min at room temperature (20 ºC). Then, okara dispersion was mixed with high-speed blender (Polytron, PT 3000, Cinematica, Littau, Swiss) at 8000 rpm for 1 min. The resulted dispersion was processed with HPH. The untreated sample was used as control (untreated).

High pressure homogenization (HPH)

Dispersion containing 10% (w/w) okara were treated by the high-pressure homogenizer represented in Figure 12B and described in § 5.1.2. The first valve was the actual homogenization stage and was set at increasing pressure up to 150 MPa. The second valve was set at the constant value of 5 MPa. Aliquots of 150 mL of okara dispersion were subjected to single-pass at pressure 50, 100 and 150 MPa and also 5 passes at 150 MPa (750 MPa). The sample temperature was measured immediately after HPH treatment by a copper constantan thermocouple probe (Ellab, Hillerød, Denmark) immersed in the fluid, connected to a portable data logger (mod. 502A1, Tersid, Milan, Italy). After treatments, all the samples were cooled at room temperature (20 ºC).

Analytical determinations

Chemical composition

Moisture, fat, protein and ash content of okara were analysed by the reference AOAC method (AOAC, 1997). Total polyphenolic content (TPC) was determined using Folin-Ciocalteau reagent as described in § 3.1. The reaction mixture contained 50 µL of supernatant, 2 mL distilled water and 250 µL of the Folin-Ciocalteau reagent. Soluble (SDF) and insoluble dietary fibre (IDF) of fresh okara and HPH treated okara dispersions were also analysed according to AOAC method (AOAC, 1997) using a total dietary fibre assay kit (TDF-100A, Sigma-Aldrich, St. Louis, Missouri, USA). The SDF/TDF and IDF/TDF ratio was reported as g/100 g fibre.

Physical stability

To monitor physical stability of okara dispersion treated by HPH, samples were transferred into 20 mL glass tube and images were then acquired as described in § 5.1.2.

pH measurement

The pH of samples was recorded at 20 ºC by using a Basic 20 pH meter (Crison Instruments, S.A., Barcelona, Spain) equipped with a combination of glass electrodes and a temperature probe.

Particle size distribution

The particle size distribution of okara dispersions was measured by using the dynamic light scattering instrument Zetasizer Nano ZS (Malvern, Milan, Italy). Samples were diluted 1:10 (v/v) in deionized water prior to the analysis to avoid multiple scattering effects. The angle of observation was 173º. Solution refractive index and viscosity were set at 1.333 and 0.88 cP, respectively, corresponding to the values of pure water at 25 °C. Particle mean diameter and polydispersity index (PDI) corresponding to intensity distribution was then measured.

Optical and polarized light microscopy

One droplet of each sample was placed on a glass slide, covered, and observed at room temperature as described in § 5.1.2.

Viscosity

Viscosity determination was performed using a bob-cup geometry at 20 °C, as described in § 5.1.2. In particular, the flow behaviour of samples was measured by recording apparent viscosity against shear rate from 0.1 to 200 s^{-1}. The relationship between apparent viscosity and shear rate was described by Ostwald-de-Waele model (eq. 3):

$$\eta_{app} = K \cdot \dot{\gamma}^{n-1} \qquad \text{(eq. 3)}$$

where η_{app} is the apparent viscosity (Pa·s); $\dot{\gamma}$, the shear rate (s^{-1}); K, the consistency index (Pa· s^n) and n, the flow behaviour index (dimensionless). Model fitting was performed using the software Haake Rheowin v.4.60.0001 (Thermo Fisher Scientific).

Protein extraction yield

The 10% (w/w) okara dispersions were centrifuged at 12,000 g for 10 min at 4 °C (Beckman, Avanti TM J-25, Palo Alto, CA, USA). The protein content of the supernatant was determined by Kjeldahl method (AOAC, 1997). Protein extraction yield (g/100 g) was calculated as reported in eq. 4:

$$Protein\ extraction\ yield = \frac{protein\ in\ the\ supernatant}{okara\ protein} \times 100 \qquad \text{(eq. 4)}$$

Absorbance at 280 nm

Okara dispersions were centrifuged at 12,000 g for 10 min at 4 ºC (Beckman, Avanti TM J-25, Palo Alto, CA, USA). The supernatant was collected and diluted to 1:200 in order to be sufficient for UV absorbance measurement. UV absorbance was measured at 280 nm using UV-2501 PC UV-VIS (Shimadzu, Kyoto, Japan) spectrophotometer connected to a CPS-240A thermoelectrically temperature-controlled cell holder (Shimadzu).

Determination of sulfhydryl content

The concentration of free sulfhydryl groups (SH) of the okara dispersion was determined using Ellman's reagent (5′,5-dithiobis (2-nitrobenzoic acid), DTNB) (Sigma-Aldrich. Milan, Italy). Changes in free sulfhydryl groups were measured based on the method of Beveridge, Toma & Nakai (1974). Briefly, a Tris-Glycine-EDTA (TGE) buffer was prepared by dissolving Tris (10.4 g), glycine (6.9 g) and EDTA (1.2 g) (Sigma-Aldrich. Milan, Italy) in 1 L of distilled water with adjusting pH to 8.0. A working SDS-TGE solution was freshly prepared by mixing 45 ml of TGE with 5 ml SDS stock solution (25% w/v). The solution was degassed in an ultrasonic bath for 30 min, and flushed with nitrogen during stirring for 15 min. 100 mg okara dispersion was mixed in 3 ml TGE-SDS buffer and vortexed every 10 min for 30 min. 0.06 Ellman's reagent (4 mg ml^{-1} DTNB in dimethylformamide) was added and the mixture was held for 15 min. This was followed by centrifugation at 12,000 g for 10 min at 4 ºC (Beckman, Avanti TM J-25, Palo Alto, CA, USA). The absorbance of supernatant was measured at 412 nm by a UV–VIS spectrophotometer (UV-2501 PC, Shimadzu Kyoto, Japan). Concentration of free sulfhydryl groups (μM g^{-1}) was calculated from eq. 5:

$$SH = \frac{73.53 \,.\, A_{412} \,.D}{C} \qquad \text{(eq. 5)}$$

where A_{412} is the absorbance at 412 nm; C is protein concentration (mg mL^{-1}); D is the dilution factor; and 73.53 is derived from $= \frac{10^6}{1.36.10^4}$; $1.36.10^4$ and is the molar absorptivity (Ellman, 1959).

HPLC-gel permeation analysis

Okara dispersions were analysed using a HPLC system Varian ProStar (model 230, Varian Associates Ltd., Walnut Creek, CA, USA) equipped with a UV/VIS detector. Two columns were used: BioSep-SEC-S 3000, 30 cm length, 7.80 mm internal diameter and BioSep-SEC-S 2000, 30 cm length, 7.80 mm internal diameter, 5 μm granulometry, 125 Å porosity with separation range among 5 and 670 kDa. Samples were filtered on 0.2 μm porosity filters (Econofilters, Agilent Technologies, Cenusco sul Naviglio, Italy). Injection volume was 20 μL and the mobile phase, delivered at a flow rate of 0.6 mL min^{-1}, was 1 M potassium phosphate buffer pH 7.0 in isocratic conditions. The detection wavelength was 220 nm. Catalase (250 kDa), Glucose oxidase (160 kDa), lipoxidase (108 kDa), lysozyme (14.3 kDa) and insulin (5.8 kDa) (Sigma, St. Louis, MO, USA) were used as calibration standards. A linear relation (R^2=0.98) was found between retention time and molecular weight of standard proteins. Peaks integration was performed by CHROM-CARD for Windows software (1.19 version).

Data analysis

Analyses were carried out at least three times in two replicated experiments. Analysis of variance ($p<0.05$) and linear regression analysis were performed using R (The R foundation for statistical

computing, v.3.1.1). Bartlett's test was used to check the homogeneity of variance, one-way ANOVA was carried out and Tukey test was used to determine statistically significant differences among means ($p<0.05$).

5.2.3 Results and discussion

Table 9 shows the chemical composition of okara obtained from the waste stream of soy milk processing. As expected, okara presented a high moisture content and was particularly rich in insoluble fibre, proteins and lipids. Interestingly, it also contained significant amounts of polyphenols. These data are in the range of proximal composition analysis reported in literature by different authors (Guimarães et al., 2018; Vong & Liu, 2016).

Table 9. Chemical composition of soy okara.

Parameter	**Amount**
Moisture (g/100 g)	76.22 ± 0.40
Protein (g/100 g)	6.53 ± 0.01
Lipid (g/100 g)	1.57 ± 0.06
Total dietary fibre (g/100 g)	12.50 ± 0.05
Insoluble fibre (g/100 g)	12.19 ± 0.04
Soluble fibre (g/100 g)	0.31 ± 0.01
Total phenolic content (mg GAE/g dry matter)	1.92 ± 0.04
Ash (g/100 g)	0.59 ± 0.05

A 10% (w/w) okara aqueous dispersion was submitted to HPH by applying pressures up to 150 MPa and number of passes up to 5. The temperature of the treated samples increased with the treatment intensity up to 63 °C (Table 10). As well known, these temperature changes are attributable to the mechanical stresses suffered by the sample during the passage through the homogenization valve (Hayes & Kelly, 2003).

Table 10. Temperature of 10% (w/w) untreated okara aqueous suspension and samples submitted to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa with 5 passes.

HPH Treatment	**Number of passes**	**Temperature (ºC)**
Untreated	-	21.3 ± 1.13 ^{d}
50 MPa	1	28.65 ± 1.91 ^{c}
100 MPa	1	36.90 ± 2.69 ^{b}
150 MPa	1	42.95 ± 1.20 ^{b}
150 MPa	5	62.80 ± 0.85 ^{a}

$^{a, b, c, d}$: In the same column, means indicated by different letters are significantly different ($p<0.05$)

The visual observation of the samples revealed that the physical stability of HPH treated dispersions was higher than that of the untreated one (Figure 9). In particular, no evident separation of treated dispersions was noticed after 1 day of storage, with the only exception of the samples treated at 50 MPa, which showed a beginning of phase separation.

Figure 9. Phase separation of 10% (w/w) untreated okara aqueous dispersion and samples submitted to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa with 5 passes during 1day storage at 4 ºC.

These results can be attributed to HPH-induced modifications of the structure of okara constituents. To study these modifications, particle size distribution of treated samples was determined (Figure 10).

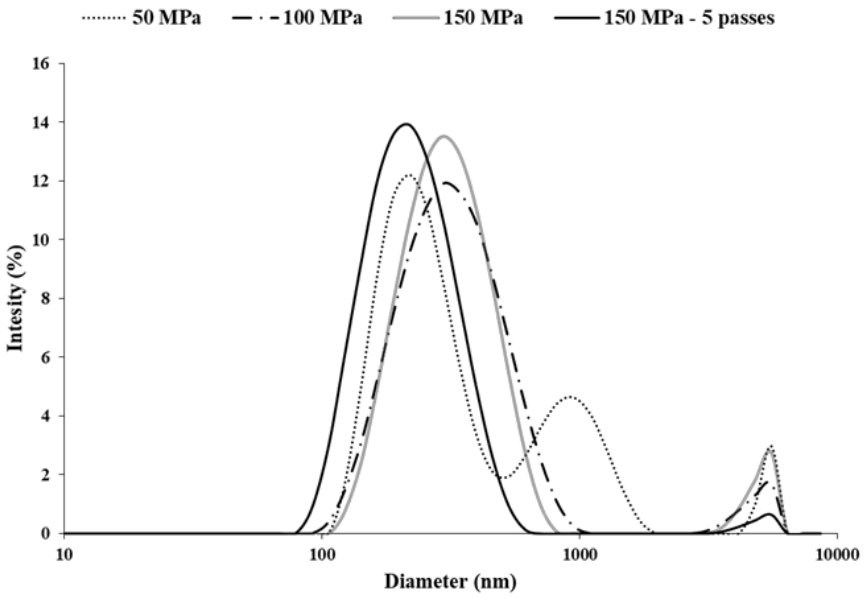


Figure 10. Particle size distribution of 10% (w/w) okara aqueous dispersion submitted to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa with 5 passes.

Untreated okara dispersions were not analysed, since quickly undergoing phase separation. Okara dispersions treated at 50 MPa showed a trimodal distribution, indicating that about 70% of the particles had mean diameter of around 200 nm, 25% showed mean diameter around 750 nm and, finally, the diameter of the rest 3% of the particles was approximately 5000 nm (Table 11).

Table 11. Mean particle size diameter and peak area (%) of 10% (w/w) okara aqueous suspension submitted to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa with 5 passes.

HPH treatment	**Mean particle diameter (nm)**			**Peak area (%)**		
	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
50 MPa	216.4 ± 45.8 ^{c}	765.9 ± 237.2	5370.0 ± 42.2 ^{a}	71.5 ± 1.3 ^{c}	25.7 ± 0.3	2.8 ± 1.6 ^{b}
100 MPa	354.8 ± 19.7 ^{a}	n.d.	4057.0 ± 789.1 ^{a}	88.8 ± 1.8 ^{b}	n.d.	11.2 ± 1.7 ^{a}
150 MPa	344.3 ± 6.6 ^{ab}	n.d.	4875.0 ± 311.1 ^{a}	95.5 ± 0.4 ^{a}	n.d.	4.5 ± 0.3 ^{b}
150 MPa-5 passes	241.2 ± 12.0 ^{bc}	n.d.	5180.5 ± 282.1 ^{a}	98.8 ± 0.2 ^{a}	n.d.	1.3 ± 0.1 ^{b}

n.d. not detected

$^{a, b, c, d}$: In the same column, means indicated by different letters are significantly different ($p<0.05$)

The application of increasing pressure led to a progressive particle downsizing with the disappearance of the intermediate peak, a reduction of the largest particles and a concomitant increase (more than 90%) of particles with 350 nm mean diameter. These results agree with literature data relevant to the effect of HPH on the particle size of different vegetable derivatives, including tomato juice, soy protein isolate, and hazelnut milk (Augusto, Ibarz, & Cristianini, 2012; Gul, Turker, Mortas, Atalar, & Yazici, 2017; Song, Zhou, Fu, Chen, & Wu, 2013). The decrease in particle size can be attributed to the intense stresses delivered by HPH (elongational stresses, sudden pressure drops, rapid fluid acceleration, shear stress, turbulence and cavitation), able to disrupt soy components. The disruptive ability of HPH can be well noted observing the microscopy images reported in Figure 11.

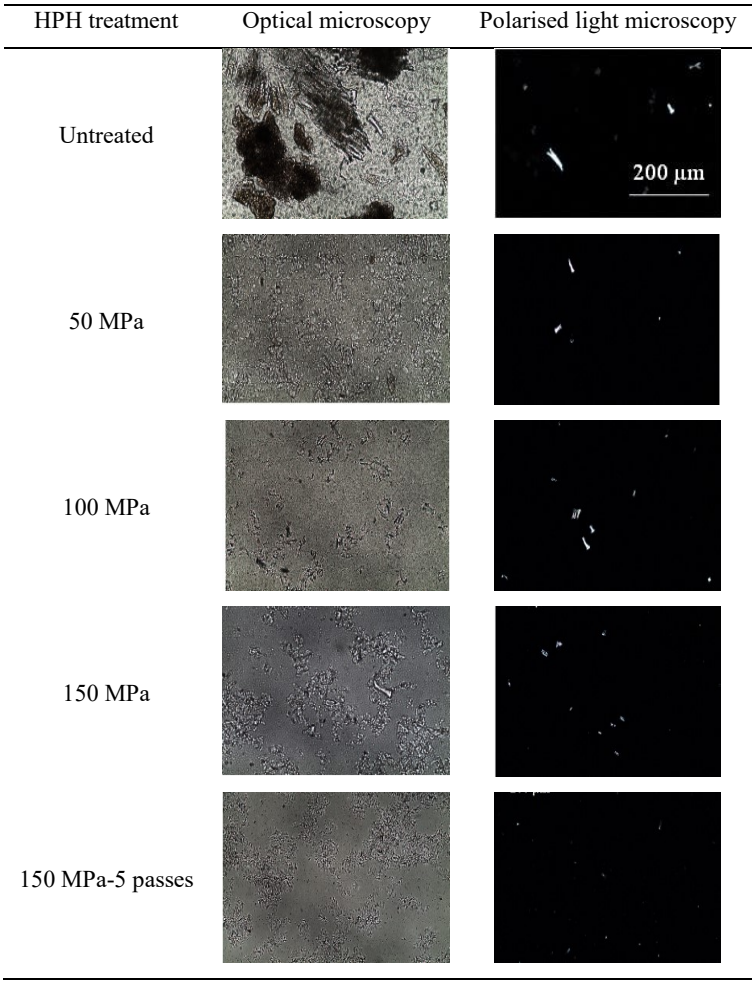


Figure 11. Optical and polarised light microscopy of 10% (w/w) untreated okara aqueous dispersion and samples submitted to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa for 5 passes.

Untreated okara dispersion showed a dense microstructure with colloidal material dispersed throughout the aqueous environment. This material is mainly formed by partially denatured proteinaceous materials. Soy okara is actually produced by heating soybeans at 80 °C, which is a temperature higher than that required for thermal denaturation of the main soy storage protein β-conglycinin (74-77 °C) (Kinsella, 1979; Wang, Qin, Sun, & Zhao, 2014). Untreated okara also showed clearly visible aggregates of fragmented fibrous cell material. As reported by Preece et al. (2015), okara is composed of intact cotyledon cells, walls of disrupted cells and other protein-polysaccharide agglomerated materials. The latter partially retain the original crystalline structures, as also observed by polarized light microscopy images, in agreement with literature data (Liu, Chien, & Kuo, 2013).

As can be seen in Figure 11, HPH treatment at 50 MPa induced the breakage of these large aggregates into smaller ones resulting in a more homogeneous dispersion of particles. The further increase of homogenization pressure caused a progressive reduction in dimension and number of insoluble particles.

To study the macroscopic effect of these microstructural changes, flow curves of okara dispersions were determined. Data were elaborated with the Ostwald-de-Waele model (R^2>0.94) and the estimated parameters reported in Table 12. Except for the sample obtained upon 50 MPa, all samples exhibited a shear thinning flow behaviour (n<1) and the application of more intense treatments increased both consistency index (K) and apparent viscosity (η_{100}).

Table 12. Flow behavior index (n), apparent viscosity at 100 s^{-1} (η_{100}), consistency index (K) of 10% (w/w) okara aqueous dispersion submitted to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa with 5 passes.

HPH treatment	**n**	**η_{100} (Pa s)**	**K (Pa s^n)**
50 MPa	1.054 ± 0.031 [a]	0.001 ± 0.00 [c]	0.001 ± 0.000 [c]
100 MPa	0.512 ± 0.022 [b]	0.010 ±0.001 [b]	0.111 ±0.020 [b]
150 MPa	0.494 ± 0.001 [b]	0.012 ± 0.000 [b]	0.140 ± 0.003 [b]
150 MPa-5 passes	0.309 ± 0.025 [c]	0.029 ± 0.00 [a]	0.803 ± 0.104 [a]

n.d. not detected

[a, b, c]: In the same column, means indicated by different letters are significantly different (p<0.05)

Samples treated at 150 MPa for 5 passes revealed an apparent viscosity about 3 times higher than that of the sample treated at 100 MPa. This result can be due to different phenomena. From one side, the system viscosity can rise as a consequence of the increased crowding associated to the progressively higher number of small particles; from the other side HPH-induced cell breakage is expected to promote extraction of okara components, leading to a higher content of soluble materials in the dispersions (Preece et al., 2017). To better understand the nature of the extracted material, samples were analysed for total (TDF), insoluble (IDF) and soluble (SDF) dietary fibre (Table 13). TDF content decreased with the increase of HPH intensity. A concomitant increase in the ratio between soluble and total dietary fibre was also observed. The redistribution of fibres in favour of the soluble fraction has been reported for different vegetable matrices submitted to homogenization or micronisation treatments. To this regard, Huang, Chen, and Wang (2010) reported a 30-50% increase in soluble/insoluble dietary fibre ratio in taro, potato and yam peels submitted to ball milling. Similar results were also reported by Chau et al. (2007) and Hu, Zhang, Adhikari, and Liu (2015) upon the application of microfluidification at 80 MPa and high pressure homogenization at 100 MPa of carrot pomace and wheat bran, respectively. The observed changes in fibre content and distribution between soluble and insoluble fraction (Table 13) can be attributed to the progressive rupture of the fibrous material and the solubilization of soybean soluble polysaccharides. This structure breakage was also associated to the release of organic acids, accounting for the pH decrease, and polyphenols held in cotyledon cells (Table 13).

Table 13. pH, total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) /TDF ratio and total phenolic compounds (TDC) of 10% (w/w) untreated okara aqueous dispersion and samples submitted to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa with 5 passes.

HPH treatment	**pH**	**TDF** (g/100 g dm)	**IDF/TDF** (g/100 g fibre)	**SDF/TDF** (g/100 g fibre)	**TPC** (mg GAE/ g dm)
Untreated	8.29 ± 0.06 ^{a}	52.57 ± 0.18 ^{a}	97.51 ± 0.061 ^{a}	2.49 ± 0.06 ^{c}	2.58 ± 0.03 ^{c}
50 MPa	8.22 ± 0.02 ^{a}	51.49 ± 2.45 ^{a}	96.37 ± 0.30 ^{ab}	3.63 ± 0.30 ^{bc}	5.08 ± 0.06 ^{b}
100 MPa	8.11 ± 0.04 ^{a}	45.82 ± 1.06 ^{ab}	94.77 ± 0.99 ^{b}	5.23 ± 0.99 ^{b}	5.11 ± 0.05 ^{b}
150 MPa	7.90 ± 0.05 ^{b}	47.56 ± 3.14 ^{ab}	94.93 ± 0.42 ^{b}	5.07 ± 0.42 ^{b}	5.31 ± 0.05 ^{b}
150 MPa – 5 passes	7.67 ± 0.02 ^{c}	41.82 ± 2.52 ^{b}	89.28 ± 0.32 ^{a}	10.72 ± 0.32 ^{a}	8.08 ± 0.35 ^{a}

dm, dry matter

$^{a, b, c, d}$: In the same row, means indicated by different letters are significantly different ($p<0.05$)

Moreover, protein extraction yields dramatically increased from 11 (g/100 g protein) to about 90 (g/100 g protein) with the increase of pressure and number of passes (Table 14). It is likely that proteins, not extracted during the soy milk process, were entrapped in the soybean cells or engaged in protein-fibre complexes. Proteins were thus analysed for conformational changes by determining the absorbance at 280 nm and free sulfhydryl group content (Table 14). The increase in HPH pressure significantly increased absorbance at 280 nm and free SH groups of proteins in okara dispersions. This increase is consistent with the change in protein content and conformation, resulting in an increased exposure of aromatic and SH groups of amino acids on the protein surface and rupture of S-S bonds within protein molecules. However, the application of the most intense treatment was associated to a decrease in both indexes. This is generally associated to reassembling phenomena of extracted proteins, probably by both inter- and intra-molecular interactions (Yu, 2018).

To confirm this hypothesis, okara dispersions were analysed by HPLC-gel permeation analysis (Table 14). The chromatogram relevant to untreated okara dispersion showed 4 main protein fractions (19, 70, 110, 290 kDa). The most abundant protein fraction (70 kDa) can be attributed to α and α' subunits of β-conglycinin (Cole & Cousin, 1994; Stanojevic, Barac, Pesic, & Vucelic-Radovic, 2012). The fraction corresponding to 19 kDa can be associated to the basic polypeptide of glycinin. The largest protein fraction (290 kDa) was represented by soy 11S globulin which is made up of acid and alkaline sub-units (Chen, Liu, Wu, & Ma, 2015). Finally, lipoxygenase was also present (110 kDa) (Cole & Cousin, 1994; Stanojevic et al., 2012). HPH treatments resulted in a progressive increase of abundance of peaks corresponding to β-conglycinin, lipoxygenase and globulin, supporting the hypothesis of protein release from the fibrous matrix upon HPH. However, in the samples treated at the most intense treatments (150 MPa for 1 and 5 passes), the polypeptide band of glycinin was no more present, suggesting its embedding into multimeric aggregates. This result might be consistent with the occurrence of a new peak, not observed in the untreated sample (166 kDa), probably resulting from protein reassembling, as also suggested by the decrease in SH groups and absorbance at 280 nm.

Table 14. The protein extraction yield, absorbance at 280 nm, free sulfhydryl groups and peak areas relevant to proteins with a molecular weight of 19, 70, 110, 166 and 290 kDa of 10% (w/w) untreated okara aqueous dispersion and samples submitted to HPH treatments at 50, 100, 150 MPa pressures and 150 MPa with 5 passes.

HPH treatment	**Protein extraction yield (g/100 protein)**	**Absorbance at 280 nm**	**Free sulfhydryl groups (µM g^{-1})**	**Peak area of proteins with different MW (arbitrary absorbance unit × 10^4)**				
				19 kDa	70 kDa	110 kDa	166 kDa	290 kDa
Untreated	11.49 ± 0.19 ^{d}	0.224 ± 0.014 ^{d}	15.77 ± 0.47 ^{c}	51.7 ± 16.5 ^{b}	189.7 ± 3 1.0 ^{b}	53.1 ± 0.3 ^{c}	n.d.	54.9 ± 0.2 ^{d}
50 MPa	37.11± 1.09 ^{c}	0.428 ± 0.002 ^{c}	51.42 ± 2.93 ^{a}	886.8 ± 96.81 ^{a}	168.1 ± 42.7 ^{b}	200.4 ± 42.7 ^{bc}	n.d.	321.1 ± 8.3 ^{bc}
100 MPa	61.14 ± 1.16 ^{b}	0.653 ± 0.004 ^{a}	51.67 ± 3.74 ^{a}	672.6 ± 54.9 ^{a}	63.9 ± 29.9 ^{b}	125.4 ± 33.4 ^{c}	n.d.	182.9 ± 76.5 ^{cd}
150 MPa	65.94 ± 2.70 ^{b}	0.646 ± 0.006 ^{a}	47.49 ± 0.87 ^{a}	n.d.	290.4 ± 82.4 ^{b}	374.7 ± 82.4 ^{b}	111.3 ± 15.5 ^{a}	433.8 ± 64.4 ^{b}
150 MPa-5 passes	89.69 ± 2.24 ^{a}	0.577 ± 0.002 ^{b}	20.70 ± 1.13 ^{b}	n.d.	830.9 ± 3.3 ^{a}	620.3 ± 3.3 ^{a}	907.7 ± 11.9 ^{a}	831.0 ± 63.0 ^{a}

MW, molecular weight and n.d., not detected

$^{a, b, c, d}$: In the same row, means indicated by different letters are significantly different ($p<0.05$)

5.2.4 Conclusions

Results obtained in this study highlighted that HPH can be used as an efficient tool to induce a progressive disruption of okara native structure, leading to the release of entrapped proteins and soluble fibres. For instance, by using this strategy at adequate intensity level, the recovery of proteins from okara can be considered almost complete. HPH might thus be applied as pre-treatment to favour extraction of proteins and fibres, allowing okara by-product to be turned into value-added ingredients for the food industry. Moreover, the possibility to directly exploit HPH-treated okara dispersions to develop physically stable soy-based beverages cannot be underestimated. The valorisation of okara by-product by its complete re-use in novel functional products could actually represent an interesting market opportunity.

5.3 Valorisation of lettuce waste by means of ultrasound and high pressure homogenisation assisted extraction of antioxidant polyphenols

In this paragraph, the potentialities of ultrasounds (US) and high pressure homogenisation (HPH) were evaluated with reference to the extraction of polyphenols from fresh-cut lettuce waste, to be used as dietary supplements or food antioxidants. To this purpose, lettuce waste was submitted to extraction with ethanolic food-grade solutions by: (i) traditional solid-liquid extraction at 50 °C for increasing time up to 60 min; (ii) US treatments for increasing time up to 120 s; (iii) HPH at increasing pressure up to 100 MPa as pre-treatment to sonication. Extraction yields were assessed by analysing total phenols, phenol profile and antioxidant capacity. Results were discussed in relation to the effect of the treatments on cell structure and polyphenoloxidase activity.

5.3.1 Introduction

Previous studies have demonstrated that lettuce might be an interesting and cheap source of health-promoting antioxidant polyphenols (Llorach et al., 2008, 2004). To this regard, discarded external leaves of lettuce have been reported to show higher phenol content than the edible portions, due to the intense secondary metabolism activated by external stresses (Viacava, Gonzalez-Aguilar, & Roura, 2014). Fresh-cut processing further promotes polyphenol production in lettuce tissue, as a response to cell injury such as leaf cutting or shredding (Cantos et al., 2001). Literature attention has been mainly focused on the extraction of lettuce polyphenols by traditional solid-liquid extraction with organic solvents at concentrations of about 50-80% (DuPont, Mondin, Williamson, & Price, 2000; Llorach et al., 2008; Viacava et al., 2014; Viacava, Roura, & Agüero, 2015). In these works, methanol and acetone came up as suitable solvents to reach good phenolic extraction yields. Procedures reported in the literature usually require the homogenization of lettuce with the extractive solution, followed by the maintenance at temperature usually in the range 4-50 °C for increasing time up to 48 h. Besides being time consuming, these procedures do not allow obtaining food-grade polyphenol extracts (Gil-Chávez et al., 2013). To solve these issues, along with the use of non-toxic and GRAS (generally recognized as safe) extraction solvents such as ethanol, alternative “green” extraction technologies might be exploited.

High energy ultrasounds (US) exploit low frequency sound waves (usually 24-50 kHz) to enhance extraction efficacy due to cavitation phenomena. The latter refer to the formation and subsequent collapse of cavitation bubbles produced during the propagation of sound waves into the extractive solutions. Cavitation bubble implosion generates microjets and solvent flows, which favour cell rupture and mass transfer, leading to an enhanced release of target bioactive compounds (Dranca & Oroian, 2016; Espada-Bellido et al., 2017). US have been demonstrated to be an efficacious technology for obtaining phenolic antioxidant extracts from different vegetable by-products,

including apple pomace, orange peel and spent coffee grounds (Al-Dhabi, Ponmurugan, & Maran, 2017; Khan, Abert-Vian, Fabiano-Tixier, Dangles, & Chemat, 2010; Pingret, Fabiano-Tixier, Bourvellec, Renard, & Chemat, 2012). In the case of lettuce, US have been mainly indirectly delivered to sample through a sonication bath (Parente, Lima, Moreira, Barros, & Guido, 2013; Viacava et al., 2014). However, times longer than 30 min have been reported for this indirect US procedure, due to energy attenuation through bath medium and sample container walls. The application of direct US procedures, by using a probe, is expected to reduce extraction time, being US energy fully delivered to the sample (Chemat, Rombaut, Sicaire, et al., 2017).

Although representing an additional processing step, possibly enhancing global processing time and energy, preliminary cell disruption has been reported to largely improve extraction efficacy (Meullemiestre, Breil, Abert-Vian, & Chemat, 2016). High pressure homogenization (HPH) (§ 5.1) has been reported as pre-treatment for the enhancement of the extraction of different target compounds such as proteins and lipids (Cho et al., 2012; Dong et al., 2011; Safi et al., 2014; Samarasinghe et al., 2012). Despite these evidences, no indications are available about HPH pre-treatments in combination with US for the extraction of polyphenols.

5.3.2 Materials and methods

Crude extract preparation

Lettuce waste was prepared as described in § 3.1. To reduce sample variability without affecting phenol composition, lettuce waste was submitted to freeze-drying. In particular, waste was frozen in single layers at -80 °C for 24 h and freeze dried for 72 h at 4053 Pa by using the pilot plant model Mini Fast 1700 (Edwards Alto Vuoto, Milan, Italy). Waste was then finely ground using a ball mill (MM2, Retsch, Hann, Germania) for 5 min. Ground lettuce waste was then dispersed in hydro-alcoholic solutions at 500 and 750 mL/L ethanol concentration (Carlo Erba, Milan, Italy) and with a lettuce/solvent ratio of 0.02 g/mL, at 20 °C. Lettuce waste dispersions were then immediately submitted to extraction protocols, filtered using 0.45 μm membrane filters (GVS, Meckenheim, Germany) and stored at 4 °C until use.

Traditional solid-liquid extraction

Lettuce waste dispersions were blended using a high-speed homogenizer (Polytron, PT 3000, Cinematica, Littau, Swiss) at 4,000 rpm for 30 s. Then, 5 identical 100 mL samples, each in separate glass vessels (250 mL capacity, 110 mm height, 60 mm internal diameter) were maintained at 50 °C under gentle mixing. At defined time interval (2, 15, 30, 45 and 60 min), one of the samples was randomly selected and analysed.

Ultrasound assisted extraction

An ultrasonic processor (Hieschler Ultrasonics GmbH, mod. UP400S, Teltow, Germany) with a titanium horn tip diameter of 22 mm was used. The instrument operated at constant ultrasound amplitude, power and frequency of 100 µm, 400 W and 24 kHz, respectively. As schematically shown in Figure 12, aliquots of 100 mL of lettuce dispersion were introduced into 250 mL capacity (110 mm height, 60 mm internal diameter) glass vessels. The tip of the sonicator horn was placed in the centre of the solution, with an immersion depth in the fluid of 50 mm. The ultrasound treatments were performed for 20, 70 and 120 s. The temperature was controlled using a cryostatic cooling system set at 4 °C to dissipate the heat generated during the treatment, allowing sample temperature to be maintained at values lower than 50 °C.

High pressure homogenization

The continuous lab-scale high-pressure homogenizer described in § 5.1.2 and schematically shown in Figure 12, was used to treat 150 mL of lettuce dispersion for 1 pass. The first valve was set at increasing pressure of 50 and 100 MPa. The second valve was set at the constant value of 5 MPa.

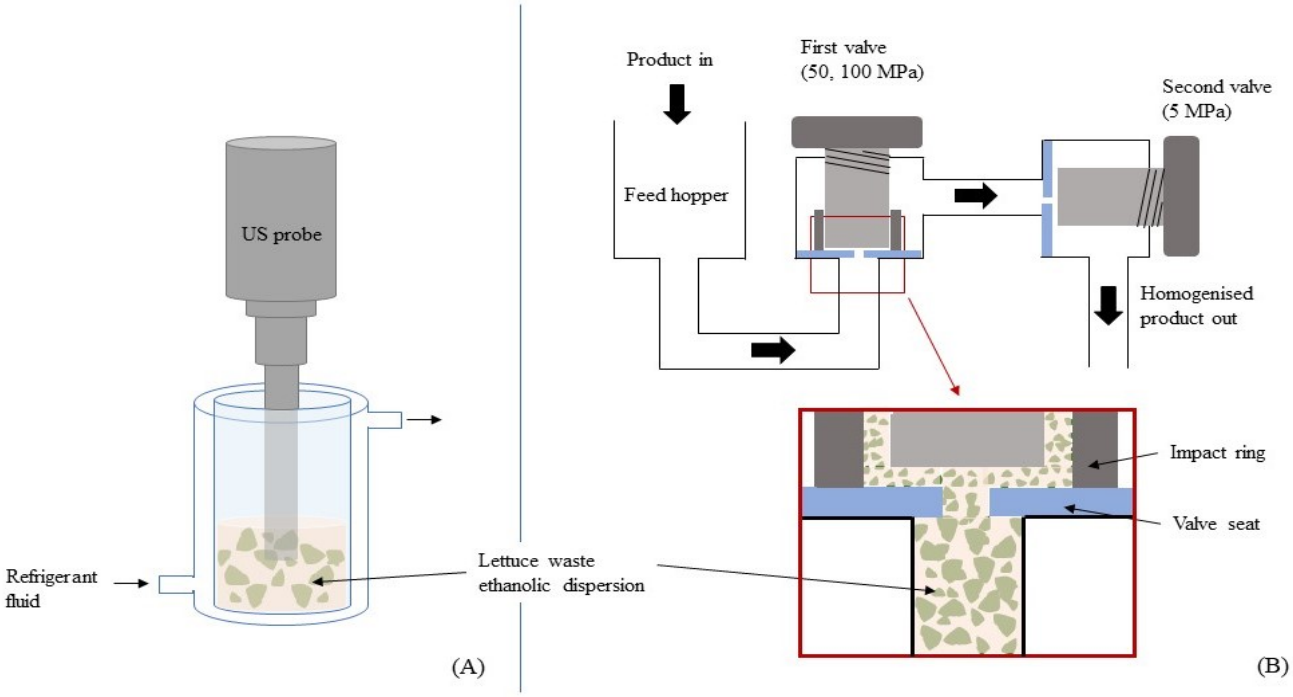


Figure 12. Schematic diagram of the ultrasonication device (A) and of the continuous lab-scale high-pressure homogenizer supplied with two homogenization valves (B) used for the treatment of lettuce waste ethanolic dispersions.

Combined treatments

Lettuce dispersions (150 mL) were subjected to HPH (50 and 100 MPa, 1 pass), 100 mL of sample were collected and immediately subjected to US (20, 70, 120 s). The time between the two treatments did not exceed 10 s.

Analytical determinations

Temperature measurement

Temperature was measured with a copper-constantan thermocouple probe (Ellab, Hillerød, Denmark) connected to a portable data logger (mod. 502A1, Tersid, Milan, Italy).

Optical microscopy

Two droplets of each sample were placed on a glass slide, covered, and observed at room temperature as described in § 5.1.2.

HPLC analysis

Extracts were analysed using a HPLC system equipped with a Prostar 230 pump (Varian, Walnut Creek, USA) and a Prostar 330 diode array detector (Varian, Walnut Creek, California, USA). To this aim, 20 µL extract was injected in a C18 column (Alltima, 5 µm, 250 × 4.6 mm, Grace, Lokeren, Belgium). The mobile phase was water with 50 mL/L formic acid (Fluka, St. Louis, Missouri, USA) (solvent A) and HPLC grade methanol (Chromasol≥99.9%, Sigma-Aldrich St. Louis, Missouri, USA) (solvent B) at a flow rate of 1 mL/min. The linear gradient started with 10% B in A to reach 20% B at 25 min, 50% B at 40 min, 50% B at 45 min and 90% B at 60 min (Llorach et al., 2004). Chromatograms were recorded at 335 nm. Data elaboration was performed by Polyview program (v. 5.3). Phenolic compounds identification was based on their UV spectra and retention times (DuPont et al., 2000; Llorach et al., 2004; Mai & Glomb, 2013; Tomás-Barberán, Loaiza-Velarde, Bonfanti, & Saltveit, 1997). Chicoric acid was quantified (Lee & Scagel, 2013) using an external standard while other compounds were quantified as 3-O-caffeoylquinic acid by comparison with external standard (Sigma-Aldrich, St. Louis, Missouri, USA). To this aim, seven-point external standard calibration curves (concentration 0.4-10 µg/mL) were produced, whose linearity was acceptable (R^2=0.994 and 0.990 for 3-O-caffeoylquinic acid and chicoric acid, respectively).
The total content of polyphenols in the extracts was determined as the sum of the amount of the individually quantified compounds and expressed as µg per mL of extract.

Polyphenoloxidase activity (PPO)

The polyphenoloxidase (PPO) activity was assayed as described in § 5.1.2. The reaction was started by the addition of 500 µL of extract to 2 mL of 0.1 mol/L potassium phosphate buffer pH 7 and $1.5 \cdot 10^{-3}$ mol/L L-Dopa (Carlo Erba, Milan, Italy). PPO specific activity was defined as the amount of enzyme that converted 1 µmol of substrate per min (U), expressed per mg of protein (U/mg). The latter was determined based on *Iceberg* lettuce protein content of official databases (USDA, 2018).

Antioxidant activity (DPPH· assay)

The chain-breaking activity was measured following the bleaching rate of a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH·) in the presence of the sample (Manzocco, Mastrocola, & Nicoli, 1998). A volume of 150 µL of 6.1×10^{-5} mol/L DPPH· (Sigma-Aldrich, St. Louis, Missouri, USA) methanol solution was used. The reaction was started by the addition of 10 µL of lettuce waste extract. DPPH· bleaching was followed using a microplate reader at 515 nm (Sunrise™, Tecan, Männedorf, Switzerland) at 25 °C for 10 min. DPPH· bleaching rate was proportional to sample concentration. Trolox (Sigma-Aldrich, St. Louis, Missouri, USA) was used as the standard for the calibration curve in the assay (5-150 µg/mL, R^2=0.999), and the antioxidant capacity was expressed as µg of Trolox equivalents (TE) per mL of extract.

Energy density

The energy density (E_v, J/mL) transferred from the ultrasound probe to the sample was determined calorimetrically by recording the temperature (T, K) increase during the homogenization process (Raso, Mañas, Pagán, & Sala, 1999). The following eq. 6 was used:

$$E_V = \frac{mC_p(\partial t/\partial T)}{V} \times t \qquad \text{(eq. 6)}$$

where m is the sample mass (g), C_p is the solvent specific heat (2.89 $Jg^{-1}°C^{-1}$), V is the sample volume (mL), δT/δt (°C/s) is the heating rate during the treatment, and t (s) is treatment time.
The energy density transferred from the homogenization valve to the sample was determined as described by Stang, Schschmann, and Schubert (2001), according to eq. 7:

$$E_V = \Delta P \qquad \text{(eq. 7)}$$

where ΔP is the pressure difference operating at the nozzles (MPa).

Statistical analysis

For each set of processing conditions, the experiments were performed in duplicate and each collected sample was analysed in triplicate. The mean values and standard deviations of experimental data were calculated. Statistical analysis was performed by using R (The R foundation for statistical computing, v.3.1.1). Bartlett's test was used to check the homogeneity of variance, one-way ANOVA was carried out and Tukey test was used to determine statistically significant differences among means ($p<0.05$).

5.3.3 Results and discussion

Traditional solid-liquid extraction

Lettuce waste was maintained in contact with hydro-alcoholic solutions at 500 and 750 mL/L ethanol concentration at 50 °C for up to 60 min. In agreement with literature data, these preliminary trials showed that the highest extraction yields were obtained at 750 mL/L ethanol concentration, which was thus selected for further experimentation (DuPont et al., 2000; Llorach et al., 2004; Mai & Glomb, 2013; Viacava et al., 2014, 2015). The chromatographic profile of 750 mL/L hydro-alcoholic extracts obtained by 60 min traditional solid-liquid extraction is reported in Figure 13. The identified phenolic compounds were mainly represented by caffeoylquinic and caffeoyltartaric acid derivatives and, among them, the main derivative identified was dicaffeoyltartaric acid (chicoric acid) (peak 4), followed by caffeoyl tartaric acid (peak 2), in agreement with literature data (Llorach et al., 2008, 2004; Mai & Glomb, 2013). In addition, an isomer of chlorogenic acid (3-O-caffeoylquinic acid, peak 1) and isochlorogenic acid (peak 3) as well as flavonoid compounds (luteolin and quercetin derivatives, peak 5 and 6) were identified. These compounds have been previously reported in *Iceberg* lettuce (Llorach et al., 2008, 2004; Tomás-Barberán et al., 1997).

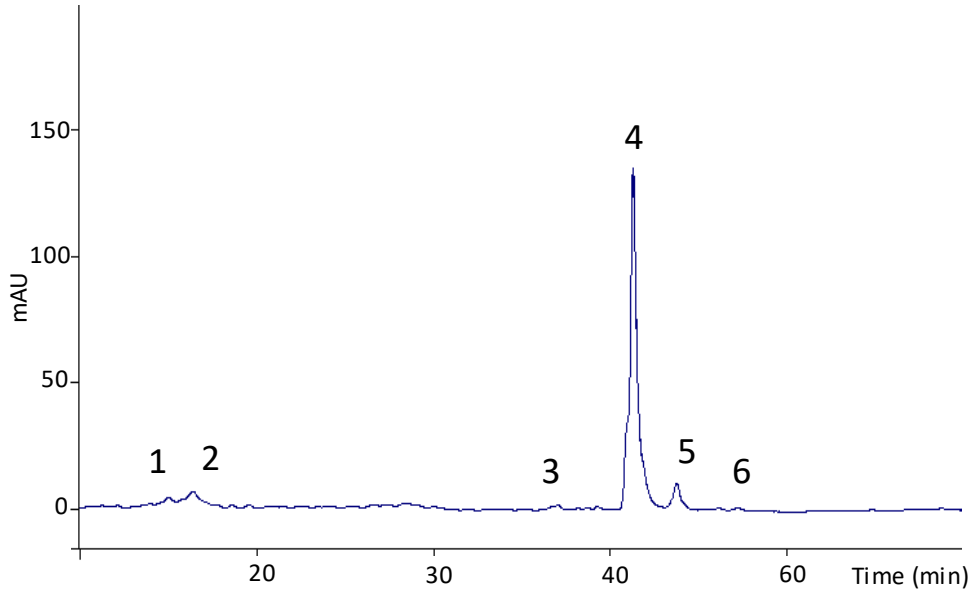


Figure 13. HPLC profile of lettuce waste extract obtained by traditional solid-liquid extraction with 750 mL/L ethanol aqueous solution for 60 min. Peak identification: (1) 3-O-caffeoylquinic acid; (2) caffeoyltartaric acid; (3) isochlorogenic acid; (4) chicoric acid; (5) luteolin-7-O- glucuronide; (6) quercetin 3-O-glucuronide. AU, arbitrary units.

Independently on extraction time up to 60 min, all samples submitted to traditional solid-liquid extraction showed similar phenolic profiles (data not shown). However, extraction time differently affected the amount of the individual phenolic compounds (Table 15). 3-O-caffeoylquinic acid was progressively extracted up to 60 min while the concentration of caffeoyltartaric acid, chicoric acid and luteolin was maximized after 15-30 min extraction. Similarly, the concentration of isochlorogenic acid and quercetin increased up to 45 min extraction while a decrease in their concentration was observed by prolonging extraction time (Table 15). These results can be explained by the possible degradation of phenolic compounds with the increase in extraction time. This agrees with literature studies reporting a maximum increase in extraction efficacy in the first stage of solid-liquid extraction, followed by a decrease, due to the counterbalancing effect of bioactive extraction and degradation (Amendola, De Faveri, & Spigno, 2010; Silva, Rogez, & Larondelle, 2007). As a result, a maximum total phenolic amount of about 50 µg/mL (corresponding to 12.5 mg/100 g fresh weight) was obtained after 15 min of extraction, which was thus selected as control extraction procedure. Despite the inherent vegetable variability and the application of different extraction parameters and quantification methods, the obtained extraction yield is consistent with literature data relevant to traditional solid-liquid extraction of phenols from green-leaf lettuce. For example, Llorach et al. (2008) obtained a total identified phenolic content of 18.2 mg/100 g fresh weight upon extraction with 50% methanol of *Iceberg* lettuce; similarly, Llorach et

al. (2004) obtained a total identified phenolic content of 24 and 14 mg/100 g fresh weight upon thermal extraction (100 °C) of *Iceberg* lettuce waste in water and methanol, respectively. By extracting butterhead lettuce in 70% ethanol solutions, Viacava et al. (2015) obtained a total phenolic content of 44 mg/100 g fresh weight, quantified with Folin-Ciocalteau method. Although this is one of the most used methods for determining TPC in vegetable matrices, it is not specific for phenolic compounds. Rather, it measures the ability of both phenolic and non-phenolic compounds in alkaline medium to reduce the phosphomolybdic/phosphotungstic acid reagent, possibly leading to an overestimation of TPC value (Singleton, Orthofer, & Lamuela-Raventos, 1999). The antioxidant activity of the extracts obtained by the application of traditional solid-liquid extraction was determined using DPPH· method. Consistently with literature data, a positive correlation (R^2=0.81) was found between the phenolic content and the antioxidant activity of the extracts. It is well known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to donate hydrogen atoms or electrons and scavenge free radicals (Altemimi, Choudhary, Watson, & Lightfoot, 2015; Chen et al., 2018; Llorach et al., 2004; Vijayalaxmi, Jayalakshmi, & Sreeramulu, 2015). A maximum antioxidant activity of 88 µg TE/mL (corresponding to 17.7 µmol TE/g and 4.4 mg TE/g of lettuce dry weight) was exerted by the extract obtained upon 15 min extraction (Control). Such value is consistent with values commonly found in green-leaf lettuces.

In this regard, extracts obtained from external leaves of butterhead lettuce by Viacava et al. (2014) resulted of about 12 µmol TE/g dry weight. Similarly, Llorach et al. (2004) found a DPPH· scavenging activity around 20 mg TE/g dry weight in *Iceberg* lettuce waste extracts obtained by thermal extraction up to 60 min.

Table 15. Individual and total (TPC) polyphenol content, and antioxidant activity of lettuce waste extracts obtained by the application of traditional, ultrasound (US) and high pressure homogenisation (HPH) treatments.

Extraction	Time (min)	HPH (MPa)	US (s)	3-O-caffeoylquinic acid (µg/mL)	Caffeoyltartaric acid (µg/mL)	Isochlorogenic acid (µg/mL)	Chicoric acid (µg/mL)	Luteolin 7-O-glucuronide (µg/mL)	Quercetin-3-O-glucuronide (µg/mL)	TPC (µg/mL)	Antioxidant activity (µg TE/mL)
Traditional	2	-	-	2.67 ± 0.01 ^{d}	7.05 ± 0.37 ^{f}	1.39 ± 0.19 ^{ab}	35.26 ± 0.10 ^{de}	2.37 ± 0.01 ^{d}	0.68 ± 0.01 ^{bc}	49.42 ± 0.69 ^{cd}	74.60 ± 1.30 ^{bc}
	15 (Control)	-	-	3.45 ± 0.01 ^{f}	8.06 ± 0.07 ^{g}	1.38 ± 0.34 ^{ab}	38.09 ± 0.17 ^{fg}	3.00 ± 0.09 ^{e}	0.70 ± 0.02 ^{bc}	54.68 ± 0.70 ^{f}	88.42 ± 1.77 ^{ef}
	30	-	-	3.46 ± 0.03 ^{f}	7.84 ± 0.04 ^{g}	1.43 ± 0.03 ^{ab}	34.42 ± 0.29 ^{de}	4.24 ± 0.01 ^{g}	0.60 ± 0.03 ^{ab}	51.09 ± 0.45 ^{de}	87.70 ± 1.90 ^{ef}
	45	-	-	3.84 ± 0.05 ^{g}	6.47 ± 0.05 ^{de}	2.25 ± 0.1 ^{b}	36.13 ± 0.49 ^{ef}	1.57 ± 0.02 ^{c}	0.94 ± 0.06 ^{ef}	51.20 ± 0.80 ^{de}	77.11 ± 2.73 ^{cd}
	60	-	-	5.10 ± 0.17 ^{i}	6.33 ± 0.24 ^{de}	1.19 ± 0.01 ^{a}	34.62 ± 0.19 ^{de}	0.47 ± 0.01 ^{a}	0.84 ± 0.05 ^{cd}	48.55 ± 0.28 ^{cd}	64.11 ± 2.27 ^{a}
US	-	-	20	3.04 ± 0.04 ^{e}	6.65 ± 0.02 ^{ef}	2.12 ± 0.14 ^{b}	39.20 ± 0.49 ^{g}	0.51 ± 0.01 ^{a}	0.59 ± 0.01 ^{a}	52.11 ± 0.70 ^{ef}	92.71 ± 3.26 ^{fg}
	-	-	70	8.72 ± 0.0 ^{j}	9.61 ± 0.01 ^{i}	3.80 ± 0.24 ^{c}	44.52 ± 0.13 ^{h}	1.69 ± 0.01 ^{c}	0.88 ± 0.01 ^{cd}	69.72 ± 0.49 ^{h}	99.00 ± 1.90 ^{gh}
	-	-	120	9.11 ± 0.17 ^{k}	12.37 ± 0.01 ^{j}	4.93 ± 0.43 ^{d}	49.02 ± 0.01 ^{i}	4.05 ± 0.08 ^{g}	1.14 ± 0.19 ^{fg}	80.62 ± 0.89 ^{i}	101.31 ± 2.45 ^{h}
HPH	-	50	-	2.54 ± 0.01 ^{d}	6.10 ± 0.14 ^{d}	1.92 ± 0.34 ^{ab}	25.32 ± 0.17 ^{ab}	0.47 ± 0.09 ^{a}	0.95 ± 0.02 ^{ef}	37.30 ± 0.77 ^{a}	81.88 ± 0.27 ^{de}
	-	100	-	2.12 ± 0.02 ^{c}	5.97 ± 0.06 ^{cd}	1.69 ± 0.11 ^{ab}	29.09 ± 0.29 ^{c}	1.03 ± 0.01 ^{b}	1.30 ± 0.04 ^{g}	41.40 ± 0.31 ^{b}	88.18 ± 2.01 ^{ef}
HPH-US	-	50	20	2.14 ± 0.0.02 ^{c}	7.12 ± 0.01 ^{f}	1.90 ± 0.05 ^{ab}	24.45 ± 0.30 ^{a}	3.45 ± 0.15 ^{f}	0.69 ± 0.01 ^{bc}	39.84 ± 0.52 ^{ab}	71.69 ± 0.82 ^{ab}
	-	50	70	1.57 ± 0.03 ^{a}	7.17 ± 0.03 ^{f}	1.99 ± 0.24 ^{ab}	25.13 ± 0.81 ^{ab}	5.70 ± 0.03 ^{h}	0.70 ± 0.01 ^{bc}	42.27 ± 1.07 ^{b}	70.67 ± 1.09 ^{ab}
	-	50	120	1.65 ± 0.06 ^{a}	5.39 ± 0.13 ^{b}	2.09 ± 0.43 ^{ab}	25.05 ± 1.38 ^{ab}	8.41 ± 0.16 ^{i}	0.63 ± 0.05 ^{ab}	43.22 ± 1.84 ^{b}	89.00 ± 2.46 ^{ef}
	-	100	20	1.73 ± 0.01 ^{ab}	4.81 ± 0.20 ^{a}	4.37 ± 0.05 ^{cd}	29.38 ± 0.48 ^{c}	5.87 ± 0.02 ^{h}	1.58 ± 0.09 ^{h}	47.74 ± 0.11 ^{c}	84.96 ± 1.79 ^{df}
	-	100	70	2.00 ± 0.10 ^{bc}	5.47 ± 0.02 ^{bc}	4.41 ± 0.18 ^{cd}	26.91 ± 0.71 ^{b}	8.72 ± 0.01 ^{j}	1.29 ± 0.02 ^{g}	48.80 ± 1.04 ^{cd}	90.54 ± 4.08 ^{fg}
	-	100	120	4.23 ± 0.19 ^{h}	8.61 ± 0.01 ^{h}	3.57 ± 0.03 ^{c}	32.92 ± 1.20 ^{d}	9.55 ± 0.04 k	1.29 ± 0.03 ^{g}	60.77 ± 1.50 ^{g}	92.65 ± 2.67 ^{fg}

$^{a-k}$: In the same column, mean values indicated by different letters are significantly different ($p<0.05$)

Ultrasound assisted extraction

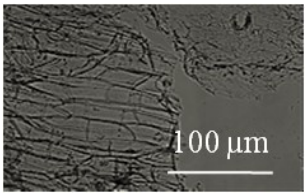
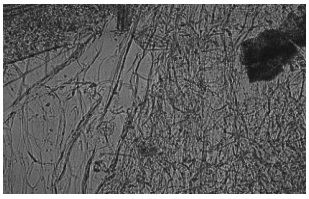
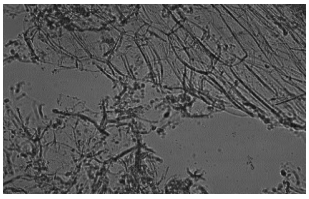
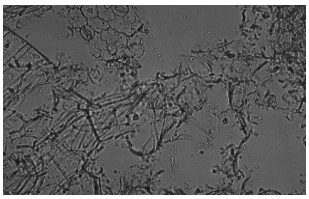
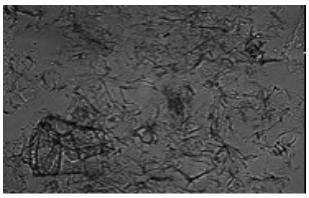
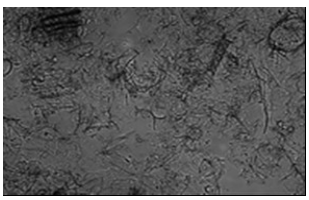
Data showing the effect of US treatments at increasing time (20, 70, 120 s) on phenolic concentration of lettuce waste extracts are reported in Table 15. The 20 s US treatment resulted in a total polyphenol amount not significantly different from that of the control extract obtained by traditional extraction carried out for 15 min. However, the application of US treatments for 70 and 120 s resulted in a 28 and 47% increase in phenolic extraction (Table 15). Low frequency US, usually in the range 20-50 kHz, have been widely reported as efficient strategy for phenol extraction from different vegetable waste materials (Kahn, 1985; Pingret et al., 2012). Microscopic images of lettuce tissue submitted to traditional control extraction and to US treatments for increasing time are reported in Table 16. Compared to control sample, which showed well-maintained cell structures, a progressive loss of cell integrity was observed with the increase in US treatment. A similar progressive destruction of cellular organization upon US application was also observed on other vegetable materials such as tomato, microalgal biomass and onion (Anese, Mirolo, Beraldo, & Lippe, 2013; Halim, Rupasinghe, Tull, & Webley, 2013; Rajewska & Mierzwa, 2017). Since the main accumulation sites of soluble phenols in lettuce are represented by vacuoles (Goupy, Varoquaux, Nicolas, & Macheix, 1990), it can be concluded that US promoted the permeabilization of these cellular structures, leading to phenol extraction. In this regard, recent studies conducted on rosemary leaves and eggplant peels proved that the effects of US are not limited to vegetable surface but involve several subsequent mechanisms, leading to the gradual physical damage of vegetable tissue and thus intra-cellular organelles (Ferarsa et al., 2018; Khadhraoui et al., 2018). Such chain of mechanisms promoted by US cavitation starts with the erosion of surface leaf structures (e.g. waxy cuticles, intact and broken trichomes, stomata) and proceeds with the deformation of remaining surface structures by US shear forces, generating micro-fractures. The latter increase in size and number (sonoporation), finally leading to tissue fragmentation. The resulting increase in surface area exposed to the extraction solvent favours its capillary penetration into inner structures, enhancing phenol extraction. Upon further US treatment, complete tissue destructuration is obtained (Khadhraoui et al., 2018). Based on microscopic analysis (Table 16), the application of US treatments up to 120 s was not able to cause complete lettuce tissue destructuration, since many intact cells were still present. It can be thus hypothesized that a further increase in US time would lead to more intense destructuration and thus to higher phenolic concentrations. However, literature data indicate that the increase in US treatment time is usually associated to an initial fast extraction phase and a subsequent decrease in extraction rate until reaching a plateau value, possibly followed by a the decrease of the target compound concentration (Chan, See, Yusoff, Ngoh, & Kow, 2017; Chan, Yusoff, & Ngoh, 2014; Pan, Qu, Ma, Atungulu, & McHugh, 2012). This has been attributed to phenol degradation during US, due to extreme pressure and temperature conditions reached in the hot-spots generated by cavitation bubble collapse as well as to the loss of metal by the sonication probe surface, which can induce radical formation (Meullemiestre et al., 2016). In our experimental

conditions, a progressively increasing phenolic concentration of the extracts, despite the increase in treatment time, was observed. This can be attributed to the mild conditions applied in the present work (times shorter than 120 s and temperature values lower than 50 °C), possibly accounting for reduced phenol degradation phenomena. This hypothesis is further supported by the absence of changes in the phenolic profile (data not shown) as compared to that of the sample obtained by traditional extraction (Figure 13).

It can be inferred that treatments more energetic than those here applied would be required to reach the extraction plateau. In this regard, Chan et al. (2017) reported energy density (E_V) (eq. 6) higher than 250 J/mL to be required for reaching the maximum extraction of Java tea bioactive compounds by using a 70% ethanolic solution. In our conditions, the E_V transferred to samples by 20, 70 and 120 s US treatments resulted lower than 150 J/mL (Table 16), suggesting that further increase in treatment energy could lead to higher extraction performances.

Consistently with polyphenolic extraction data, the antioxidant activity of samples obtained by US treatments progressively increased with sonication time (Table 15). Extracts obtained by the application of 20 s showed an antioxidant activity similar to that of the control sample ($p \geq 0.05$). Upon 70 and 120 s US treatment, extracts showed an increase in antioxidant activity of 12 and 15%, respectively. Also in this case, a strong positive correlation ($R^2=0.98$) was found between TPC and antioxidant activity of sonicated lettuce waste extracts.

Table 16. Microscopic images and polyphneoloxidase (PPO) activity of control lettuce waste extracts and of extracts obtained by the application of ultrasounds (US) for 20, 70 and 120 s, and high pressure homogenisation (HPH) at 50 and 100 MPa. The energy density (E_V) of HPH and US treatments is also reported.

Extraction	**HPH (MPa)**	**US (s)**	**Ev (J/mL)**	**Microscopic image**	**PPO activity (U/mg)**
Traditional (15 min, control)	-	-	-		3.49 ± 0.03 [a]
US	-	20	23.4		3.43 ± 0.05 [a]
	-	70	81.9		3.45 ± 0.07 [a]
	-	120	140.4		3.37 ± 0.05 [a]
HPH	50	-	50.0		4.82 ± 0.07 [b]
	100	-	100.0		4.63 ± 0.26 [b]

[a-b]: In the same column, mean values indicated by different letters are significantly different ($p<0.05$)

Effect of high pressure homogenization pre-treatment

HPH has been widely reported to increase the extractability of plant bioactive compounds by disrupting vegetable tissues (Guan et al., 2016; Velázquez-Estrada, Hernández-Herrero, Rüfer, Guamis-López, & Roig-Sagués, 2013). For this reason, it has been suggested as a pre-treatment for increasing the yield of target compounds during a subsequent extraction process (Cho et al., 2012; Safi et al., 2014; Spiden et al., 2013). Table 16 reports the light microscope images of lettuce waste extracts obtained upon 50 and 100 MPa HPH treatment. The disruption efficacy of HPH increased with the applied pressure. Samples treated at 50 MPa still presented a number of intact cells, although the broken cell material was the most abundant. By contrast, no intact cells were observed in the samples submitted to 100 MPa, in which the broken cell material was quite uniformly distributed. The increase of HPH pressure results in an increase of energy density (E_V) transferred to the sample (eq. 7, Table 16), due to the intensification of forces experienced by vegetable particles. In this regard, several mechanisms underly HPH efficiency in cell breakage, favouring the release of target compounds. A pressure increase resulted in a higher pumping speed of the lettuce ethanolic dispersion and thus in a more intense collision of suspended particles with valve seat (Figure 12). During the passage through the valve, sample particles underwent fluid dynamic stresses, initially leading to their deformation and, beyond a certain pressure value, to their breakage. The sudden pressure drop at the valve exit induced rapid sample acceleration and cavitation, leading to high kinetic energy, which is responsible for intensive collisions among particles and between particles and instrument walls (especially the so-defined impact ring, Figure 12) (Coccaro, Ferrari, & Donsì, 2018). Since in this experimentation a two-stage homogenizer was used (Figure 12), also the back pressure (5 MPa) applied to the second valve allowed increasing cavitation intensity and breaking aggregates, possibly formed by inter-particle collisions (Freudig, Tesch, & Schubert, 2003; Sharabi, Okun, & Shpigelman, 2018). Differently from US, thus, HPH-induced tissue disruption is not gradual, but involves concomitant events, so that energy density lower than that applied by US was required to obtain even higher tissue destructuration levels (Table 16).

Although tissue disruption induced by HPH resulted much more intense than that promoted by US (Table 16), HPH carried out as a pre-treatment to US extraction resulted in lower phenolic yields as compared to US solely (Table 15). For example, the 50 and 100 MPa HPH pre-treatments associated to 120 s resulted in a TPC value about 46 and 25% lower than that obtained by the application of 120 s US treatment alone. These results suggest that cellular disruption solely cannot account for a higher efficacy of US assisted extraction.

The inefficacy of HPH treatments can be possibly explained by the decompartmentalising effect of this treatment on the oxidative enzymes entrapped in the plant matrix. As already discussed in § 5.1.3, it can be inferred that, upon cell disruption induced by HPH (Table 16), polyphenoloxidase (PPO), which has been reported to be highly active in *Iceberg* lettuce, was no longer separated from

its phenolic substrates, which were thus oxidised (Butz, Koller, Tauscher, & Wolf, 1994; Guan et al., 2016; Mai & Glomb, 2013). In addition, cell wounding could promote the release of proteases, responsible for the activation of latent PPO which, differently from the free soluble one, is bounded to the cellular membrane. In particular, latent PPO forms have been reported to account for about 50% of total PPO in *Iceberg* lettuce (Cantos et al., 2001). To confirm this hypothesis, PPO activity upon extraction treatments was determined. The average PPO activity in control extract was found to be about 3.5 U/mg of proteins (Table 16). This value resulted much lower than that found by Mai and Glomb (2013) in the external leaves of *Iceberg* lettuce (about 90 U/mg of proteins), probably due to the procedure used for preparing the extract. The latter, in fact, were obtained from freeze-dried lettuce and presented a 750 mL/L ethanol liquid phase. Both frozen storage and ethanol presence have been reported to hinder PPO activity (Cantos et al., 2001; Lerici & Manzocco, 2000). US treatments did not modify this activity value ($p \geq 0.05$), suggesting that applied US treatments did not cause changes in PPO conformation. In this regard, PPO inactivation induced by US has been attributed to the modification of secondary and tertiary protein structure upon localized increase of pressure and temperature, and strong shear stress associated to acoustic cavitation. Obtained results confirm literature evidences, in which times longer than 5 min in the same US conditions applied in this study (400 W, 24 kHz, temperature lower than 50 °C) were required to promote a slight decrease in PPO activity (Bot et al., 2018).

By contrast, HPH treatments at 50 and 100 MPa promoted the activation of PPO, which resulted 40 and 34% higher than that of control extract, respectively (Table 16). This activation can be attributed not only to the disruption of cellular structures that physically separate phenols from PPO but also to the possible effect of HPH on PPO conformation and activity. In this regard, a progressive PPO activation was observed also in Chinese pear and mushroom submitted to high pressure microfluidisation at pressures in the range from 80 to 200 MPa (Liu, Liu, Liu, et al., 2009; Liu, Liu, Xie, et al., 2009), while inactivation has been mostly reported only upon multiple passes in the homogenization valve, due to the combined effect of pressure and thermal effect of these treatments (Bot et al., 2018). The effect of HPH on the specific phenolic compounds (Table 15) could represent a further evidence of the implication of PPO in phenol extraction yield. HPH pre-treatment reduced the extraction of caffeoyl phenolic compounds (3-O-caffeoylquinic acid, caffeoyltartaric acid, isochlorogenic acid, chicoric acid) to a higher extent than the flavonoids luteolin-7-O-glucuronide and quercetin-3-O-glucuronide. This can be attributed to the higher affinity of PPO towards caffeoyl derivatives, which are much better substrates for PPO than flavonoids (Goupy et al., 1990). As expected, the loss of phenolic compounds upon HPH pre-treatments negatively affected the antioxidant activity of the extracts, leading to values 10-30% lower than those obtained without applying HPH before sonication (Table 15).

5.3.4 Conclusions

Lettuce waste can be considered a cheap and always available source of antioxidant polyphenols, to be potentially used in the food and pharmaceutical sectors. Although further optimisation of processing parameters (e.g. solvent/sample ratio, temperature, time) is needed, ultrasounds can be efficaciously exploited to rapidly prepare food-grade polyphenol extracts from lettuce waste.

Despite the complete disruption of cellular organization, the application of high pressure homogenization pre-treatments can be detrimental for ultrasound extraction yields, probably due to the activation of oxidative enzymes.

The application of alternative pre-treatments able to enhance polyphenolic yield by disrupting cell integrity while inactivating polyphenoloxidase should be considered to further enhance ultrasound extraction efficacy. In this regard, blanching was shown in § 5.1 to favour tissue homogenisation, possibly enhancing phenol extraction. Similarly, microwaves have been reported to be an efficient extraction technique and their efficacy in the extraction of functional compounds from peach pomace is reported in § 5.4.

5.4 Valorisation of peach pomace by means of optimized microwave and ultrasound assisted extraction of bioactive compounds

Based on results presented in § 5.3 relevant to the extraction of antioxidant compounds from lettuce waste by using ultrasounds, the possibility to exploit ultrasound assisted extraction (UAE) on peach pomace was investigated. In addition, another innovative technology, based on microwave assisted extraction (MAE) was tested. In particular, the aim of the present study was to identify optimal conditions of MAE and UAE of antioxidant bioactive compounds from the industrial discard resulting from the extraction of peach juice, either frozen or dried. To this aim, frozen and air-dried peach waste were submitted to 70%-ethanol extraction assisted either by MAE or UAE. MAE power, UAE amplitude and time were optimized using a 2^2-factorial design. Extracts were analysed for colour, browning index, content of polyphenols, flavonoids, anthocyanins, vitamin C, and antioxidant activity. In addition, the effects of MAE and UAE were compared based on the energy density involved in the extraction process, to possibly identify the more sustainable process. This part of the Ph.D. project was conducted at the University of Lleida (Spain), taking advantage of the scientific collaboration with prof. Olga Martín-Belloso.

5.4.1 Introduction

Based on its rich composition (Table 4), peach waste has been exploited for the extraction of bioactive compounds such as polyphenols, anthocyanins and carotenoids (Adil et al., 2007; Grigelmo-Miguel et al., 1999; Redondo, Venturini, Luengo, Raso, & Arias, 2018; Vargas et al., 2017; Xu, Jiao, Yuan, & Gao, 2015). As reported for other fruit and vegetable waste, the main issue related to peach waste management is its high moisture content, making it quickly prone to microbial spoilage (Ajila et al., 2012). For this reason, peach waste must be frozen or dried to be available for further processing. Both strategies present advantages and drawbacks. Freezing is expected to maintain bioactive compounds of peach waste but requires low-temperature for its storage and transport, leading to high management costs (Li et al., 2013). By contrast, peach waste flour is expected to be microbiologically stable at ambient temperature and have lower volume, thus reducing packaging, storage and transport costs (Karam, Petit, Zimmer, Djantou, & Scher, 2016). However, peach waste drying is energy consuming, due to the high moisture content that must be removed (Ferreira et al., 2015; Nilnakara, Chiewchan, & Devahastin, 2009). Moreover, the drying process is expected to significantly reduce peach waste bioactive content, due to thermal damage caused by the high temperatures reached (Ratti, 2001).

The extraction of bioactive compounds from frozen or dry peach waste could be carried out by the application of energy-efficient, rapid, inexpensive and environmentally friendly extraction techniques (Kumari, Tiwari, Hossain, Brunton, & Rai, 2018). Microwaves have been reported to be an efficient method for the extraction of bioactive compounds from vegetable matrices (Ameer, Shahbaz, & Kwon, 2017). Microwave assisted extraction (MAE) is based on electromagnetic radiation and usually operates at the frequency of 2.45 GHz. Microwave treatments favour extraction from plant materials due to two basic principles: ionic conduction and dipole rotation. Ionic conduction refers to the migration of the charge species, such as ions, under the effect of the microwave-induced electric field. Dipole rotation involves dipolar molecules, such as water, attempting to align themselves with the alternating electric field under microwave treatment. Both ion migration and dipole oscillation generate a "friction" between the moving molecules and the medium enhancing the extraction (Chan et al., 2017; Chan, Yusoff, & Ngoh, 2013; Vinatoru, Mason, & Calinescu, 2017). The increase of internal pressure of the cell, can disrupt plant structure, favouring the release of target bioactive compounds (Chen & Spiro, 1994).

Ultrasound assisted extraction (UAE) has been also widely exploited in the last years as an efficient extraction technique. As previously detailed in § 5.3, UAE exploits the propagation of high energy sound waves (20-24 kHz) into the extraction solvent (Medina-Torres, Ayora-Talavera, Espinosa-Andrews, Sanchez-Contreras, & Pacheco, 2017), leading to alternate phases of compression and rarefaction, promoting cavitation. Acoustic cavitation, associated with power dissipation, is considered the driving force in sonochemical induced effects. This phenomenon refers to the formation, growing and subsequent collapse of cavitation bubbles (Canselier, Delmas, Wilhelm, Abismail, & Abismaïl, 2002). The implosion, in turn, is related to extremely high energies producing local increase of pressure and temperature, as well as microjets and shockwaves that can disrupt external structure of plant tissues, effectively releasing target bioactive compounds (Chan et al., 2017).

UAE and MAE were thus applied to peach pomace and compared to identify the more efficient technique.

5.4.2 Materials and methods

Reagents

The used reagents were absolute ethanol (Scharlau, Barcelona, Spain.), bidestilled water (Milli-Q system, Millipore, Bedford, USA), Folin-Ciocalteau reagent (Sigma Aldrich, St. Louis, U.S.A.), Sodium carbonate (Carlo Erba, Milan, Italy) gallic acid 97% (Sigma Aldrich, St. Louis, U.S.A.), Sodium acetate anhydrous (Sigma Aldrich, St. Louis, U.S.A.), Aluminium chloride (Sigma Aldrich, St. Louis, U.S.A.), Quercetin: 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (Sigma Aldrich, St. Louis, U.S.A.), 1,4-dithiothreitol (DTT) (Sigma Aldrich, St. Louis, U.S.A.), Potassium chloride (Sigma Aldrich, St. Louis, U.S.A.), Meta-phosphoric acid (Sigma Aldrich, St. Louis, U.S.A.), ABTS: 2,2'azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (Sigma Aldrich, St. Louis, U.S.A.), Trolox: (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 97% (Sigma Aldrich, St. Louis, U.S.A.).

Peach waste

The peach waste, residue from peach juice extraction, either frozen (-18 °C) or dried (140 °C) and ground was kindly furnished by Indulleida S.A. (Alguaire, Lleida, Spain). Moisture content of the wastes were determined by placing about 2 g of waste in previously dried and weighed containers and keeping them at 103 ± 5 °C until constant weight (AOAC, 1997). Frozen peach waste was thawed at 4 °C prior to use. Both frozen and dry peach wastes were used at room temperature (20 °C).

Extract preparation

Peach waste was prepared as described in § 3.1. Based on the humidity content of frozen and dry peach waste (84.5 ± 0.3%, w/w and 4.0 ± 1.0%, w/w, respectively), peach waste dispersions at 70% (w/w) ethanol concentration were obtained. Frozen peach waste dispersions were manually mixed for 1 min and submitted to different extraction protocols. After that, dispersions were filtered using 1.2 µm filters (Sartorius 17593-100 cellulose acetate 25 mm/1.20 µm, Filtros, Anoia, Spain) to remove the solid residue, obtaining the extracts. The latter were stored in the dark at -18 °C until analysis.

Ultrasound-assisted extraction (UAE)

An ultrasonic processor (Hieschler Ultrasonics GmbH, mod. UP400S, Teltow, Germany) with a titanium horn tip diameter of 10 mm was used. The instrument operated at constant sound wave frequency of 24 kHz. Peach waste suspensions were introduced into 250 mL capacity plastic truncated vessels (110 mm height, 90 mm upper internal diameter, 60 mm lower internal diameter). The tip of the sonicator horn was placed in the centre of the sample, with an immersion depth in the fluid of 20 mm. Treatments were performed on 120 g of peach waste dispersions at different

amplitude (12, 69 and 125 µm) and time (up to 16 min and 36 s. Samples were covered with a plastic cap to avoid solvent evaporation during treatments.

Microwave-assisted extraction (MAE)

A domestic microwave (Mig225, Orbegozo, Murcia, Spain) was used. Peach waste suspensions were introduced into 1000 mL capacity glass bekers (145 mm height, 110 mm internal diameter). Treatments were performed on 120 g of peach waste dispersions at different nominal power (180, 540 and 900 W) and time (up to 4 min and 12 s). Samples were covered with a plastic cap to avoid solvent evaporation during treatments.

Analytical determinations

Temperature measurement

Sample temperature was measured during extraction for thermal control and UAE, just before and immediately after MAE process by a copper-constantan thermocouple probe connected to a portable data logger (mod. TP100, XS Instruments, Carpi, Italy).

Colour

Colour was determined with a light source C with a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan). The instrument was set up for illuminant D65 and 10º observer angle and standardized against a white reflector plate (CR-A43 Calibration Plate, Minolta, Osaka, Japan) before use. A volume of 3 mL of extract was poured in a CM-A128 petri dish (Minolta, Osaka, Japan) and the latter was then positioned on the measuring head (CR-A33b Light Projection Tube, Minolta, Osaka, Japan). Colour was expressed in L*, a* and b* CIELAB scale parameters.

Browning index (BI)

Browning index (BI) was obtained by measuring absorbance values at 420 nm of extracts (UV/VIS Thermo Multiskan Spectrum spectrophotometer, Thermo Scientific, Waltham, USA), diluted with water to obtain absorbance signals within the scale. BI was calculated as the measured absorbance multiplied by the dilution factor (Buera, Chirife, Resnik, & Wetzler, 1987).

Total phenolic content (TPC)

Total phenolic content was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1985), adapted to a 96-well microplate. A portion of 20 µL of ethanolic extract was mixed with 100µL of Folin-Ciocalteau reagent (100 mL/L) and 80 µL of Na_2CO_3 solution (0.15 g/mL). Samples were mixed and stored at room temperature in darkness for 90 min. Absorbance was measured at 750 nm using a UV/VIS Thermo Multiskan Spectrum spectrophotometer (Thermo Scientific, Waltham, USA). Ethanol blanks (70%, w/w) were run in each assay. Calibration curve was built

with gallic acid (0–500 mg/L). Results were expressed as mg of gallic acid equivalents (GAE) per 100 g of dry matter (dm).

Total flavonoid content (TF)

Total flavonoids were evaluated using the method of Humadi and Istudor (2008), adapted to a 96-well microplate. An amount of 25µL of the ethanolic extract was added with140 µL of deionized water, 10µL of $C_2H_3NaO_2$ (1 M) and 10 µL of $AlCl_3$ (0.1 g/mL). The mixture was mixed and stored in the dark for 40 min. The absorbance was determined at 405 nm using a UV/VIS Thermo Multiskan Spectrum spectrophotometer (Thermo Scientific, Waltham, USA). Blanks containing water instead of $C_2H_3NaO_2$and$AlCl_3$were run in each assay. Calibration curve was built with quercetin (0–1000 mg/L). Results were expressed as mg of quercetin equivalents (QE) per 100 g (dm).

Total anthocyanin content (TA)

Total anthocyanin content was evaluated by differential pH method (Chaovanalikit & Wrolstad, 2004). A portion of 2.5 mL of extract was added to 2.5 mL of 45 g/L metaphosphoric acid solution containing 1,4-Dithiothreitol (7.2 g/L). An aliquot of the sample (1 mL) was added with 1 mL of pH 1.0 buffer (0.025M potassium chloride). Similarly, 1 mL of the sample was added with1 mL of pH 4.5 buffer (0.4 M sodium acetate). Absorbance (A) was measured using a CECIL 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) at 520 and 700 nm. Absorbance was calculated as reported in (eq. 1):

$$A = [(A_{520} - A_{700})pH1.0] - [(A_{520} - A_{700})pH4.5] \quad \text{(eq. 8)}$$

The total anthocyanidin content (TA, mg/L) was calculated using (eq. 2):

$$TA = \frac{A \times W \times DF \times 1000}{\varepsilon \times L} \quad \text{(eq. 9)}$$

where A is absorbance, εis the cyanindin-3-glucoside molar absorption coefficient (26,900 $L \cdot mol^{-1} \cdot cm^{-1}$), L is the cell path length (1 cm), W is the molecular weight of cyanindin-3-glucoside (449.2 Da), DF is the dilution factor. Data were expressed as mg of cyanindin-3-glucoside equivalents (CGE) per 100 g (dm).

Vitamin C content (VIT C)

The extraction was based on the procedure proposed by Odriozola-Serrano, Hernández-Jover and Martín-Belloso (2007). A portion of 2.5 mL of extract was added to 2.5 mL of 45 g/L metaphosphoric solution containing 1,4-Dithiothreitol (7.2 $g \cdot L^{-1}$). The mixture was homogenized, passed through a Millipore 0.45 µm membrane and injected in the HPLC system. The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA) working at 245 nm. Samples were introduced onto the column through a manual injector equipped with a

sample loop (20 ll). The flow rate was fixed at 1.0 mL/min at room temperature. A reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6 mm 250 mm) was used as stationary phase. The mobile phase was a 0.1 g/L solution of sulphuric acid adjusted to pH 2.6. Calibration curve was built with L-ascorbic acid (0-50 mg/L). Results were expressed as mg of VIT C per 100 g (dm).

Antioxidant activity (AA)

TEAC assay was used to assess extract antioxidant activity. The assay was performed according to Al-Duais, Müller, Böhm and Jetschke (2009) with some modifications. An amount of 25 mL of $ABTS^{\cdot+}$ radical cation aqueous solution (7 mM) was added with 25 mL of $K_2S_2O_8$ aqueous solution (2.45 mM) and stored in the dark for 16 h at room temperature. The solution was diluted with ethanol up to an absorbance at 750 nm of 0.700 (± 10%) and maintained at 30 °C. An amount of 10 µL of ethanolic extract was added with the prepared diluted solution and mixed. Absorbance was read at 750 nm after 5 min using a UV/VIS Thermo Multiskan Spectrum spectrophotometer (Thermo Scientific, Waltham, USA) and 70% (w/w) ethanol blanks were run in each assay. Calibration curve was built with trolox (0.005–0.250 mg/L). Results were expressed as mmol of trolox equivalents (TE) per 100 g dm).

Energy density

As anticipated in § 5.3.2, energy density (E_V, J/mL) can be defined as the total heating energy experienced by a unit volume of extraction solvent during microwave or ultrasonic treatment and can be calculated according to eq. 10 (Chan et al., 2017):

$$E_V = P_V \cdot t \qquad \text{(eq. 10)}$$

where P_V is the power density (W/mL) and t is the treatment time (s). Power density indicates the heating power experienced by a unit volume of extraction solvent under microwave or ultrasonication (eq. 11).

$$P_V = \frac{m\, C_P \left(\frac{\partial T}{\partial t}\right)}{V} \qquad \text{(eq. 11)}$$

where m is the sample mass (g), C_p is the solvent specific heat (2.964 $Jg^{-1}°C^{-1}$), V is the sample volume (mL) and δT/δt (°C/s) is the heating rate during the treatment.

Experimental design

A response surface methodology (RSM) was used to evaluate the effect of MAE and UAE on TPC, TF, TA, VIT C and AA of frozen and dry peach waste extracts. A 2^2-factorial design was proposed, considering MAE power and treatment time or UAE amplitude and treatment time. For each factor, extreme lower and upper values were identified and combined to form the factorial part of the design (4 factorial points). MAE power was set at 180 and 900 W, MAE time at 10 and 50 s, UAE

amplitude at 12 µm and 125 µm, UAE time at 20 and 120 s. To complete the design, 1 central point (combination of the intermediate values of the two factors) was defined. For MAE the central point conditions were 540 W and 30 s; for UAE the central point conditions were 69 µm amplitude and 70 s. All the factorial points were replicated twice, and the two replicates were assigned to two different blocks. Every analytical analysis was carried out in triplicate. The central point was replicated 3 times in each block. The full set of sampling points for frozen and dry peach pomace is reported in Table 17, Table 18, Table 19 and Table 20. RSM was chosen to analyse the experimental results because it offers a large amount of information with a smaller number of experiments, with respect to other traditional experimental designs, and it allows observing the interaction effect of the independent parameters on the response (Baş & Boyaci, 2007). Experimental data were fitted to a polynomial response surface. The response function was predicted by the following eq. 12:

$$Y_i = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k-1} \sum_{j>1}^{k} \beta_{ij} X_i X_j \qquad \text{(eq. 12)}$$

where Y is the response, B_0, B_i and B_{ij} are the constant, linear and interaction regression coefficients, respectively, and X_i represents the independent variables. RSM was employed for the experimental design, data analysis, model building, and contour plot generation using the Design Expert 7.01 software (Stat Ease Inc., Minneapolis, MN, USA).

Validation, optimization of the predictive models and comparison of MAE and UAE

A set of 12 experiments was carried out to validate the developed predictive models. The determination coefficients between the predicted and the experimental data were taken as indicator of prediction accuracy. An optimization in the range of the studied parameters was carried out according to the method described by Derringer and Suich (1980). The highest desirability represented the most adequate condition to reach the highest levels of TPC, TF, TA, VIT C and AA in the extracts. Extracts obtained by MAE and UAE under the optimized conditions were compared to determine differences among treatments, using a one-way analysis of variance (ANOVA) ($p<0.05$), performed using R (The R foundation for statistical computing, v.3.1.1). Optimal MAE and UAE treatments were compared based on developed energy density (eq. 10).

5.4.3 Results

Temperature

Temperatures of extracts obtained from frozen and dry peach waste after the application of MAE or UAE are shown in Table 17, Table 18, Table 19 and Table 20. Both frozen and dry peach wastes extracts showed the same temperature pattern when submitted to MAE or UAE treatments, reaching higher temperatures at longer treatment time (Figure 14, Figure 15) As it can be observed in Table 21, extract temperature significantly increased with time, MAE power and UAE amplitude ($p<0.001$). Based on the adjusted determination coefficient (R^2 adj), a strong positive correlation was found between extract temperature and extraction parameters. Temperatures higher than 70 °C and 50 °C were attained in the extracts after MAE and UAE treatments, respectively. Heating rate increased with the applied microwave power and ultrasonication amplitude, leading to the highest power density (P_v) values at 900 W and 125 µm, respectively. Being energy density (E_v) function of P_v and treatment time, the highest E_v was developed by MAE at 900 W for 50 s and UAE at 125 µm for 120 s.

Browning and colour parameters

CIELAB parameters (L*, a* and b*) of frozen and dry peach waste extracts resulted significantly different ($p<0.05$). L* of extracts obtained by frozen peach waste (26.04 ± 0.16) was higher than that of dry waste extracts (24.31 ± 0.83), while a* and b* parameters were found to be lower in frozen waste extracts (-0.48 ± 0.09 and 4.17 ± 0.17) than in those obtained from dried waste (0.93 ± 0.14 and 9.32 ± 0.21). However, CIELAB parameters did not show significant differences ($p\geq0.05$) between extracts obtained from frozen and dry peach waste for any of MAE or UAE treatments. BI values of dry peach waste extracts resulted 5-10 times higher than those from frozen peach waste extracts, showing the same tendency than CIELAB parameter values (Table 17, Table 18, Table 19 and Table 20). BI increased with MAE power ($p<0.001$), UAE amplitude ($p<0.05$) and treatment time ($p<0.001$), or, in other words, with the applied E_v, in both frozen and dry peach waste extracts (Figure 14, Figure 15 and Table 21). In addition, MAE treatments, which were characterised by higher E_v than those of UAE, resulted in BI values higher than those observed in extracts obtained by UAE.

Bioactive extraction and antioxidant activity

Total phenolic compounds

Total phenolic content (TPC) of extracts obtained from frozen and dry peach waste submitted to MAE or UAE is reported in Table 17, Table 18, Table 19 and Table 20. The effects of independent variables and their interactions on the extraction of TPC can also be observed on contour plots reported in Figure 14 and Figure 15. Phenolic extract content increased with MAE power, UAE amplitude and treatment time, and thus with E_v. The highest TPC values of both fresh and dry peach waste extracts were obtained by applying 900 W for 50 s for MAE (Table 17 and Table 18) and 125 µm for 120 s for UAE (Table 19 and Table 20). In Table 21, the effect of each independent variable over the different evaluated response parameters is shown. In particular, TPC was always significantly affected by treatment time and MAE power, while UAE amplitude had a significant effect only in frozen extracts. Neglecting the non-significant parameters, the equations to predict TPC as a function of MAE and UAE parameters were obtained (eq. 13, eq. 14, eq. 15 and eq. 16):

$$TPC_{MAE}(frozen) = 60.49 + 0.12\,P + 2.14\,t \quad \text{(eq. 13)}$$

$$TPC_{MAE}(dry) = 249.15 + 0.05\,P + 0.29\,t + 0.008\,P \times t \quad \text{(eq. 14)}$$

$$TPC_{UAE}(frozen) = 204.02 + 0.50\,A + 0.75\,t \quad \text{(eq. 15)}$$

$$TPC_{UAE}(dry) = 575.38 + 0.19\,t \quad \text{(eq. 16)}$$

where TPC_{MAE}(frozen), TPC_{MAE}(dry), TPC_{UAE}(frozen), TPC_{UAE}(dry) were total phenolic compounds, for frozen and dry peach pomace submitted to MAE and UAE, P was the microwave power (W), A was the ultrasound amplitude (µm) and t the treatment time (s).

Based on the R^2 adj coefficient, experimental data showed a correlation higher than 68% ($p=0.05$) with calculated models, which were thus adequate to explain the variation of TPC with process parameters. The TPC obtained by MAE from frozen peach waste resulted to be a function of power and extraction time ($p<0.01$). The TPC of dry waste extracts significantly depended not only on power and the time ($p<0.001$) but also on the interaction between these two parameters ($p<0.01$). In the case of wastes treated with UAE, the TPC of frozen waste extracts was significantly affected by amplitude ($p<0.05$) and time ($p<0.01$). By contrast, TPC of extracts obtained by UAE from dry waste only depended on the extraction time ($p<0.01$).

Total flavonoids

Total flavonoid (TF) content in both frozen and dry peach waste extracts increased with MAE power, UAE amplitude and extraction time and, consequently, with the Ev absorbed by the samples (Figure 14 and Figure 15). Upon both MAE and UAE, TF content resulted 1.5-2.0 times higher in extracts from frozen wastes (Table 17 and Table 19) as compared to those from dry peach waste (Table 18 and Table 20). ANOVA reported in Table 21 showed that MAE power and UAE amplitude were the most significant parameters affecting TF content of frozen peach waste extracts ($p<0.001$), followed by treatment time ($p<0.01$). Models presented R^2 adj higher than 0.80, indicating a close agreement between experimental data and predictive values. In dry peach waste extracts, MAE extraction time ($p<0.01$) and its interaction with power ($p<0.05$) significantly affected extract TF content; in the case of UAE both amplitude and time were found to have a significant effect on TF extraction from dry waste ($p<0.05$). However, the extraction parameters showed R^2 adj values lower than 0.60, indicating a weak correlation between data and calculated models (Table 21). Equations from eq. 17 to eq. 20 represent the model equations:

$$TF_{MAE}(frozen) = 28.17 + 0.08\,P + 1.28\,t \quad \text{(eq. 17)}$$

$$TF_{MAE}(dry) = 17.47 + 1.27t + 0.001\,P \times t \quad \text{(eq. 18)}$$

$$TF_{UAE}(frozen) = 38.91 + 0.71\,A + 0.37\,t - 0.003\,A\ \times t \quad \text{(eq. 19)}$$

$$TF_{UAE}(dry) = 22.57 + 0.49\,A + 0.44\,t \quad \text{(eq. 20)}$$

where TF_{MAE}(frozen), TPC_{MAE}(dry), TF_{UAE}(frozen) and TF_{UAE}(dry)were total flavonoids, respectively for frozen and dry peach pomace submitted to MAE and UAE, P was the MAE power (W), A was the ultrasound amplitude (μm) and t the treatment time (s).

Total anthocyanins and Vitamin C

Total anthocyanins (TA) and vitamin C (VIT C) were not detectable in extracts obtained from dry matrix. As clearly shown in Figure 14 and Figure 15, the maximum amount of TA was obtained at low MAE power or UAE amplitude applied for long time, corresponding to E_v values of 75.4 and 44.4 J/mL respectively for MAE and UAE. In the same way, ANOVA showed MAE power and UAE amplitude as well as the interaction of these parameters with extraction time to be highly significant ($p<0.001$) in determining frozen peach waste content of TA (Table 21). The following equations (eq. 21 and eq. 22) express TA extraction from frozen peach waste as a function of MAE and UAE significant parameter, identified by ANOVA (Table 21):

$$TA_{MAE} = 1.31 - 0.005\,P + 0.27\,t - 0.0003\,P\ \times t \quad \text{(eq. 21)}$$

$$TA_{UAE} = 4.73 - 0.003\,A + 0.04\,t - 0.0003\,A \times t \quad \text{(eq. 22)}$$

where TA_{MAE} and TA_{UAE} were total anthocyanins for frozen peach pomace submitted to MAE and UAE, P was the MAE power (W), A was the ultrasound amplitude (μm) and t the treatment time (s). Calculated models presented a value of R^2 adj higher than 0.96, indicating a high correlation with experimental data.

As in the case of TA, VIT C was well extracted at low MAE power applied for long time. MAE time ($p<0.01$) and its interaction with power ($p<0.05$) significantly reduced frozen peach waste content of this bioactive compound (Figure 14). A well fit model was obtained, presenting a 61% correlation with experimental data, as indicated by the R^2 adj (Table 21). The eq. 23 expresses VIT C content on extracts from frozen peach waste as a function of significant MAE parameters, as indicated by ANOVA reported in Table 5:

$$VIT\ C_{MAE} = 61.71 + 1.16 \times t - 0.001\ P \times t \qquad \text{(eq. 23)}$$

where VIT C_{MAE} was total vitamin C for frozen pomace, P was the MAE power (W) and t the treatment time (s).

By contrast, the model expressing the extraction of VIT C from frozen peach waste as a function of UAE amplitude and time resulted not significant ($p\geq0.05$) and able to explain only 31% of the experimental data, as indicated by R^2 adj value (Table 21). This indicates that the increase in sonication amplitude and time did not significantly affect the extraction efficacy of this compound.

Antioxidant activity

As shown by the relevant contour plots reported in Figure 14 and Figure 15, the antioxidant activity (AA) of frozen and dry peach waste extracts increased with MAE power, UAE amplitude and time, and thus with the E_v absorbed by the samples. In both frozen and dry peach waste extracts, AA reached a maximum value of about 2.2-2.3 mmol TE on 100 g of dry weight by the application of the most intense MAE (900 W for 50 s) and UAE treatments (125 μm for 120 s) (Table 17, Table 18, Table 19 and Table 20). ANOVA was performed to identify factors mostly affecting extract AA, leading to well-adjusted models with R^2 adj values reported in Table 21. In all cases, treatment time significantly affected AA of extracts ($p<0.01$ and 0.05 for frozen and dry waste extracts, respectively). In the case of MAE, power significantly influenced AA of both frozen ($p<0.01$) and dry ($p<0.05$) peach waste extracts, while a significant effect of UAE amplitude was obtained only on dry peach waste extract ($p<0.001$). Equations from eq. 24 to eq. 27 express AA of extracts from frozen and dry peach waste as a function of significant MAE and UAE parameters, as identified by ANOVA (Table 21).

$$AA_{MAE}(frozen) = 1.81 + 3.30 \times 10^{-5}P + 0.0002\,t + 8.76 \times 10^{-6}P \times t \quad \text{(eq. 24)}$$

$$AA_{MAE}(dry) = 1.69 + 0.0004P + 0.007t \quad \text{(eq. 25)}$$

$$AA_{UAE}(frozen) = 5.35 + 15.55\,t \quad \text{(eq. 26)}$$

$$AA_{UAE}(dry) = 1.42 + 0.007\,A + 0.004\,t \quad \text{(eq. 27)}$$

where AA_{MAE}(frozen), AA_{MAE}(dry), AA_{UAE}(frozen) and AA_{UAE}(dry) were the antioxidant activity, respectively for frozen and dry peach pomace submitted to MAE and UAE, P was the MAE power (W), A was the ultrasound amplitude (μm) and t the treatment time (s) for frozen and dry pomace, P was the microwave power (W) and t the treatment time (s).

Validation and optimization

In the range of the studied MAE and UAE parameters, treatment conditions that exhibited the maximum TPC, TF, TA, VIT C content associated to the maximum AA were estimated by the desirability function. In the case of frozen waste, MAE treatment at 540 W and 50 s and UAE treatment at 14 μm amplitude and 120 s, with a desirability value of 0.630 and 0.658 respectively, were identified (Table 22). In the case of dry wastes, the desirability function identified the optimal MAE treatment at 900 W and 50 s and the UAE treatment at 125 μm amplitude and 120 s, with a desirability of 0.829 and 0.919, respectively. These optimal MAE and UAE treatment conditions were thus applied, and their extraction performances were evaluated in terms of TPC, TF, TA, VIT C and AA of frozen waste extract (Table 22). The correlation between observed and predicted values in the optimal treatment conditions resulted always higher than 0.88, indicating that expressions obtained for each assay (from eq. 13 to eq. 27) were adequate to fit the experimental results. In both frozen and dry peach waste extracts, no significant differences were found between the extraction performances of optimized MAE and UAE (Table 22).

Power and energy density of optimal processes

Power and energy densities (P_v and E_v) of optimal MAE and UAE treatments were calculated. As shown in Table 22, the P_v values of MAE optimal treatment were about 4.0 and 8.0 W/mL for frozen and dry wastes, respectively, which resulted much higher than those obtained for UAE optimal treatment (about 0.5 and 1.0 W/mL for frozen and dry wastes, respectively). For frozen and dry wastes, the E_v values for MAE and UAE are 210 and 380 J/mL, respectively. These values resulted 4 times higher than those of optimal UAE conditions in frozen wastes and more than double in dry wastes.

Table 17. Experimental design for the independent variables and corresponding response values for microwave assisted extraction from frozen peach waste. T temperature (°C), BI browning index, TPC total phenolic content (mg GAE/100 g), TF total flavonoids (mg QE/100 g), TA total anthocyanins (mg CGE/100 g, VIT C vitamin C (mg/100 g, AA antioxidant activity (mmol TE/100 g), P_v power density (W/mL), E_v energy density (J/mL).

Independent variables						Response values						
Assay no.**	**Block**	**Power (W)**	**Time (s)**	**P_v (W/mL)**	**E_v (J/mL)**	**T**	**BI**	**TPC***	**TF***	**TA***	**VIT C***	**AA***
1	1	180	10	1.507	15.1	30.7 ± 1.3	0.20 ± 0.04	100 ± 3	46 ± 06	4.19 ± 0.09	69 ± 13	1.9 ± 0.3
2	2	180	10	1.507	15.1	31.5 ± 1.0	0.212 ± 0.005	114 ± 25	62 ± 09	4.61 ± 0.31	84 ± 4	1.79 ± 0.15
3	1	900	10	7.577	75.8	33.1 ± 0.5	0.16 ± 0.06	145 ± 3	102 ± 22	5.7 ± 0.6	98 ± 22	1.82 ± 0.15
4	2	900	10	7.577	75.8	32.8 ± 1.3	0.163 ± 0.015	272 ± 321	112 ± 26	6.4 ± 0.4	81 ± 3	1.91 ± 0.18
5	1	180	50	1.507	75.4	36.7 ± 1.3	0.429 ± 0.008	177 ± 29	87 ± 17	12.3 ± 0.4	119 ± 9	1.85 ± 0.05
6	2	180	50	1.507	75.4	37.6 ± 0.5	0.431 ± 0.008	230 ± 19	111 ± 17	13.54 ± 0.29	112 ± 17	1.93 ± 0.23
7	1	900	50	7.577	378.9	76.5 ± 1.5	0.56 ± 0.04	283 ± 25	132 ± 16	5.08 ± 0.12	103 ± 7	2.1 ± 0.5
8	2	900	50	7.577	378.9	75.6 ± 1.3	0.605 ± 0.008	415 ± 4	124 ± 12	6.6 ± 0.8	91 ± 12	2.26 ± 0.15
9	1	540	30	4.228	126.8	48.0 ± 1.3	0.428 ± 0.008	213 ± 7	103 ± 18	4.4 ± 0.5	99 ± 8	1.91 ± 0.03
10	1	540	30	4.228	126.8	47.7 ± 0.5	0.43 ± 0.14	277 ± 29	84 ± 17	4.2 ± 0.5	90 ± 10	1.96 ± 0.05
11	1	540	30	4.228	126.8	48.6 ± 0.3	0.40 ± 0.08	309 ± 25	106 ± 03	4.3 ± 0.5	95 ± 5	2.01 ± 0.10
12	2	540	30	4.228	126.8	47.7 ± 0.3	0.43 ± 0.03	243 ± 20	113 ± 03	4.4 ± 0.6	112 ± 7	2.01 ± 0.21
13	2	540	30	4.228	126.8	48.0 ± 1.3	0.420 ± 0.110	264 ± 07	99 ± 03	4.4 ± 0.5	99 ± 12	1.94 ± 0.21
14	2	540	30	4.228	126.8	48.6 ± 0.5	0.410 ± 0.120	289 ± 7	122 ± 25	4.7 ± 0.4	99 ± 4	2.14 ± 0.08

**Assay order was randomized; * Expressed as dry matter

Table 18. Experimental design for the independent variables and corresponding response values for microwave assisted extraction from dry peach waste. T temperature (°C), BI browning index, TPC total phenolic content (mg GAE/100 g), TF total flavonoids (mg QE/100 g), AA antioxidant activity (mmol TE/100 g), P_v power density (W/mL), E_v energy density (J/mL).

	Independent variables					Response values						
Assay no.**	**Block**	**Power (W)**	**Time (s)**	**P_v (W/mL)**	**E_v (J/mL)**	**T**	**BI**	**TPC***	**TF***	**AA***	**TA**	**VIT C**
1	1	180	10	1.507	15.1	31.5 ± 0.8	1.60 ± 1.10	235 ± 8	40 ± 18	1.77 ± 0.13	ND	ND
2	2	180	10	1.507	15.1	32.1 ± 0.5	1.62 ± 0.12	315 ± 42	36 ± 10	1.88 ± 0.13	ND	ND
3	1	900	10	7.577	75.8	33.5 ± 1.3	2.25 ± 0.14	409 ± 19	64 ± 10	2.12 ± 0.10	ND	ND
4	2	900	10	7.577	75.8	33.5 ± 1.3	2.21 ± 0.20	324 ± 6	70 ± 7	2.1 ± 0.5	ND	ND
5	1	180	50	1.507	75.4	37.4 ± 1.5	3.81 ± 0.11	361 ± 28	83 ± 25	2.09 ± 0.10	ND	ND
6	2	180	50	1.507	75.4	38.3 ± 0.3	3.8 ± 0.7	327 ± 3	77 ± 14	2.03 ± 0.23	ND	ND
7	1	900	50	7.577	378.9	77.3 ± 0.3	4.6 ± 0.3	681 ± 8	80 ± 6	2.15 ± 0.03	ND	ND
8	2	900	50	7.577	378.9	76.2 ± 0.5	4.0 ± 0.4	652 ± 15	70 ± 9	2.22 ± 0.26	ND	ND
9	1	540	30	4.228	126.8	48.5 ± 1.5	3.45 ± 0.11	364 ± 27	69 ± 5	2.20 ± 0.02	ND	ND
10	1	540	30	4.228	126.8	48.4 ± 0.5	3.60 ± 0.15	310 ± 21	84 ± 5	2.10 ± 0.28	ND	ND
11	1	540	30	4.228	126.8	49.3 ± 1.3	3.65 ± 0.19	334 ± 24	60 ± 16	2.10 ± 0.05	ND	ND
12	2	540	30	4.228	126.8	48.4 ± 1.0	3.51 ± 0.20	381 ± 22	97 ± 12	2.16 ± 0.08	ND	ND
13	2	540	30	4.228	126.8	48.8 ± 0.8	3.2 ± 0.3	370 ± 25	87 ± 20	2.22 ± 0.08	ND	ND
14	2	540	30	4.228	126.8	49.2 ± 0.5	3.43 ± 0.15	329 ± 19	82 ± 16	1.97 ± 0.13	ND	ND

**Assay order was randomized; * Expressed as dry matter; ND not detected

Table 19. Experimental design for the independent variables and corresponding response values for ultrasound assisted extraction from frozen peach waste. T temperature (°C), BI browning index, TPC total phenolic content (mg GAE/100 g), TF total flavonoids (mg QE/100 g), TA total anthocyanins (mg CGE/100 g), VIT C vitamin C (mg/100 g), AA antioxidant activity (mmol TE/100 g), P_v power density (W/mL), E_v energy density (J/mL).

		Independent variables				**Response values**						
Assay no.**	**Block**	**Amplitude (µm)**	**Time (s)**	**P_v (W/mL)**	**E_v (J/mL)**	**T**	**BI**	**TPC***	**TF***	**TA***	**VIT C***	**AA***
1	1	12	20	0.370	7.4	26.5 ± 0.8	0.317 ± 0.008	213 ± 23	53 ± 1	5.00 ± 0.23	120 ± 15	2.10 ± 0.05
2	2	12	20	0.370	7.4	26.8 ± 0.8	0.32 ± 0.14	250 ± 10	65 ± 21	5.6 ± 0.8	114 ± 6	2.13 ± 0.08
3	1	125	20	1.168	23.4	31.3 ± 1.3	0.38 ± 0.09	215 ± 11	105 ± 7	4.3 ± 1.4	122 ± 14	2.06 ± 0.05
4	2	125	20	1.168	23.4	31.0 ± 1.3	0.39 ± 0.06	345 ± 6	117 ± 22	4.75 ± 1.10	102 ± 10	2.22 ± 0.08
5	1	12	120	0.370	44.4	35.0 ± 0.8	0.44 ± 0.13	311 ± 28	92 ± 19	8.00 ± 1.10	127 ± 13	2.23 ± 0.18
6	2	12	120	0.370	44.4	35.2 ± 0.3	0.46 ± 0.06	324 ± 16	88 ± 13	8.8 ± 0.8	137 ± 9	2.26 ± 0.03
7	1	125	120	1.168	140.2	58.4 ± 0.3	0.89 ± 0.11	362 ± 13	115 ± 9	5.0 ± 0.3	109 ± 6	2.25 ± 0.05
8	2	125	120	1.168	140.2	58.3 ± 0.3	0.85 ± 0.16	462 ± 23	116 ± 19	4.8 ± 0.7	111 ± 20	2.27 ± 0.05
9	1	69	70	0.682	47.7	37.4 ± 0.5	0.46 ± 0.05	346 ± 16	93 ± 8	4.6 ± 1.4	128 ± 12	2.22 ± 0.28
10	1	69	70	0.682	47.7	37.9 ± 0.8	0.45 ± 0.13	280 ± 21	92 ± 19	4.2 ± 0.8	139 ± 10	2.18 ± 0.05
11	1	69	70	0.682	47.7	37.6 ± 1.3	0.44 ± 0.16	310 ± 24	92 ± 17	4.4 ± 0.4	151 ± 22	2.25 ± 0.13
12	2	69	70	0.682	47.7	37.4 ± 1.3	0.456 ± 0.105	349 ± 20	104 ± 4	5.20 ± 1.20	139 ± 25	2.18 ± 0.08
13	2	69	70	0.682	47.7	37.8 ± 0.5	0.48 ± 0.08	298 ± 24	80 ± 20	4.6 ± 1.0	131 ± 3	2.18 ± 0.03
14	2	69	70	0.682	47.7	37.6 ± 0.3	0.50 ± 0.04	358 ± 15	91 ± 21	4.6 ± 1.3	145 ± 17	2.23 ± 0.08

**Assay order was randomized; * Expressed as dry matter

Table 20. Experimental design for the independent variables and corresponding response values for ultrasound assisted extraction from dry peach waste. T temperature (°C), BI browning index, TPC total phenolic content (mg GAE/100 g), TF total flavonoids (mg QE/100 g), AA antioxidant activity (mmol TE/100 g), P_v power density (W/mL), E_v energy density (J/mL).

Independent variables						Response values						
Assay no.**	**Block**	**Amplitude (µm)**	**Time (s)**	**P_v (W/mL)**	**E_v (J/mL)**	**T**	**BI**	**TPC***	**TF***	**AA***	**TA**	**VIT C**
1	1	12	20	0.370	7.4	27.2 ± 0.5	3.2 ± 0.4	563 ± 45	50 ± 4	1.71 ± 0.10	ND	ND
2	2	12	20	0.370	7.4	27.4 ± 0.8	3.1 ± 0.4	593 ± 41	31 ± 11	1.51 ± 0.13	ND	ND
3	1	125	20	1.168	23.4	31.7 ± 1.0	3.4802 ± 0.0021	560 ± 32	78 ± 7	2.09 ± 0.10	ND	ND
4	2	125	20	1.168	23.4	31.7 ± 1.0	3.4 ± 0.4	582 ± 29	76 ± 9	2.08 ± 0.05	ND	ND
5	1	12	120	0.370	44.4	35.7 ± 0.8	3.70 ± 0.4	607 ± 19	72 ± 14	2.00 ± 0.10	ND	ND
6	2	12	120	0.370	44.4	35.8 ± 0.3	3.6 ± 0.4	605 ± 46	91 ± 13	1.80 ± 0.23	ND	ND
7	1	125	120	1.168	140.2	59.0 ± 0.3	3.68 ± 0.11	639 ± 38	92 ± 9	2.10 ± 0.03	ND	ND
8	2	125	120	1.168	140.2	58.9 ± 0.3	3.83 ± 0.03	630 ± 33	116 ± 5	2.16 ± 0.26	ND	ND
9	1	69	70	0.682	47.7	37.8 ± 0.5	3.72 ± 0.13	584 ± 33	84 ± 1	1.92 ± 0.05	ND	ND
10	1	69	70	0.682	47.7	38.5 ± 1.3	3.66 ± 0.20	612 ± 35	79 ± 6	2.09 ± 0.05	ND	ND
11	1	69	70	0.682	47.7	38.3 ± 1.3	3.64 ± 0.17	587 ± 49	116 ± 11	2.15 ± 0.03	ND	ND
12	2	69	70	0.682	47.7	38.1 ± 0.8	3.84 ± 0.05	602 ± 26	59 ± 4	2.05 ± 0.26	ND	ND
13	2	69	70	0.682	47.7	38.6 ± 0.5	3.6 ± 0.4	602 ± 11	81 ± 9	2.14 ± 0.08	ND	ND
14	2	69	70	0.682	47.7	38.0 ± 0.3	3.7143 ± 0.0021	622 ± 09	101 ± 8	2.07 ± 0.05	ND	ND

**Assay order was randomized; * Expressed as dry matter; ND not detected

Table 21. ANOVA of the first-order polynomial models for extracts obtained by microwave (MAE) and ultrasound assisted extraction (UAE) from frozen and dry peach waste. Adjusted determination coefficient (R^2 adj) for evaluating model goodness-of-fit is also reported. P power (W), A amplitude (μm), t time (s), T temperature (°C), BI browning index, TPC total phenolic content (mg GAE/100 g dm), TF total flavonoids (mg QE/100 g dm), TA total anthocyanins (mg CGE/100 g dm), VIT C vitamin C (mg/100 g dm), AA antioxidant activity (mmol TE/100 g dm).

	F-value						
	T	**BI**	**TPC**	**TF**	**TA**	**VIT C**	**AA**
MAE frozen							
Model	3637.86 ***	360.2 ***	9.40 **	18.14 ***	242.81 ***	6.8 *	11.4 **
P	3325.88 ***	26.41 ***	14.20 **	31.36 ***	123.76 ***	0.15	11.47 **
t	4838.36 ***	965.72 ***	13.53 **	20.29 **	288.84 ***	14.13 **	16.05 **
P * t	2749.33 ***	88.46 ***	0.49	2.77	315.82 ***	6.11 *	6.67 *
R^2 adj	0.99	0.99	0.70	0.82	0.99	0.61	0.74
MAE dry							
Model	3683.01 ***	147.35 ***	41.51 ***	6.79 *	ND	ND	6.98 *
P	3334.03 ***	28.92 ***	59.2 ***	2.88	ND	ND	11.28 *
t	4915.99 ***	412.72 ***	46.98 ***	11.87 **	ND	ND	8.37 *
P * t	2799.00 ***	0.42	18.36 **	5.64 *	ND	ND	1.29
R^2 adj	0.99	0.98	0.92	0.61	ND	ND	0.62
UAE frozen							
Model	9866.18 ***	363.61 ***	8.88 **	25.44 ***	89.96 ***	2.66	5.35 *
A	9526.55 ***	342.4 ***	7.81 *	57.6 ***	130.54 ***	5.06	0.46
t	15722.75 ***	569.66 ***	18.03 **	12.26 **	85.44 ***	1.06	15.55 **
A * t	4349.23 ***	178.77 ***	0.79	6.46 *	53.89 ***	1.86	0.04
R^2 adj	0.99	0.99	0.68	0.87	0.96	0.31	0.54
UAE dry							
Model	6477.38 ***	17.3 ***	9.30 **	4.39 *	ND	ND	12.26 **
A	6194.37 ***	7.08 *	1.32	5.54 *	ND	ND	27.18 ***
t	10363.71 ***	43 ***	23.24 **	7.33 *	ND	ND	6.21 *
A * t	2874.05 ***	1.82	3.35	0.31	ND	ND	3.40
R^2 adj	0.99	0.82	0.69	0.48	ND	ND	0.75

*p<0.05, **p<0.01, ***p<0.001; ND not detected

Table 22. Process parameters, extraction performances and energy of optimized microwave and ultrasound treatments applied to frozen and dry peach waste extracts. MAE microwave assisted extraction, UAE ultrasound assisted extraction, TPC total phenolic content (mg GAE/100 g), TF total flavonoids (mg QE/100 g), TA total anthocyanins (mg CGE/100 g), VIT C vitamin C (mg/100 g), AA antioxidant activity (mmol TE/100 g), P power, A amplitude, t time, P_v power density, E_v energy density.

	Frozen		**Dry**	
	MAE	**UAE**	**MAE**	**UAE**
Process parameters				
P(W)	540	-	900	-
A (µm)	-	14	-	125
t (s)	50	120	50	120
Desirability	0.630	0.658	0.829	0.919
Extraction performances				
TPC*	309.14 ± 3.22	317.33 ± 2.91	666.41 ± 20.62	636.77 ± 3.24
TF*	120.47 ± 4.34	89.93 ± 2.69	74.75 ± 7.19	97.75 ± 8.37
TA*	8.95 ± 0.32	8.39 ± 0.56	ND	ND
VIT C*	108.04 ± 1.30	131.93 ± 7.40	ND	ND
AA*	2.14 ± 0.03	2.24 ± 0.02	2.19 ± 0.05	2.12 ± 0.02
Process energy				
P_v (W/mL)	4.228	0.377	7.577	1.168
E_v (J/mL)	211.4	45.24	378.85	140.16

*Expressed as dry matter; ND not detected

No significantly different means were identified by t-test comparing extraction performances of MAE and UAE applied to frozen and dry peach waste ($p \geq 0.05$)

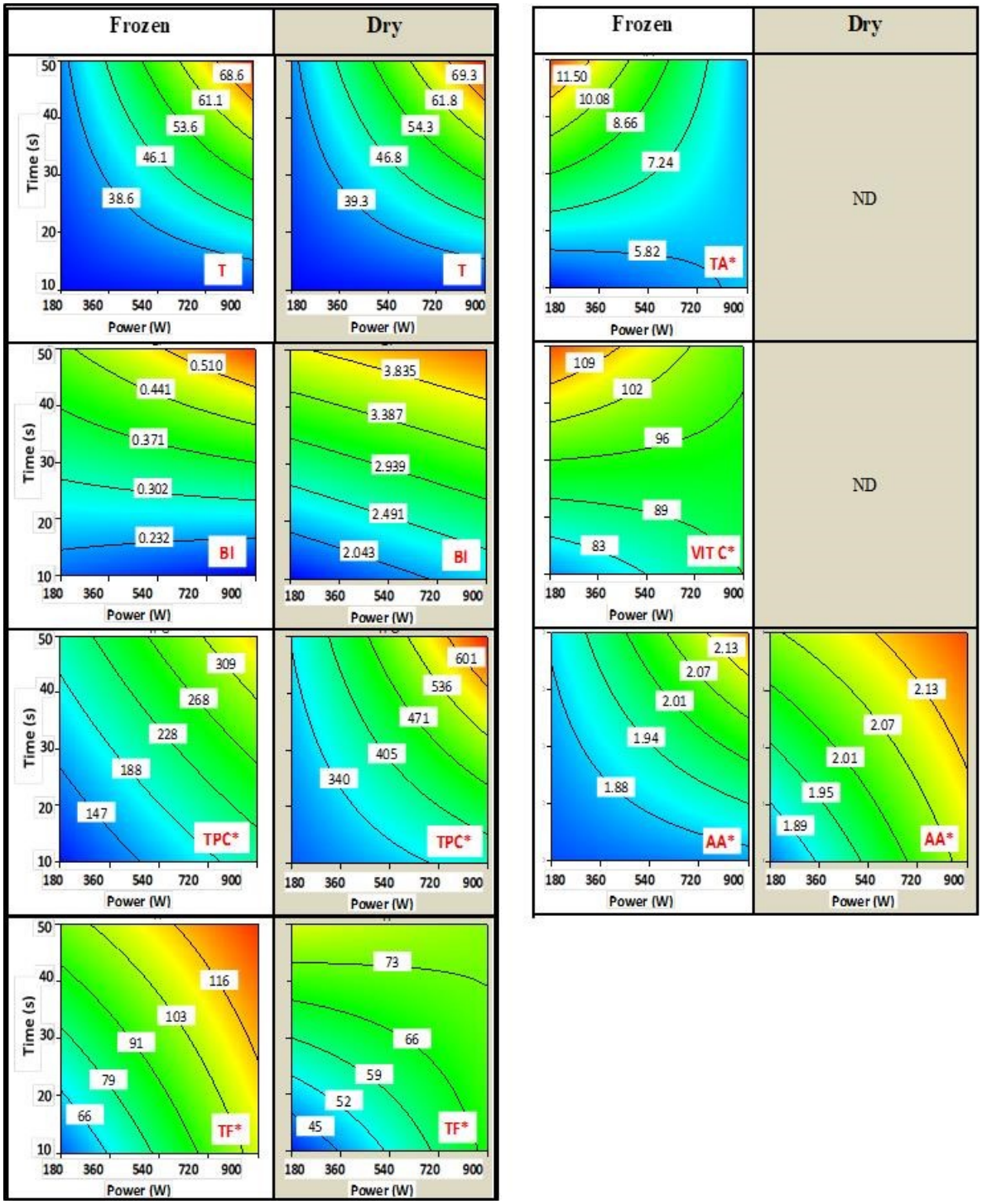


Figure 14. Effect of microwave power (W) and time (s) on temperature (T, °C), browning index (BI), total phenolic content (TPC, mg GAE/100 g), total flavonoids (TF, mg QE/100 g), total anthocyanins (TA, mg CGE/100 g), vitamin C (VIT C, mg/100 g) and antioxidant activity (AA, mmol TE/100 g) of frozen and dry peach waste extracts.*Expressed as dry matter; ND not detected

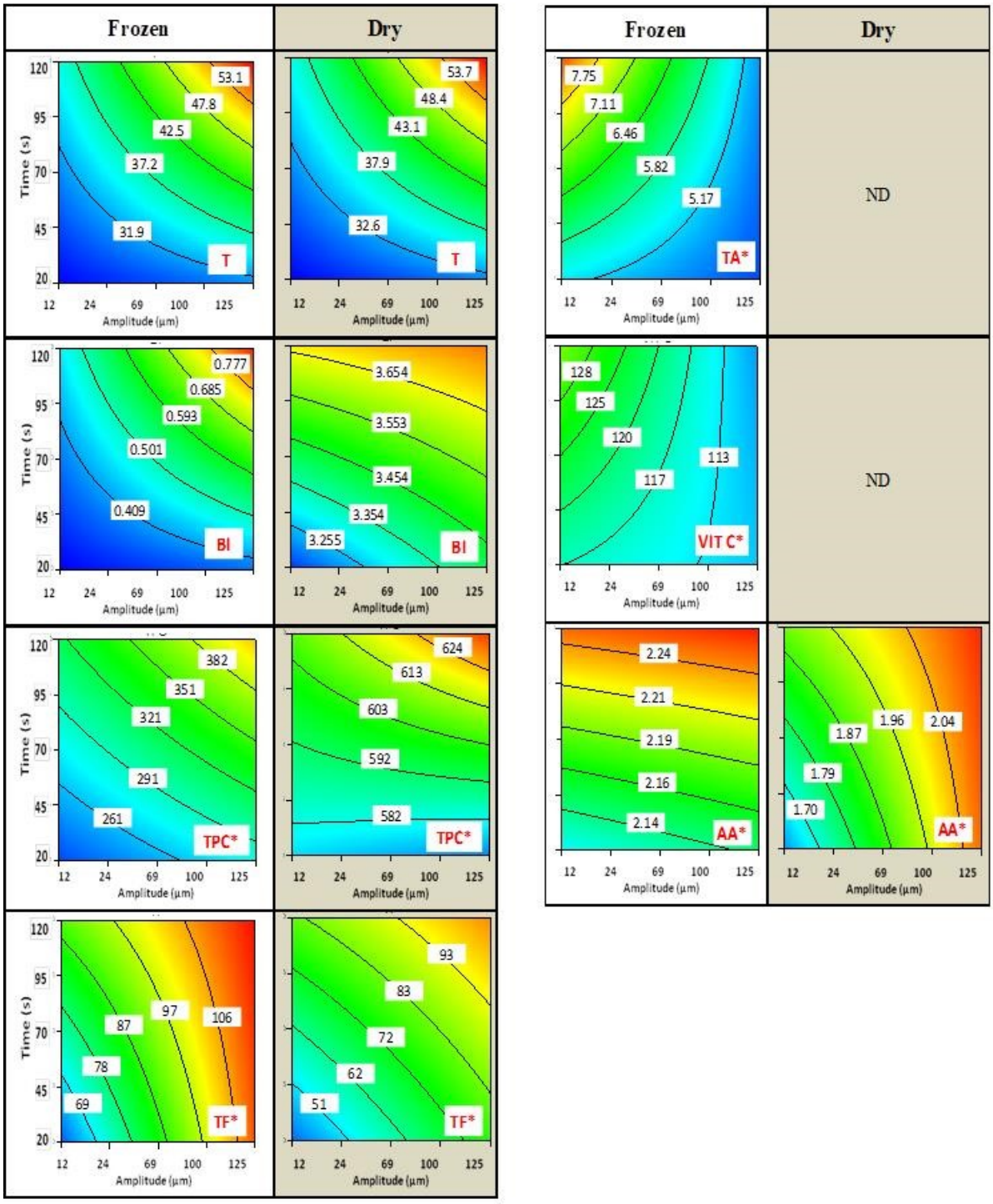


Figure 15. Effect of ultrasound amplitude (µm) and time (s) on temperature (T, °C), browning index (BI), total phenolic content (TPC, mg GAE/100 g), total flavonoids (TF, mg QE/100 g), total anthocyanins (TA, mg CGE/100 g), vitamin C (VIT C, mg/100 g) and antioxidant activity (AA, mmol TE/100 g) of frozen and dry peach waste extracts.*Expressed as dry matter; ND not detected

5.4.4 Discussion

Results obtained in the present study indicate that TPC extraction from peach waste was maximized increasing process energy density (E_V), by increasing MAE power, UAE amplitude and, in both cases, treatment time. A similar increase in the TPC content with MAE nominal power and treatment time was observed in extracts from *Orthosiphon stamineus* and citrus mandarin peel (Chan et al., 2017; Hayat et al., 2009). Similarly, UAE amplitude and treatment time were reported to have an important influence on TPC extracted from *Orthosiphon stamineus* (Chan et al., 2017), orange peel (Khan et al., 2010) and acerola fruit (Le & Le, 2012). However, it must be highlighted that literature data relevant to MAE and UAE extraction of TPC showed, in most cases, that the extraction efficiency increases only until an optimum temperature is reached. Upon a further temperature increase, extraction efficacy has been reported to slow down and then decrease. This reduction in treatment efficacy at high temperature has been attributed to thermal degradative reactions of phenols beyond a certain temperature treshold (Nayak et al., 2015; Pingret, Fabiano-Tixier, & Chemat, 2013). In the case of UAE, another drawback in employing high temperatures would be a decrease in the extraction rate constant, due to the reduction in cavitation intensity as a result of the lower surface tension and the increased vapour pressure of the cavitation bubbles (Tao, Zhang, & Sun, 2014). Based on this considerations, the intensity of treatments applied in this study, was below the intensity treshold leading to TPC degradation.

Although temperatures higher than 50 °C have been reported to promote flavonoid degradation, the highest TF values were obtained increasing MAE and UAE applied energy density, which resulted in temperatures of about 70 and 50 °C, respectively (Figure 14 and Figure 15). These results can be explained considering that both MAE and UAE can generate heating in a reduced time, leading to a so-defined high temperature-short time treatment, which has been reported to increase extraction rate without damaging flavonoids in different fruit derivatives (Saikia, Mahnot, & Mahanta, 2015; Salazar-González, San Martín-González, Vergara-Balderas, López-Malo, & Sosa-Morales, 2014). To this regard, Raner, Strauss, Vyskoc and Mokbel (1993) reported that MAE power variation from 500 to 1000 W on a model system had no significant effect on the flavonoids if the applied treatment time allowed maintaing temperature at values lower than 110 °C. Similarly, TF degradation in hawthorn seed extracts obtained by UAE were observed only at temperatures higher than 90 °C (Pan, Yu, Zhu, & Qiao, 2012).

The efficacy of high-energy MAE and UAE procedures in extracting TPC and TF can be attributed to the thermal effect of the applied treatments, which increased with the increase of MAE power, UAE amplitude and treatment time. Such thermal effect was expected, since an increase in these parameters is known to increase the energy amount delivered to the treated sample (E_V), leading to a more intense heating (Chan et al., 2017). To this regard, temperature increase in MAE is controlled by incident microwave power that is converted into heat energy into the sample (Ameer

et al., 2017). Similarly, with the increase in UAE amplitude, the number of compression and rarefaction cycles of ultrasonic waves increases, leading to an intensification of cavitation-induced phenomena, such as development of microjets, increase in local pressure and temperature (Al-Dhabi et al., 2017). In any case, an increase in extract temperature is expected to promote extraction efficacy, due to tissue softening and a concomitant increase in diffusion rate (Zheng et al., 2013).

Interestingly, the TPC value of extracts from dry peach waste resulted about double than those of frozen peach wastes. Similar results were also observed by Londoño-Londoño et al. (2010) on citrus peel submitted to UAE at 60 kHz and 40 °C for 30 min. These authors attributed the more efficient extraction obtained from dry than fresh matrix to its higher porosity, leading to an increased extractive surface. A further possible explanation is the thermal-induced formation of novel compounds during peach waste drying, such as Maillard reaction derivatives, that may react with Folin reagent (Echavarria, Pagán, & Ibarz, 2013; Mrkìc, Cocci, Dalla Rosa, & Sacchetti, 2006). By contrast, the TF content resulted 1.5-2.0 times higher in extracts obtained from frozen wastes as compared to extracts obtained from dry peach waste. This suggests drying to be responsible for a reduction of peach waste flavonoid content. To this regard, flavonoid degradation upon air-drying was observed in different vegetables such as *Centella asiatica* and onions (Sharma et al., 2015; Zainol, Abdul-Hamid, Bakar, & Dek, 2009). Consequently, the compositional differences between fresh and dry peach waste extracts submitted to the same extraction procedure should be probably mainly attributed to matrix drying treatment rather than to the specific extraction effect.

Colour differences (L*, a* and b*) highlighted that dry peach waste extracts resulted more brownish than frozen peach waste extracts, confirming the possible presence of Maillard compounds formed during drying (Giusti & Wrolstad, 2001). However, colour determination did not highlight significant differences among extracts submitted to different MAE or UAE treatments. Extract BI confirmed colour differences between frozen and dry peach waste extracts and was also able to highlight the effect of different MAE or UAE extraction procedures. Interestingly, BI mimicked the TPC and TF trend, suggesting phenols as the main responsible for extract brown colour. In fact, the increase of extract BI with MAE and UAE energy density can be attributed to the accumulation of brown compounds, such as polymerized polyphenols, presenting absorption in the 400-440 nm range (Echavarria et al., 2013).

With regards to AA of the extracts, the highest values were obtained by the application of the highest MAE or UAE energy densities. Being efficiently extracted in these conditions, TPC and TF should be probably mainly accounted for extract AA, as also reported in other studies (Li et al., 2012). Naturally occurring phenolic compounds are expected to be responsible for the AA detected in frozen peach waste extracts. By contrast, the AA of dry peach waste extracts should be mainly attributed to thermal-induced compounds, formed during drying, such as partially oxidized polyphenols and Maillard reaction products, identified by the Folin-Ciocalteau assay used for TPC determination (Nicoli, Anese, & Parpinel, 1999). Although it is one of the most used methods for determining TPC in vegetable matrices, Folin-Ciocalteau method is not specific for phenolic compounds. Rather, it measures the ability of both phenolic and non-phenolic compounds in alkaline medium to reduce the phosphomolybdic/phosphotungstic acid reagent to blue complexes that are detected spectrophotometrically (Singleton et al., 1999). Therefore, it would be convenient to determine specific bioactive compounds present in the peach waste extracts. For this reason, total anthocyanins and vitamin C contents were determined in frozen and dry peach waste extracts obtained by MAE and UAE treatments.

In frozen peach waste extracts, the highest TA content was obtained upon the application of low MAE or UAE power densities (P_V) applied for long times. As also reported for other matrices such as blueberry and orange (Vikram, Ramesh, & Prapulla, 2005; Zheng et al., 2013), this result can be attributed to the reduced temperature reached during these treatments, limiting damage of these thermolabile bioactive compounds. Consequently, an increase in MAE power or UAE amplitude (and thus of P_V) would be detrimental for the TA content of the extract, due to heat energy accumulation, leading to a fast anthocyanin thermal degradation (Le & Le, 2012; Sang, Sang, Ma, Hou, & Li, 2017). Our results indicated a low efficacy of sonication in VIT C extraction. This can be explained by the counterbalancing effect of UAE-induced extraction and rapid VIT C oxidation during the treatment. Similar results were also observed in orange and strawberry juice (Tiwari, O'Donnell, Muthukumarappan, & Cullen, 2009; Tiwari, O'Donnell, Patras, & Cullen, 2008). VIT C is well known for its preferential reaction with ·OH radicals generated during sonication (Sprinz, Beckert, & Brede, 1998). Even if ultrasound propagation results in a reduction in sample oxygen content, which positively affects VIT C stability (Solomon, Svanberg, & Sahlström, 1995), hydroxyl radical formation was found to increase with degassing, thus favouring VIT C oxidation (Portenlänger & Heusinger, 1992). Although this is responsible for VIT C depletion into the sample, it may also be regarded as a positive mechanism, which would preserve phenols from hydroxylation (Ashokkumar et al., 2008). TA and VIT C were not detectable in dry peach waste extracts, probably due to their thermal lability, leading to an almost complete degradation during drying at 140 °C. In this regard, Kara and Erçelebi (2013) found a 90% anthocyanin reduction in mulberry juice upon 10 h thermal treatment at 80 °C. Similarly, Vikram et al. (2005) reported a 50% VIT C degradation in orange juice upon only 3 min conventional heating at 90 °C.

In order to identify the most efficient extraction treatment, energy density (E_V) developed by MAE and UAE optimized treatments was calculated. Results indicated that the E_V applied by MAE optimized treatment resulted higher than that developed by UAE optimal treatment, suggesting UAE to be more efficient, since a lower energy was required to reach the same extraction performances of MAE. However, it must also be underlined that UAE optimal treatment was characterized by an extraction time (120 s) more than double than that required by MAE optimal treatment (50 s). These results agree with those obtained by Hayat et al. (2009) and Chan et al. (2017) on citrus mandarin peel and *Orthosiphon stamineus* fruit, respectively. These authors found no significant differences in bioactive extraction performances of optimized MAE and UAE treatments, but highlighted MAE treatment time to be much lower (4-50 times) than that of UAE. This can be attributed to the fact that microwave process is a volumetric treatment. By contrast, ultrasound propagation does not allow a homogeneous energy distribution into the treated sample, since energy is located around the sonication probe, leading to the need for longer treatments (Ameer et al., 2017; Canselier et al., 2002).

5.4.5 Conclusions

The MAE and UAE of bioactive compounds from frozen and dry peach waste was successfully optimized by identifying optimal microwave power, ultrasound amplitude and treatment time. Frozen peach waste resulted richer than dry matrix in specific compounds such as flavonoids, anthocyanins and vitamin C, due to thermal damage induced by drying.

Both assisted extraction processes can be optimized to give comparable extraction performances in terms of total phenolic compounds, flavonoids, anthocyanins and antioxidant activity. The only exception was vitamin C, which was successfully extracted only by MAE, due to oxidative vitamin C degradation occurring during UAE.

The selection of one extraction technology over the other should be driven by an accurate consideration where process efficiency and time are considered. Even if energy density developed by UAE treatment resulted lower than that developed by MAE, the latter required a much lower time than UAE to give analogous extraction performances without degrading vitamin C. Thus, MAE can be considered a more feasible and applicable technology for valorisation of peach waste, leading to antioxidant extracts.

5.5 Valorisation of lettuce waste into dried food ingredients rich in antioxidants and innovative materials with high solvent loading capacity

In the previous paragraphs, the possibility to exploit fresh lettuce waste to produce fresh homogenates (§ 5.1) and extraction of antioxidant compounds (§ 5.3) was evaluated. Due to the high moisture content and proneness to microbial spoilage of lettuce waste, these valorisation processes would require a fast waste transformation, limiting this application to processing plants located near the waste generation site. Alternately, waste should be stored in refrigerated or freezing conditions, which would imply huge storage volumes, energy consumption and logistical costs.

To solve these issues, drying could be applied in order to obtain lettuce waste dried derivatives, which would be stable at ambient temperature and present lower volume. Based on these considerations, the aim of the present work was to investigate the possibility to valorise lettuce waste by turning it into dried materials and flour via traditional (air-drying and freeze-drying) and novel (supercritical-CO_2-drying with or without ethanol as co-solvent) drying techniques. Dried lettuce waste and flour were analysed for macro- and micro-appearance, particle size, dietary fibre, polyphenol content, antioxidant activity, water vapour sorption, water and oil holding capacity. Results were discussed to suggest possible uses of lettuce waste submitted to different drying and grinding processes in food and non-food sectors.

5.5.1 Introduction

Drying can be applied to FVW to produce value-added derivatives, such as food-grade dried materials and flour, rich in fibre and antioxidants (Ferreira et al., 2015; Galanakis, 2012). In the case of lettuce waste, dried lettuce waste derivatives are expected to be microbiologically stable and have lower volume, reducing packaging, storage and transport issues (Karam et al., 2016).

The main drawback of lettuce waste drying lays in the cost of water removal from a material containing more than 90% moisture (Strumillo & Adamiec, 1996). Nevertheless, different drying techniques could be exploited to increase process affordability. Air-drying, which is based on the contact of wet materials with a hot air flow, is energy intensive and is associated to material shrinkage, hardness, poor appearance, reduced ability to rehydrate and bioactive loss. On the other hand, it is the most commonly applied food drying technique and has limited investment costs (Ratti, 2001; Strumillo & Adamiec, 1996). On the contrary, freeze-drying produces high-quality dried products, due to water removal by sublimation of ice crystals. However, equipment is costly, drying rates are low and much energy is consumed for freezing and vacuum phases (Ratti, 2001). Novel drying techniques, such as supercritical-CO_2-drying, have been claimed to increase environmental sustainability of traditional drying processes. In this case, water is slowly removed from the food material by a continuous supercritical-CO_2 flow. Temperature and pressure

conditions are mild (20-50 °C and 10-20 MPa), guaranteeing a good bioactive retention. Moreover, co-solvents such as ethanol can be used to significantly reduce drying time (Brown et al., 2008). Supercritical-CO_2-drying avoids the formation of vapour-liquid interfaces, allowing product structure to be preserved (Brown et al., 2008; García-González, Camino-Rey, Alnaief, Zetzl, & Smirnova, 2012). Investment and running costs are high but they could be counterbalanced using non-toxic carbon dioxide, which leaves no residues and can be recycled (Viganó, Paula, & Martínez, 2014).

In this present work, the possibility to valorise lettuce waste by turning it into dried materials and flour via traditional and novel drying processes was investigated.

5.5.2 Materials and methods

Lettuce waste preparation

Lettuce waste was prepared as described in § 5.1.2 and immediately submitted to drying.

Air-drying

Lettuce waste (1 kg) was manually chopped in homogeneous pieces (about 5 x 5 cm) with a sharp knife and dried in single layers at 70±0.5 °C and at a relative humidity in the drying chamber <5% using an air-drying oven (UM100, Memmert, Schwabach, Germany).

Freeze-drying

Lettuce waste (1 kg) was manually chopped in homogeneous pieces (about 5 x 5 cm) with a sharp knife and dried in single layers and frozen at -30 °C for 24 h and then freeze dried for 72 h at 4053 Pa by using the pilot plant model Mini Fast 1700 (Edwards Alto Vuoto, Milan, Italy).

Supercritical-CO_2-drying

Lettuce waste was manually chopped in homogeneous pieces (about 5 × 5 cm) with a sharp knife. An amount of 5 g lettuce waste was then dried using supercritical-CO_2-drying with or without previous substitution of lettuce water with ethanol. In this case, lettuce waste was immersed (0.1 g/mL) in pure ethanol (J.T. Baker, Centre Valley, USA) for 24 h twice. During this time, water was progressively removed from lettuce leaves, as indicated by monitoring the decrease in the alcoholic degree of the ethanol solution by a lab alcoholmeter (Alcolyzer plus, Anton Paar, Graz, Austria). Additional samples were prepared by grinding (MC3001, Moulinex, China) the lettuce waste submitted to ethanol substitution and subsequently removing excess solvent by vacuum filtration before supercritical-CO_2-drying. Supercritical-CO_2-drying was performed by using a plant developed at the Department of Agricultural, Food, Environmental and Animal Sciences (University of Udine), previously described by Manzocco et al. (2017). Sample was placed inside the reactor in which CO_2 was then pressurized at 11 ± 1 MPa and 45 °C. The outlet flow through

the reactor was set at 6.0 L/min. This flow was selected since allowing drying time to be minimized while maintaining the structural integrity of the material as visually assessed. Samples in which water had been previously substituted with ethanol were considered dried when ethanol was no more detectable in the gaseous outlet. Decompression from 11 MPa to atmospheric pressure was then carried out in 30 min. In the case of samples not submitted to water substitution with ethanol, at increasing drying times, samples were removed from the reactor and weighted. The end of the drying process was set in correspondence of a residual moisture in the sample lower than 50 g/kg. Drying time was of 2.5, 5.0 and 1.5 hours for lettuce waste in which water was substituted with ethanol, for samples containing water and for ground samples, respectively.

Lettuce waste flour

Dried lettuce waste was finely ground using a ball mill (MM2, Retsch, Hann, Germania) for 15 min.

Sample storage

Dried lettuce waste and flour were stored at 20 °C in sealed aluminized aseptic bags until use.

Analytical determinations

Particle size distribution

20 g of flour was sieved on a set of sieves with mesh sizes of 500, 250, 125, 63 and 20 µm (Endecotts Ltd, London, UK). The amount of flour remaining in each sieve was weighted and expressed as a percentage (w/w) of the initial flour weight.

Colour determination

Colour was determined as described in § 5.1.2.

Image acquisition

Images were acquired as described in § 5.1.2.

Optical and electronic microscopy

For optical microscopy, samples were observed at room temperature, as described in § 5.1.2.
For scanning electron microscopy, samples were mounted on aluminium sample holders and sputter coated with 10 nm of gold using a Sputter Coater 108 auto (Cressington Scientific Instruments, Watford, United Kingdom). The aluminium holder was transferred to the SEM unit (EVO 40XVP, Carl Zeiss, Milan, Italy), which was at ambient temperature and under vacuum. Samples were imaged using an acceleration voltage of 20 kV and SmartSEM v. 5.09 (Carl Zeiss, Milan, Italy) application software was used to capture images of the samples. Images were taken at 1000× magnification and saved in tiff format resulting in 1696×2048 pixels.

Moisture content

Moisture content was calculated according to AOAC gravimetric method (AOAC, 1997).

Water vapour sorption

Samples (2 g) were inserted into dried weighting bottles and transferred into desiccators containing distilled water. Sample weight increase was monitored for 5 h during rehydration.

Water and oil holding capacities

Dried lettuce waste leaves (2 g) were immersed into water or sunflower oil for 24 h at room temperature under gentle mixing. Samples were accurately drained on a wire mesh for 10 min.
In the case of flour, an accurately weighted amount of sample was inserted into tared 2-mL Eppendorf tubes and added with 2 mL of distilled water or sunflower oil. Tubes were stirred using a vortex (Vortex 1, Ika, Milan, Italy) three times for 30 s and centrifuged at 1327 x g 30 min (Mikro 20, Hettich Zentrifugen, Tuttlingen, Germany). The sediment obtained after centrifugation was weighted. Water and oil holding capacities were calculated as g of water or oil held by 1 g of dried sample.

Total dietary fibre

Total dietary fibre (TDF) was calculated as described in § 3.1.

Preparation of lettuce waste extract

10 g of lettuce waste, trimmed with a sharp knife, or flour were extracted by reflux with boiling water for 60 min applying a dilution of 1:4 (w/v) and 1:20 (w/v) respectively. Extracts were cooled at room temperature, vacuum filtered thorough Whatman no. 1 filter paper (Maidstone, UK), freeze-dried at -50 °C and stored in a desiccator containing P_2O_5 at room temperature until use.

Total polyphenolic content

Total polyphenolic content (TPC) was determined using Folin-Ciocalteau reagent as described in § 3.1 (Singleton & Rossi, 1985). The reaction mixture contained 100 µL of lettuce waste extract solubilised in water (1:10 w/v), 500 µL of the Folin-Ciocalteau reagent, 4 mL of water and 2 mL of a sodium carbonate-water solution (0.15 g/mL).

HPLC

Freeze-dried extracts (10 mg) were dissolved in 1 mL of distilled water, filtered through a 0.45 µm membrane filter (GVS, Meckenheim, Germany) and analysed using a HPLC system equipped with a Prostar 230 pump (Varian, Walnut Creek, USA) and a Prostar 330diode array detector (Varian, Walnut Creek, California, USA). To this aim, 20 µL sample was injected in a C18 column (Alltima, 5 microns, 250 x 4.6 mm, Grace, Lokeren, Belgium). The mobile phase was water with 50 mL/L formic acid (Fluka, St. Louis, Missouri, USA) (solvent A) and HPLC grade methanol (Chromasol≥99.9%, Sigma-Aldrich St. Louis, Missouri, USA) (solvent B) at a flow rate of 1 mL/min. The linear gradient started with 10% B in A to reach 20% B at 25 min, 50% B at 40, 50% B at 45 min and 90% B at 60 min. Chromatograms were recorded at 335nm. Data elaboration was performed by Polyview program (v.5.3). Phenolic compounds identification was based on their UV spectra and retention times (DuPont et al., 2000; Llorach et al., 2004; Mai & Glomb, 2013; Tomás-Barberán et al., 1997). Chicoric acid was quantified (Lee & Scagel, 2013) using an external standard while other compounds were quantified as 3-O-caffeoylquinic acid by comparison with external standard (Sigma-Aldrich, St. Louis, Missouri, USA).

Chain-breaking activity (DPPH· assay)

The chain-breaking activity (CBA) was measured following the bleaching rate of 2,2-diphenyl-1-picrylhydrazyl (DPPH·) in the presence of the sample. 3 mL of 6.1×10^{-5} M DPPH· methanol solution was used. The reaction was started by the addition of 150 µL of lettuce waste extract solubilised in water (1:10 w/v). DPPH· bleaching was followed at 515 nm (UV-2501PC, UV-Vis Recording Spectrophotometer, Shimadzu Corporation, Kyoto, Japan) at 25 °C for 10 min. DPPH· bleaching rate was proportional to sample concentration. The following eq. 28 was chosen to obtain the reaction rate of DPPH· bleaching, k (Manzocco et al., 1998):

$$\frac{1}{A^3} - \frac{1}{A_0^3} = 3kt \qquad \text{(eq. 28)}$$

where A_0 is the initial optical density (OD) and A is the optical density at increasing time, t. The chain-breaking activity was expressed as k/g of dry weight ($OD^{-3}/min/g_{dw}$). The chain-breaking phenolic ratio (CBP) was also determined dividing the chain-breaking activity of the sample by its phenol content ($OD^{-3}/min/mg_{GAE}^{-1}$).

Data analysis

Analyses were carried out at least three times in two replicated experiments. Analysis of variance ($p<0.05$) and linear regression analysis were performed using R (The R foundation for statistical computing, v.3.1.1). Goodness-of-fit was evaluated based on R^2 and p-values.

5.5.3 Results and discussion

Characterization of lettuce waste

External leaves of *Iceberg* lettuce from fresh-cut processing presented the typical green colour and a moisture level exceeding 900 g/kg (Table 23). Lettuce waste resulted particularly rich in fibre, in agreement with nutritional databases relevant to edible lettuce (USDA, 2018). By contrast, lettuce waste polyphenol content (Table 23) resulted about 4 times lower than that reported by Llorach et al. (2004). Different factors, including agronomic practices, lettuce variety and extraction solvent, could significantly affect polyphenol quantification (Llorach et al., 2008). Nevertheless, due to its polyphenol content, comparable to that of grape marc, lettuce waste can be considered an always-available and cheap source of antioxidants (Bonilla, Mayen, Merida, & Medina, 1999).

Table 23. CIELAB scale colour parameters (L*, a*, b*), moisture, total dietary fibre (TDF), total polyphenolic content (TPC), chain-breaking activity (CBA) and chain-breaking phenolic ratio (CBP) of lettuce waste.

Colour			**Moisture (g/kg)**	**TDF (g/kg)**	**TPC (mg GAE/g dw)**	**CBA (OD^{-3}/min/g dw)**	**CBP (OD^{-3}/min/mg GAE)**
L*	a*	b*					
71.4 ± 1.3	-16.9 ± 1.2	31.6 ± 1.4	941 ± 12	16.1 ± 2.0	1.84 ± 0.02	6.04 ± 0.79	4.17 ± 0.54

dw, dry weight

Characterization of dried lettuce waste

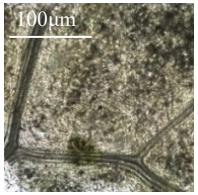
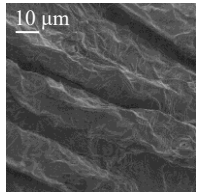
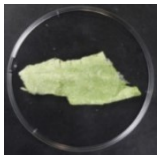
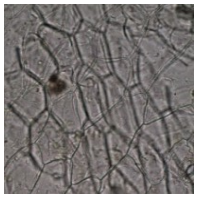
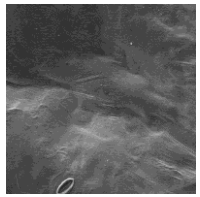
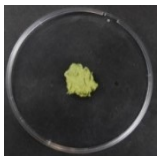
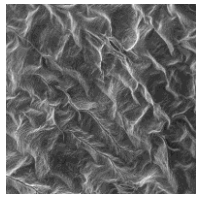
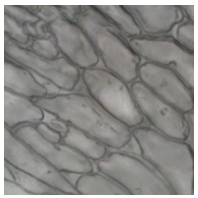
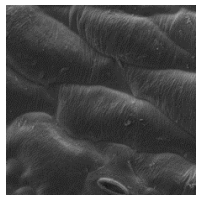
Water content of lettuce waste makes it microbiologically unstable, posing critical management issues. To increase its stability, dehydration could be performed, as proposed for other vegetable wastes (Annadurai et al., 2002; de Oliveira et al., 2009). Air-dried (AD), freeze-dried (FD), supercritical-CO_2-dried samples were thus prepared. The latter were produced in the absence (SCCD sample) or presence (SCCD-EtOH sample) of ethanol as co-solvent.

Drying techniques exerted different effects on lettuce waste colour (Table 24). AD sample appeared brown, due to enzymatic and non-enzymatic reactions, prevailing in the initial and advanced phases of the process, respectively (Adam, Mühlbauer, Esper, Wolf, & Spiess, 2000). FD leaves maintained the original colour, confirming the ability of freeze-drying to minimize quality damage (Argyropoulos, Heindl, & Mu, 2011). Similarly, SCCD samples resulted green, suggesting this technology as a valid alternative to freeze-drying (Brown et al., 2008). Interestingly, SCCD-EtOH sample completely lost the original colour, probably due to pigment extraction during lettuce immersion into ethanol. In fact, the SCCD sample, which had not been immersed in ethanol, highly retained the original colour (Table 24). Pigment extraction by supercritical-CO_2 was probably

negligible since the pressure here applied (<12 MPa) was lower than that required for chlorophyll extraction (>25 MPa) (Guedes et al., 2013).

The drying technique strongly affected sample physical structure, as shown by visual appearance and microscopic analyses (Table 24). AD samples resulted severely shrunk, since water evaporation created intense capillary tensions in cellular structure (Ahmed, 2010). On the contrary, FD samples maintained cellular organization thanks to water removal by sublimation of ice, which provides structural rigidity (Ratti, 2001). However, no clear morphology of cells was revealed by SEM, probably due to the presence of the typical wax protective layer on vegetable surface. SCCD samples appeared completely collapsed. This phenomenon was prevented by adding co-solvents during drying (Table 24). Cells of SCCD-EtOH sample were actually visible and appeared even swallowed in microscopic images. Similar effects were also observed in carrot slices and can be attributed to tissue expansion during CO_2 decompression (Brown et al., 2008). In addition, in SEM image of SCCD-EtOH sample, no protective wax layer was evident onto sample surface, probably due to its solubilization in the supercritical-CO_2 flow (Roy, Goto, Kodama, & Hirose, 1996).

Table 24. Visual appearance, CIELAB scale colour parameters (L*, a*, b*), microscopic images (optical and SEM), water and oil holding capacities (WHC, OHC) of dried lettuce waste leaves obtained using air-drying (AD), freeze-drying (FD), and supercritical-CO_2-drying without (SCCD) or with (SCCD-EtOH) ethanol.

Lettuce waste	**Visual appearance**	**Colour**			**Microscopy**		**WHC** (g H_20/g ds)	**OHC** (g oil/g ds)
		L*	a*	b*	Optical	SEM		
AD		61.2 ± 0.3 [c]	-1.5 ± 0.2 [b]	23.2 ± 0.1 [a]			5.3 ± 0.6 [b]	1.1 ± 0.2 [b]
FD		75.6 ± 0.3 [b]	-11.2 ± 0.1 [d]	18.4 ± 0.2 [b]			7.5 ± 0.4 [b]	2.1 ± 0.4 [b]
SCCD		53.4 ± 2.0 [d]	-5.2 ± 0.7 [c]	15.9 ± 2.2 [b]			4.2 ± 0.9 [b]	1.0 ± 0.4 [b]
SCCD-EtOH		85.0 ± 2.4 [a]	-0.2 ± 0.1 [a]	8.7 ± 0.4 [c]			37.1 ± 1.1 [a]	16.3 ± 1.7 [a]

[a, b, c, d]: In the same column, mean values indicated by different letters are statistically different ($p<0.05$); ds = dry sample

To better assess the effects of drying treatments on lettuce waste properties, the ability of the dried leaves to interact with water vapour was evaluated (Figure 16A). All samples showed a progressive vapour adsorption upon maintenance in a moisture-rich atmosphere. The evolution of vapour sorption was significantly affected by the drying technique. AD and SCCD samples showed a slow vapour uptake, probably due to their dense microstructure (Table 24) (Argyropoulos et al., 2011; Ratti, 2001). A faster water vapour sorption was observed for FD sample, which well-maintained structure (Table 24). The expanded SCCD-EtOH sample (Table 24) showed the fastest and highest vapour uptake. These findings suggest that drying-induced structure deeply affects the ability of samples to interact with solvents. To confirm this hypothesis, samples were analysed for water and oil holding capacity (WHC, OHC). A WHC much higher than OHC was observed for all samples (Table 24), being vegetable waste rich in hydrophilic polysaccharides (Ferreira et al., 2015). SCCD-EtOH sample showed the highest WHC and OHC values (Table 24). Excellent rehydration properties were also observed for carrot slices submitted to supercritical-CO_2-drying using ethanol as co-solvent (Brown et al., 2008). Rehydration ability was attributed to the capacity of supercritical-drying with ethanol as co-solvent to beget highly porous materials, favouring water capillary adsorption. Interestingly, the amount of water held by 1 g of SCCD-EtOH sample resulted much higher than that originally present in the fresh lettuce waste tissue (circa 16 g H_2O/g_{dw}, as computed based on moisture content, Table 23). The capacity of SCCD-EtOH sample to absorb water beyond the amount entrapped in the native plant tissue could be attributed to the expanded structure obtained by supercritical-CO_2-drying and to water solvation of polysaccharides, which would favour sample swallowing. By contrast, oil adsorption did not promote swallowing of sample, which retained circa 16 g oil/g_{dw}, indicating that oil simply substituted voids left upon water removal.

The interesting ability of dried lettuce wastes to interact with water and oil suggests their possible exploitation as ingredients in dried instant foods (e.g. soups, noodles, meat).

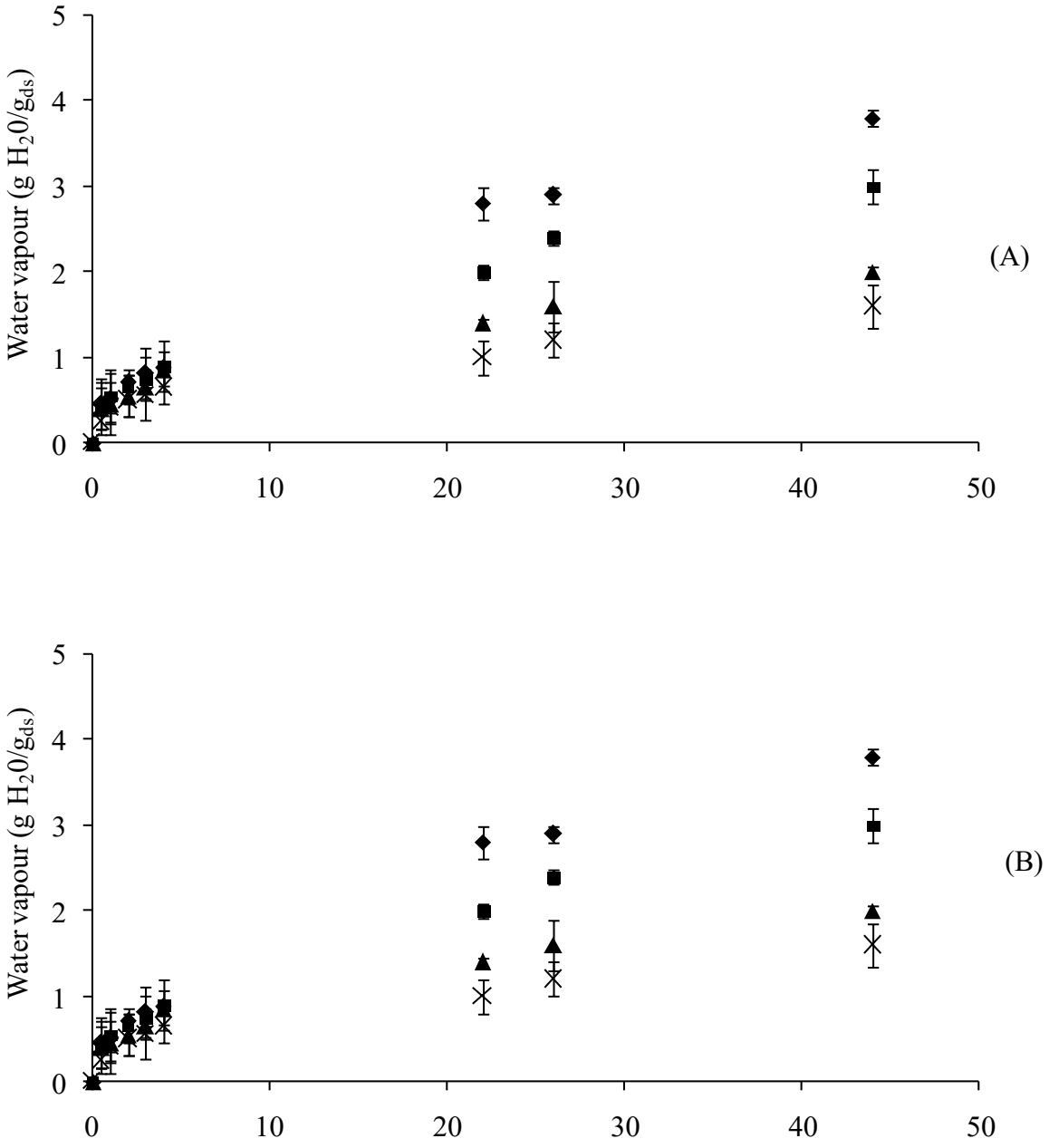


Figure 16. Adsorption of water vapour of dried lettuce waste leaves (A) and lettuce waste flour (B) submitted to air-drying (▲), freeze-drying (■), and supercritical-CO_2-drying without (×) or with (♦) ethanol. ds = dry sample

Characterization of lettuce waste flour

The possibility to valorise lettuce waste by turning it into flour was studied. The attention was focused on AD, FD and SCCD-EtOH lettuce wastes. SCCD sample was not considered since characterised by a collapsed structure with low WHC and OHC (Table 24, Figure 16A). AD and FD samples were ground to flour with a 95% yield. On the contrary, grinding yield of SCCD-EtOH sample resulted <10%, possibly due to the difficulty in grinding an expanded tissue. The flour was thus obtained by grinding lettuce waste after ethanol substitution before supercritical-CO_2-drying. All lettuce flour samples presented most particles in the range 200-250 µm (Table 25). However, a lower size particle fraction was observed in flour from AD and FD samples, confirming their grinding to be particularly efficacious. Samples showed similar moisture and fibre content (Table 25). The latter resulted higher than that of rice (21 g/100 g) and oat (15 g/100 g) bran (USDA, 2018), suggesting the possible suitability of lettuce flour as ingredients to increase fibre content of foods (e.g. instant foods, bakery products).

Table 25. Particle size distribution, moisture, total dietary fibre (TDF), total phenolic content (TPC), relevant chain-breaking activity (CBA), chain-breaking phenolic ratio (CBP) and water and oil holding capacities (WHC, OHC) of flour samples obtained using air-drying (AD), freeze-drying (FD), and supercritical-CO_2-drying with ethanol as co-solvent (SCCD-EtOH).

Lettuce flour	**Particle size (g/kg)**		**Moisture (g/kg)**	**TDF (g/kg)**	**TPC (mg GAE/ g dw)**	**CBA (OD^{-3}/min/ g dw)**	**CBP (OD^{-3}/min/ mg GAE)**	**WHC (g H_20/ g ds)**	**OHC (g oil/ g ds)**
	200-250 µm	<200µm							
AD	942 ± 9 ^{b}	61 ± 4 ^{a}	40 ± 1 ^{a}	266 ± 4 ^{a}	3.05 ± 0.08 ^{a}	27.03 ± 1.60 ^{a}	8.87 ± 0.13 ^{a}	9.1 ± 0. 7 ^{c}	2.3 ± 0.4 ^{b}
FD	928 ± 1 ^{b}	80 ± 6 ^{a}	46 ± 2 ^{a}	266 ± 4 ^{a}	1.23 ± 0.01 ^{b}	4.01 ± 0.05 ^{b}	3.22 ± 0.02 ^{c}	12.5 ± 0.6 ^{bc}	3.2 ± 0.3 ^{b}
SCCD-EtOH	996 ± 3 ^{a}	2 ± 1 ^{b}	39 ± 8 ^{a}	272 ± 3 ^{a}	0.84 ± 0.01 ^{c}	3.38 ± 0.08 ^{b}	4.04 ± 0.06 ^{c}	43.2 ± 0.4 ^{a}	35.2 ± 0.7 ^{a}

$^{a, b, c}$: In the same column, mean values indicated by different letters are statistically different ($p<0.05$); dw = dry weight; ds = dry sample

Drying treatment significantly affected both polyphenol content and antioxidant activity of flour (Table 25). AD flour showed the highest polyphenol content and antioxidant activity, which resulted significantly higher than those of fresh sample ($p<0.05$) (Table 23). This can be attributed to the formation of partially-oxidised polyphenols and Maillard reaction products with a prominent antioxidant action (Mrkìc et al., 2006). Freeze-drying allowed polyphenol content and antioxidant activity of fresh lettuce waste to be partly retained (Table 23). Due to the low process temperature and almost complete absence of oxygen, degradation reactions are minimized during freeze-drying (Michalska, Wojdyło, Lech, Łysiak, & Figiel, 2017). Nevertheless, phenols could be enzymatically oxidised upon enzyme de-compartmentalization during freezing (Chang, Lin, Chang, & Liu, 2006). SCCD-EtOH flour presented a phenolic content lower than that of FD sample, probably due to partial polyphenol extraction by supercritical-CO_2. The latter is actually applied for polyphenol extraction from vegetable matrices (Cavalcanti, Navarro-Díaz, Santos, Rostagno, & Angela, 2012; Gadkari, Balarman, & Kadimi, 2013). HPLC was performed for polyphenol qualitative (Figure 17) and quantitative (Table 26) analyses.

Table 26. Quantification of phenolic compounds identified by HPLC in fresh lettuce waste (Fresh) and in flour samples obtained using air-drying (AD), freeze-drying (FD), and supercritical-CO_2-drying with ethanol as co-solvent (SCCD-EtOH).

Phenolic compounds (mg/g_{dw})	**Retention time (min)**	**Sample**			
		Fresh	AD	FD	SCCD-EtOH
3-O-caffeoylquinic acid	10.5 ± 0.1	0.014 ± 0.004	0.002 ± 0.001	0.010 ± 0.001	ND
Caffeoyltartaric acid	12.8 ± 0.2	0.158 ± 0.003	0.060 ± 0.003	0.074 ± 0.004	0.023 ± 0.002
4-O-caffeoylquinic acid	21.5 ± 0.1	0.012 ± 0.001	ND	ND	ND
5-O-caffeoylquinic acid	22.5 ± 0.1	0.074 ± 0.003	ND	0.003 ± 0.001	ND
Caffeic acid derivative	23.3 ± 0.1	0.036 ± 0.002	ND	0.002 ± 0.001	ND
Isochlorogenic acid	33.0 ± 0.3	0.007 ± 0.001	ND	ND	ND
Chicoric acid	38.1 ± 0.2	0.187 ± 0.002	0.044 ± 0.002	0.040 ± 0.006	0.002 ± 0.001
Caffeic acid derivative	38.5 ± 0.1	0.007 ± 0.001	ND	ND	ND
Luteolin 7-O-glucuronide	42.4 ± 0.1	0.007 ± 0.001	ND	ND	ND
Quercetin 3-O-glucuronide	42.8 ± 0.1	0.011 ± 0.001	ND	ND	ND

dw = dry weight; ND = not detected

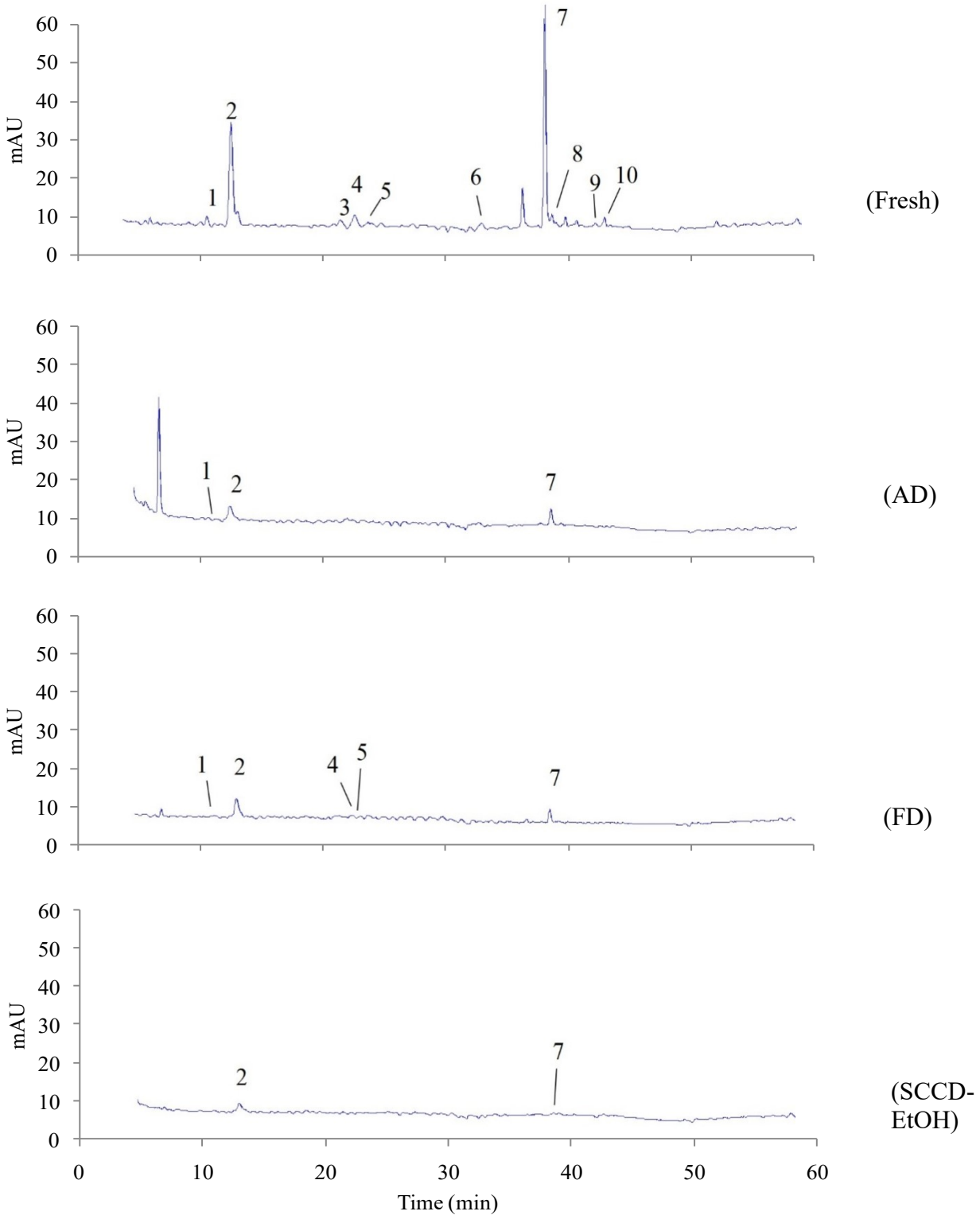


Figure 17. HPLC profiles of water extracts of fresh lettuce waste (Fresh) and flour samples obtained by air-drying (AD), freeze-drying (FD) and supercritical-CO_2-drying using ethanol as co-solvent (SCCD-EtOH). Peak identification: (1) 3-O-caffeoylquinic acid; (2) caffeoyltartaric acid; (3) 4-O-caffeoylquinic acid; (4) 5-O-caffeoylquinic acid; (5) caffeic acid derivative; (6) isochlorogenic acid; (7) chicoric acid; (8) caffeic acid derivative; (9) luteolin 7-O-glucuronide; (10) quercetin 3-O-glucuronide. AU, arbitrary units

HPLC profile of fresh lettuce waste revealed the presence of different phenolic acids, in agreement with literature (Llorach et al., 2004). The main identified phenolic acid was dicaffeoyltartaric acid (chicoric acid) (peak 7), followed by caffeoyltartaric acid (peak 2) and 5-O-caffeoylquinic acid (peak 4). The latter can isomerise in warm aqueous phase, leading to 3-O-caffeoylquinic acid (peak 1) and 4-O-caffeoylquinic acid (peak 3) (Llorach, Espín, Tomás-Barberán, & Ferreres, 2003). Flavonoid compounds, such as luteolin derivatives (luteolin 7-O-glucuronide, peak 9) and quercetin derivatives (quercetin 3-O-glucuronide, peak 10) were also detected. Independently on the applied technology, lettuce waste drying always promoted a severe decrease in the intensity of peaks relevant to naturally occurring polyphenols (Table 26). However, AD flour chromatogram also showed an intense peak at low retention times (5.75 min), probably ascribable to Maillard reaction compounds, which can account for the high antioxidant activity of this flour (Table 25) (Mrkìc et al., 2006). Drying technology thus affected not only content but also composition of flour phenols (Table 25) and, consequently, their chain-breaking activity. This was confirmed by the chain-breaking phenolic ratio (CBP, Table 25) that allows comparison of antiradical activity of samples with different phenolic content (Manzocco et al., 1998). AD flour showed the highest CBP, confirming the high antioxidant activity of compounds formed during air-drying. FD and SCCD-EtOH flour presented CBP similar to that of fresh samples ($p \geq 0.05$) (Table 23), suggesting supercritical-CO_2-drying as a suitable technology for producing high-quality dried products (Brown et al., 2008).

Lettuce waste flour samples were then evaluated for their water vapour sorption (Figure 16B). As expected, vapour uptake of flour, which has high absorptive surface, was higher than that observed in the not-ground samples (Table 23A). Flour vapour uptake was in the order AD<FD<SCCD-EtOH, in accordance with decreasing sample structural collapse upon drying (Table 24). SCCD-EtOH flour also showed the highest WHC and OHC values (Table 25). Moreover, SCCD-EtOH flour presented a similar tendency to interact with water and oil (Table 25). It can be inferred that performing grinding before supercritical-CO_2-drying allowed obtaining an extremely porous flour with excellent solvent-loading capacity and in which absorption would be mainly driven by capillary forces rather than chemical interactions. Large amounts of different solvents could be thus easily embedded into the pores of SCCD-EtOH flour. This property could have interesting practical relevance, suggesting the possible exploitation of this flour as oil spill absorber or bulking agent in food formulations. It could also be used to structure liquid oil, leading to the development of innovative materials, such as oleogels, able to simulate technological performances of fats while reducing saturated fatty acid content.

5.5.4 Conclusions

Lettuce waste drying is an interesting strategy to valorise this critical industrial discard by turning it into ingredients rich in fibre and antioxidant compounds, with tailored physico-chemical properties. These can be steered by exploiting different drying mechanisms such as evaporation, sublimation or supercritical-fluid extraction. In this latter case, grinding before drying and using ethanol as co-solvent allowed obtaining a flour with excellent ability to adsorb both water and oil. However, when intended for food-use, these ingredients should be accurately assessed for safety aspects such as microbial quality and presence of contaminants deriving from cultivation practises. In the light of the acquired results, supercritical-CO_2-dried materials presented an interesting expanded structure, suggesting their possible use not only as food ingredients but also as innovative biodegradable materials for non-food applications. The identification of the proper intended use of these materials requires a detailed characterisation of their structure and physical properties (§ 5.6).

5.6 Valorisation of lettuce waste into innovative bioaerogel-like materials via supercritical-CO_2-drying

Given the interesting expanded structure of the sample obtained by supercritical drying of lettuce waste (§ 5.5), in the present study, the possibility to further exploit supercritical-CO_2-dried lettuce waste to produce bioaerogel-like materials was investigated. Bioaerogels are interesting innovative and biocompatible materials, possibly exploitable as thermal insulators, packaging and medical engineering applications. Lettuce waste was submitted to ethanol solvent exchange and supercritical-CO_2-drying. Samples were then analysed for optical, thermal, mechanical and structural properties as well as for the ability to uptake water and oil. Analyses were performed in order to get an insight in the physical and physico-chemical properties of the material to suggest possible applications.

5.6.1 Introduction

Aerogels are defined as microporous materials entrapping a gas phase within the pores (Jones, 2007). The term is generic and refers to a wide variety of materials, presenting low density (0.0003-0.5 g/cm^3), high surface area (50-1200 m^2/g) and high porosity (70.0-99.8%) (Fricke & Tillotson, 1997). Based on their composition, aerogels can be classified into inorganic, organic, hybrid and bioaerogels. Although studies about bioaerogels are still pioneering, they have raised great interest in the last years, being produced from natural sources such as cellulose and its derivatives, marine polysaccharides, starch and proteins (Stergar & Maver, 2016). Bioaerogels are biocompatible, biodegradable and food-grade, making them potentially applicable in medical engineering, sustainable packaging production and novel food development (Maleki, 2016) To this regard, bioaerogels were proposed as carriers for different solvents, due to their ability to absorb large amounts of liquids by capillary forces (Ahmadi, Madadlou, & Akbar, 2016; Comin, Temelli, & Saldaña, 2012; Ivanovic, Milovanovic, & Zizovic, 2016). In this context, they have been suggested as food bulking agents, templates for liquid oil structuring and innovative carriers of nano-sized biochemicals (Ahmadi et al., 2016; Manzocco et al., 2017; Ubeyitogullari & Ciftci, 2017).

To obtain bioaerogels, a two-step procedure is traditionally applied. Initially, hydrogels are produced by gelation of a biopolymer in an aqueous media. Following, hydrogel solvent is slowly removed by a flow of continuous supercritical-CO_2. The latter is commonly applied since it avoids the formation of liquid-vapor interfaces and capillary tensions, thus reducing local collapse and maintaining the original hydrogel network (Maleki, 2016). The time required for supercritical-CO_2-dryingis generally reduced by an ethanol-assisted procedure. In this case, before drying, hydrogel aqueous phase is substituted with ethanol, which has a higher affinity to supercritical-CO_2 (García-González et al., 2012; Viganó et al., 2014). Supercritical-CO_2-drying is regarded as sustainable

since it is performed at mild pressure (<10 MPa) and temperature (<45 °C), often using recycled carbon dioxide (Ghafar et al., 2017).

It can be inferred that supercritical-CO_2-drying of biological systems other than biopolymer gels could also produce highly aerated structure, similar to that of bio-aerogels. For instance, moisture-rich plant tissues could represent optimal candidates for the preparation of bioaerogel-like materials. These vegetable matrices can be regarded as complex polymeric networks, mainly structured by cell wall cellulosic fibres, embedding water within intra- and inter-cellular spaces. Water removal, while maintaining cellular organization, could possibly result in an aerated fibrous network with high internal surface area and porosity. Circumstantial evidence supporting this hypothesis could be the enhanced rehydration ability of fruit and vegetables submitted to supercritical-CO_2-drying (Brown et al., 2008).

The preparation of bioaerogel-like materials from vegetable tissues could present the advantage of simplifying the conventional production process, since not requiring the gelling phase. In addition, if applied to vegetable wastes, this technological strategy could allow valorisation of industrial discards, which typically represent an environmental and economic burden (Raak, Symmank, Zahn, Aschemann-witzel, & Rohm, 2017).

5.6.2 Materials and methods

Supercritical-CO_2-dried lettuce waste preparation

Samples of supercritical-CO_2-dried lettuce waste were prepared as described in § 5.5.2 and stored in P_2O_5 at room temperature until use.

Analytical determinations

Lettuce waste composition

Moisture, ash, protein and fat content was calculated according to AOAC methods (AOAC, 1997). Total dietary fibre (TDF) was calculated according to the AOAC international method (AOAC, 1997) using a total dietary fibre assay kit (TDF – 100A, Sigma-Aldrich, St. Louis, Missouri, USA). Carbohydrates were calculated by difference from moisture, ash, fat, protein and TDF.

Total polyphenolic content

Preparation of phenolic lettuce waste extract and determination of total polyphenolic content (TPC) were carried out as described in § 5.5.2.

Chlorophyll content

Lettuce waste was homogenized with ethanol (95% w/w) applying a dilution of 1:4 (w/v) and filtered. The chlorophyll content was determined spectrophotometrically (Shimadzu UV-2501PC, UV-Vis recording spectrophotometer, Shimadzu Corporation, Kyoto, Japan), using the Lambert-Beer law and the specific absorbance coefficient (α) for chlorophyll α in 95% ethanol (84.6 $Lg^{-1}cm^{-1}$) (Lichtenthaler & Buschmann, 2001; Perucka et al., 2014). Results were expressed as µg of chlorophyll per mg of dry weight.

Colour

Colour determination was carried out as described in § 5.1.2.

Image acquisition

Images were acquired as described in § 5.1.2.

Optical and electronic microscopy

For optical microscopy, samples were observed at room temperature as described in § 5.1.2.

Sorption isotherm

Samples were weighted and transferred into dried weighting bottles. The latter were then transferred into desiccators containing P_2O_5 and LiCl, CH_3COOK, $CaCl_2$, K_2CO_3, NaCl, KCl, K_2SO_4 saturated solutions and water with equilibrium relative humidity (ERH%) values of 5, 11, 25, 31, 43, 75, 86, 96 and 100%, respectively. Samples were kept inside desiccators until constant weight was reached. The Brunauer-Emmet-Teller (BET) sorption isotherm model (eq. 29) was fitted into water sorption data (Braunauer, Emmett, & Teller, 1938).

$$\frac{a_w}{m(1-a_w)} = \frac{1}{m_0 c} + \frac{c-1}{m_0 c} a_w \qquad \text{(eq. 29)}$$

where a_w is the water activity, m is the moisture of the sample expressed as ratio between the weight (g) of absorbed water and the weight (g) of dry matter, m_0 is the moisture of the water monolayer, and c is an experimental constant.

Differential Scanning Calorimetry (DSC)

DSC analysis was carried out using a TA4000 differential scanning calorimeter (Mettler-Toledo, Greifensee, Swiss) connected to a Graph Ware software TAT72.2/5 (Mettler-Toledo). Heat flow calibration was achieved using indium (heat of fusion 28.45 J/g). Temperature calibration was carried out using hexane (m.p. -93.5 °C), water (m.p. 0.0 °C) and indium (m.p. 156.6 °C). Samples were prepared by carefully weighing around 5 mg of cut sample in 160 µL aluminium DSC pans, closed with hermetic sealing. An empty pan was used as a reference in the DSC cell.

For determination of specific heat capacity ($C_{p,}$ $Jg^{-1}K^{-1}$), dried lettuce waste was heated from 15 to 150 °C, at a constant heating rate of 10 °C/min and without purge gas. A blank curve was also recorded by submitting an empty sealed pan to the same analysis. C_p (40 °C) was determined according to the following eq. 30:

$$C_p = \frac{H}{r_T} \cdot \frac{1}{m} \qquad \text{(eq. 30)}$$

Where H is the heat flow in mW, calculated as signal difference between sample and blank curve, r_T is the sample heating rate (K/s) and m is the sample mass in mg.

Specific volume and apparent density

Samples were cut into 2 × 2 cm square shape and accurately weighted. Sample volume was then measured by a CD-15APXR digital calibre (Absolute AOS Digimatic, Mitutoyo Corporation, Kanagawa, Japan) and expressed as specific volume (cm^3/g). Sample apparent density (g/cm^3) was then calculated as the ratio between sample weight and specific volume.

Mechanical properties

Sample mechanical properties were measured by uniaxial tensile test using an Instron 4301 (Instron LTD., High Wycombe, UK). The instrumental settings and operations were accomplished using the software Automated Materials Testing System (v. 5, Series IX, Instron LTD., High Wycombe, UK). Rectangular samples (2 × 4 cm) were positioned between two clamps mounted on a 100 N tensile head at a 5 mm/min crosshead speed, with a gauge length of 1 cm. Stress-strain curves were obtained from the tensile tests. Tensile strength at break (N) and the elongation at break (mm) were used to characterize sample tensile strength.

Hygroscopicity

The hygroscopicity was determined by measuring the moisture absorption capacity of samples at a set relative humidity (RH) of 86% at 30 °C. For this purpose, saturated solution of potassium chloride (KCl) was used. Samples were kept in desiccators with the set RH and weighed over time until a constant weight was reached. The percentage of moisture sorption (MS) was calculated from eq. 31:

$$MS = \frac{W - W_0}{W_0} \times 100 \qquad \text{(eq. 31)}$$

where, W (g), and W_0 (g) are the material weight after moisture sorption and its initial weight, respectively (Su et al., 2010).

FTIR measurement

Spectra were recorded at 25 ± 1 °C using a FTIR instrument, equipped with an ATR accessory and a Zn-Se crystal that allows collection of FTIR spectra directly on sample without any special preparation (Alpha-P, Bruker Optics, Milan, Italy). The "pressure arm" of the instrument was used to apply constant pressure to the samples positioned on the top of the Zn-Se crystal, to ensure a good contact between the sample and the incident IR beam. All FTIR spectra were collected in the range from 4000 to 400 cm^{-1}, at a spectrum resolution of 4 cm^{-1} and with 32 co-added scans. Background scan of the clean Zn-Se crystal was acquired prior to sample scanning.

Porosity

Total pores

Oil imbibition was exploited for total porosity determination (Khosravi & Azizian, 2016). Samples (2 × 2 cm square shape) were immersed into oil until constant weight (§ "Water and oil absorption kinetics"). Based on oil density (0.89 g/cm^3), the oil volume absorbed by samples was calculated and used to estimate total porosity (%). In particular, the latter was calculated as the ratio between the volume of the absorbed oil and sample specific volume (§ "Specific volume and apparent density").

Mesoporous structures

A gas analyser was employed with nitrogen gas at -196 °C to investigate sample mesoporous structures, i.e. pore diameter, pore volume and specific surface area. The latter were determined based on Brunauer-Emmett-Teller (BET) model (Braunauer et al., 1938). Samples were cut using a sharp scissors and sieved to obtain particles in the range 1-2 mm and dried at 110 °C for 2 h in an oven (UM100, Memmert, Schwabach, Germany). No significant changes in sample weight was detected upon this pre-treatment. Then, about 10 mg of sample, accurately weighted, was placed inside the analyser. Pore diameter, pore volume and specific surface area of 1 g-sample were

obtained after 24 h. The ratio % between mesopore total volume and total pore volume, determined as described in § "Total pores", was used to determined mesopore total volume ratio. The latter was defined as the contribution of mesopores to sample total pore volume.

Macroporous structures

Pore dimension was estimated based on image analysis of optical microscopic images by using Image-Pro® Plus (ver. 6.3, Media Cybernetics, Inc., Bethesda, MD, USA). Images were divided into 8 sections and the longitudinal radius (µm) of 10 pores present in each section were measured by comparison with a 50 µm scale. Total macropore volume and macropore total volume ratio were estimated by the difference between data relevant to total pores and mesopores.

Water and oil absorption kinetics

Samples of 2 × 2 cm square shape were introduced into 50 mL beakers previously filled with 15 mL of water or sunflower oil and maintained at room temperature. The complete immersion of samples in the liquid phase was assured by using a plastic filter, preventing sample from floating. At defined time intervals, samples were withdrawn, wiped with absorbing paper and weighted. Absorbed water or oil was expressed as the ratio between weight gain at time t and the initial weight of the dried sample. The immersion of sample into water or oil was prolonged until a constant weight after three consequent readings was reached.

Absorption kinetic data were then elaborated by fitting a two-phase exponential decay model (eq. 32) (Blake, Co, & Marangoni, 2014).

$$y = y_{fast}\left(1 - e^{(-k_{fast}t)}\right) + y_{slow}\left(1 - e^{(-k_{slow}t)}\right) \quad \text{(eq. 32)}$$

$$y_{max} = y_{fast} + y_{slow} \quad \text{(eq. 33)}$$

where y_{fast} and y_{slow} are the asymptote values of the fast- and slow-decaying components, respectively, k_{fast} and k_{slow} are the rate constants for the fast- and slow-decaying component, respectively, and y_{max} is the maximum amount of absorbed water or oil when time t tends to infinite and is the sum of y_{fast} and y_{slow} (eq. 33). The value y_{max} can also be considered the theoretical plateau value.

Water and oil absorption capacity

Water and oil absorbing capacities of dried leaves were calculated from water and oil absorption data, as the amount of water or oil (g) present in 1 g of sample at the absorption plateau value (y_{max}).

Data analysis

All determinations were expressed as the mean ± standard error of at least two repeated measurements from two experiment replicates (n≥4). Statistical analysis was performed by using R v. 3.0.2 (The R foundation for Statistical Computing). Student's t-test was used to determine statistically significant differences among means ($p<0.05$). Non-linear regression analysis of absorbed water and oil as a function of sample mass was performed by using TableCurve2D software (Jandel Scientific, ver. 5.01). Levenberg-Marquardt algorithm was used to perform least squares function minimization and the goodness of fit was evaluated based on statistical parameters of fitting (R^2, p, standard error) and the residual analysis.

5.6.3 Results and discussion

Characterization of lettuce waste

Lettuce waste composition was determined, as shown in Table 27, to highlight the presence of ethanol CO_2-soluble compounds, which, along with water, are expected to be lost upon supercritical-CO_2-drying

Beside water, other compounds such as fat, polyphenols and chlorophylls can be easily dissolved during the supercritical-CO_2 treatment (Fiori, de Faveri, Casazza, & Perego, 2009; Guedes et al., 2013; Lan, Wu, Zhang, Hu, & Liu, 2011; Roy et al., 1996). These compounds represented about 3.87% of lettuce waste dry weight. This suggests that the change in lettuce waste properties upon supercritical-CO_2-drying should be attributed not only to water removal but also to the loss of these compounds.

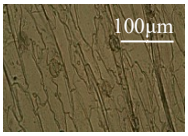
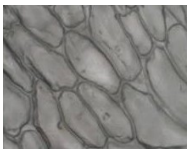
Table 27. Composition of lettuce waste.

	Amount in fresh lettuce (g/kg)
Humidity	945 ± 6
Carbohydrates	28.8 ± 0.6
Total dietary fibre	13.3 ± 0.2
Proteins	10.5 ± 0.8
Ash	2.55 ± 0.11
Fat	2.05 ± 0.07
Total polyphenols (GAE)	0.0722 ± 0.0055
Chlorophylls	0.00765 ± 0.00013

Characterization of supercritical-CO_2-dried lettuce waste

Waste external leaves from fresh-cut *Iceberg* lettuce processing were submitted to supercritical-CO_2-drying after substituting compositional water with ethanol. Table 28 shows the effect of this procedure on visual appearance, colour and microscopic structure of lettuce waste.

Table 28. Visual appearance, color and microscopic images of fresh lettuce leaves and lettuce leaves dried using supercritical CO_2.

Sample	**Visual appearance**	**Colour**			**Optical microscopy**
		L*	a*	b*	
Fresh		71.4 ± 1.3	-16.9 ± 1.2	31.6 ± 1.4	
Dried		85.0 ± 2.4	-0.2 ± 0.1	8.7 ± 0.4	

Dried lettuce waste completely lost the original green colour. Red point significantly increased while yellow point decreased. This can be attributed to tissue bleaching upon pigment extraction from lettuce leaves during immersion into ethanol. An increase in luminosity (L*) was also observed (Table 28), probably due to an increase in light scattering phenomena, generally associated to materials with a high surface roughness (Krokida, Maroulis, & Saravacos, 2001). The micrographs (Table 28) showed that fresh tissue cells were regular in shape and appeared turgid with well-defined cell wall structure. Stoma leaf cells were also evident. Supercritical-CO_2-drying did not affect cell integrity but promoted cell swelling as well as the increase of intercellular space. It is likely that cell expansion occurred during the decompression phase, which is required to remove pressurized CO_2 from the sample after drying. Swelling phenomena upon supercritical-CO_2-drying has also been reported for other vegetable matrices, such as carrot (Brown et al., 2008). By contrast, most bioaerogels have been reported to undergo severe shrinkage upon both ethanol substitution and supercritical-CO_2-drying, as a result of the different structural organization of the gel network depending on the solvent nature (Therkelsen, 1993).

To investigate the physico-chemical characteristics of the material obtained by supercritical-CO_2-drying of lettuce waste, its thermal and mechanical properties were analysed (Table 29).

Table 29. Specific heat capacity, mechanical properties (tensile strength and elongation at break), apparent density and specific volume of supercritical-CO_2-dried lettuce waste.

Specific heat capacity (Jg^{-1} K^{-1})	**Tensile strength at break (N)**	**Elongation at break (mm)**	**Apparent density (g/cm^3)**	**Specific volume (cm^3/g)**
2.617 ± 0.034	0.18 ± 0.10	0.21 ± 0.05	0.032 ± 0.004	31.3 ± 3.4

Specific heat of the sample resulted in the same range of that reported in the literature for other dried vegetable materials (Mykhailyk & Lebovka, 2014). Based on stress-strain diagrams (Figure 18), CO_2-dried lettuce waste resulted a brittle material, presenting low values of tensile strength and deformation at break. This can be regarded as a positive attribute of the material, since, differently from other food-grade bioaerogels (Manzocco et al., 2017), it is not hard and could be thus directly used in food systems.

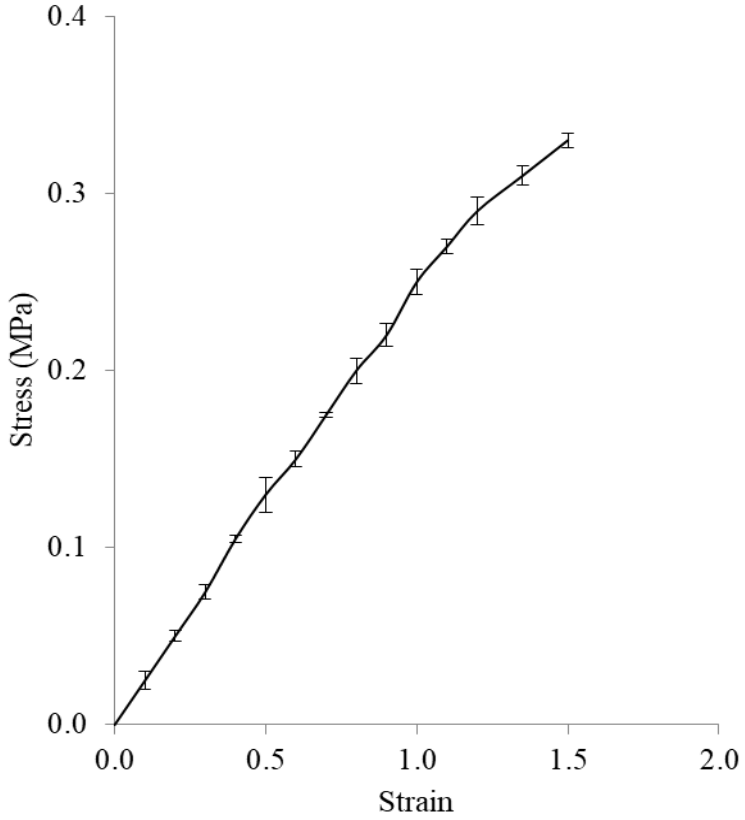


Figure 18. Stress-strain diagram of supercritical-CO_2-dried lettuce waste.

To better characterize the properties of this material, apparent density was evaluated, resulting in a very low value, corresponding to a high specific volume (Table 29). These properties suggest that a light weighted, and porous material was obtained upon supercritical drying. Total sample porosity was thus evaluated exploiting oil imbibition technique (Khosravi & Azizian, 2016). According to the latter, oil absorption into porous materials such as synthetic aerogels leads firstly to the imbibition of smaller-size pores and then to the imbibition of larger ones, until reaching a plateau. It is thus likely that the latter value can be used to estimate total pore volume. Total pore volume and porosity of the material obtained by supercritical-CO_2-drying of lettuce waste is reported in Table 30.

To get an insight into the porous structures of the obtained material, BET analysis, which is conventionally applied to analyse mesopore surface, volume and dimension was also exploited (Wiman, Dienes, Hansen, Meulen, & Zacchi, 2012) (Table 30). Figure 19 reports an example of the nitrogen isotherms obtained by BET analysis.

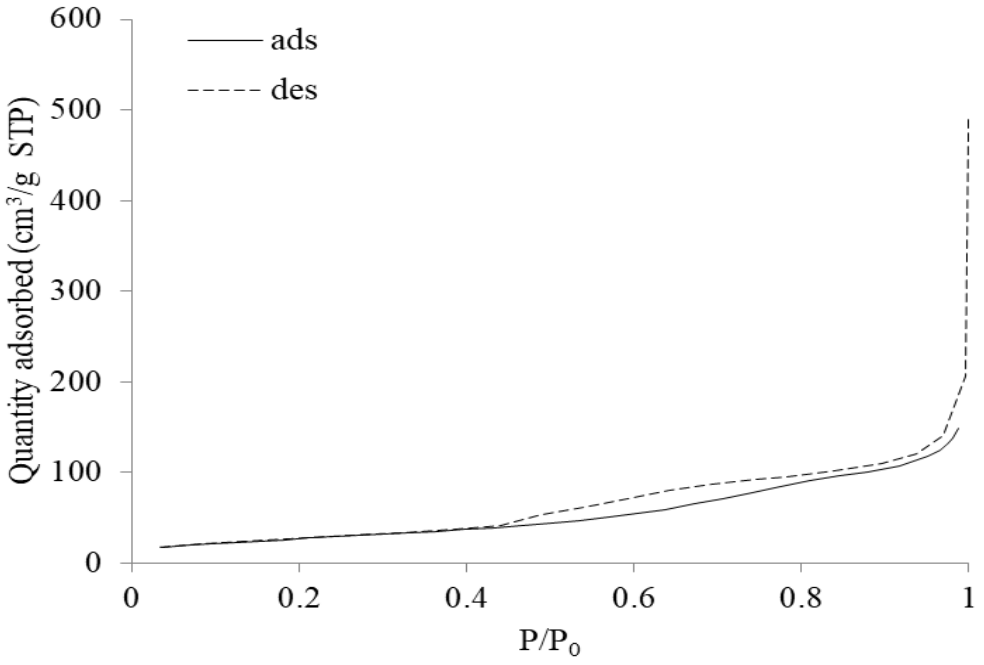


Figure 19. Nitrogen adsorption (ads) and desorption (des) isotherms of supercritical-CO_2-dried lettuce waste.

Table 30. Characteristics of mesopores, macropores, and total porosity of supercritical-CO_2-dried lettuce leaves.

Mesopores [a]				**Macropores**			**Total pores** [d]	
Surface area (m^2/g)	Total pore volume (cm^3/g)	Pore dimension (nm)	Total pore volume ratio (%)	Total pore volume [c] (cm^3/g)	Pore dimension [b] (μm)	Total pore volume ratio [c] (%)	Total pore volume (cm^3/g)	Total porosity (%)
112.8 ± 8.2	2.80 ± 0.30	46.8 ± 1.9	10.9 ± 0.2	22.8 ± 0.4	111.1 ± 35.3	89.1 ± 1.6	25.6 ± 0.4	81.7 ± 1.4

[a] estimated by BET technique, [b] estimated by image analysis of photomicrographs, [c] estimated by difference between data of total pores and mesopores, [d] estimated by oil imbibition

The latter revealed the presence of mesoporous structures presenting an average dimension of 47 nm. As expected, the value of internal surface area resulted much higher than that reported by Amin, Abkenar, and Zendehboudi (2015), for an air-dried water fern intended for oil spill absorption (4.7 m^2/g). This difference is attributable to the capacity of supercritical-CO_2-drying to hinder structural collapse, due to the absence of surface tension during the drying treatment (Michalska et al., 2017). Moreover, it can also be due to the cellular structure swelling clearly observed in microscopic images (Table 28). Interestingly, sample internal surface area resulted in the same range of that reported by Ghafar et al. (2017) and Ubeyitogullari and Ciftci (2017) for aerogels obtained via supercritical-CO_2-drying of hydrogels containing different biopolymers such as guar galactomannan (99-333 m^2/g) and nanoporous starch (60-63 m^2/g). It is noteworthy that mesopores detected by BET analysis presented an average total volume pore of about 2.8 cm^3/g, contributing to the total sample pore volume by only 11%. Based on these observations, it is likely that most pores in the sample were not accounted for by these mesopores but rather by larger macropores, corresponding to cellular voids left upon removal of water and other compounds that are soluble in ethanol or CO_2. These cellular voids were not detectable by BET technique but clearly observed in photomicrographs (Table 28). The latter actually showed these cell voids to present dimensions much higher than those estimated for mesopores by BET technique (Table 30). Based on this hypothesis, 89% of the sample pore volume, corresponding to a value of about 22.8 cm^3/g, was developed by macropores (Table 30).

It is noteworthy that the obtained values of internal surface area and total porosity, associated to density lower than 0.5 g/cm^3 (Table 29) are typical of aerogels (Fricke & Tillotson, 1997). Based on these data, the sample obtained by supercritical-CO_2-drying of lettuce waste leaves can be regarded as a bioaerogel-like material.

Interaction of supercritical-CO_2-dried lettuce waste with solvents

The large specific surface area of bioaerogels can be exploited for sorption of bioactive compounds and has been proposed for controlled release in pharmaceutical, food applications and innovative active food packaging. The sorption isotherm of the bioaerogel-like material obtained from lettuce waste was assessed to obtain preliminary information about the possibility of the material to interact with hydrophilic volatiles (Figure 20).

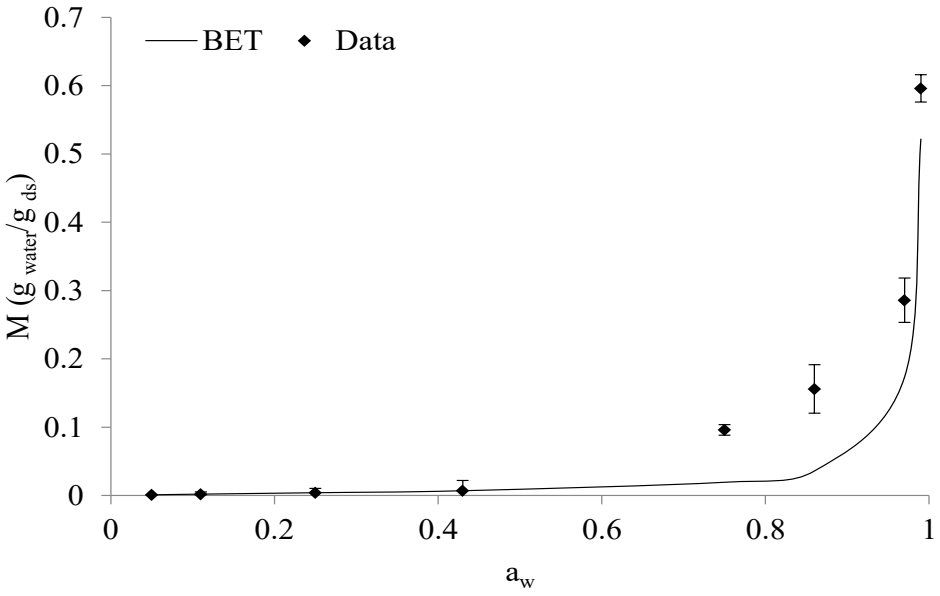


Figure 20. Isotherm of supercritical-CO_2-dried lettuce waste. Fitting curve of BET model (BET) to experimental data (Data) is also reported. ds =dry sample.

It must be noted that BET model is known to be valid for low a_w values, as well appreciable in Figure 20 (van den Berg, 1985). Regression analysis in the a_w range 0.05-0.43 provided good model fitting to data (R^2=0.999; $p<0.05$). The isotherm shape revealed the presence of a type III isotherm. This is typical of materials able to only weakly interact with water, leading to a strong increase in a_w values upon a reduced moisture increase (Figure 20). In particular, the material obtained by supercritical-CO_2-drying of lettuce waste is expected to mainly interact with water through capillarity phenomena in its porous structure as well as through surface interactions with the hydrophilic residues present on cellulosic fibres, which are the main constituents of vegetable cell walls (Al-Muhtaseb, McMinn, & Magee, 2002; Brunauer, Deming, Deming, & Teller, 1940). Given the limited capacity to interact with water vapor, the material obtained by submitting lettuce waste to supercritical-CO_2-drying would be physically stable under a wide range of humidity values (Figure 20). This property suggests a possible exploitation of this material for different applications, including biodegradable and edible packaging.

To better characterize material interactions with water vapor, its hygroscopicity was evaluated by moisture sorption kinetics (Figure 21).

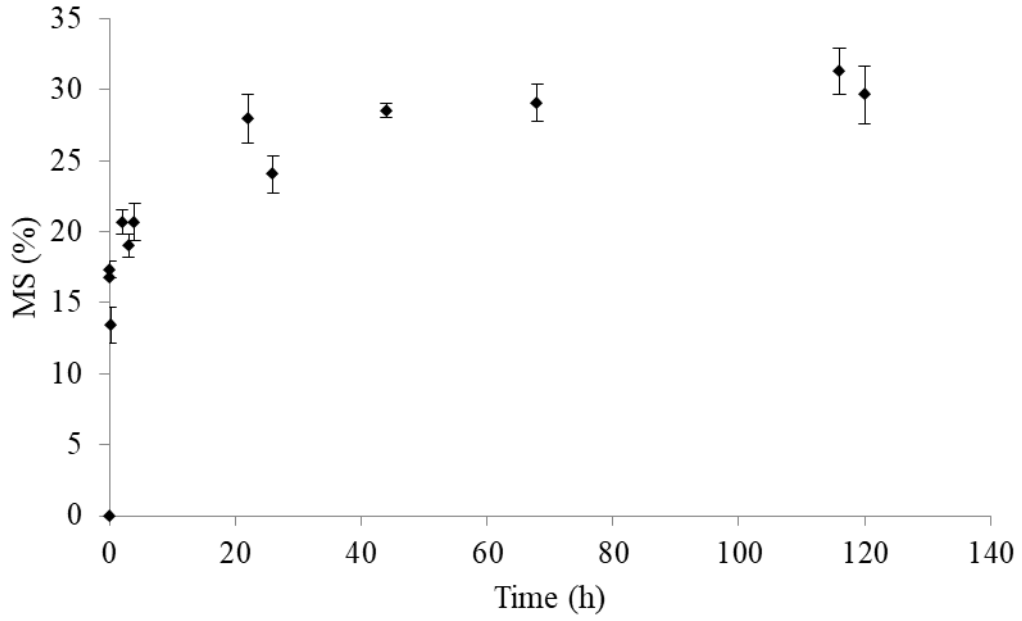


Figure 21. Moisture sorption (MS) versus time diagram of the supercritical-CO_2-dried lettuce waste.

Moisture sorption evolved rapidly and reached a final value of about 30% within the first 20 h. This can be related to the inherent hygroscopicity of the material, rich in hydrophilic residues (Ahmadi et al., 2016), as confirmed by the FTIR spectra of the material (Figure 22).

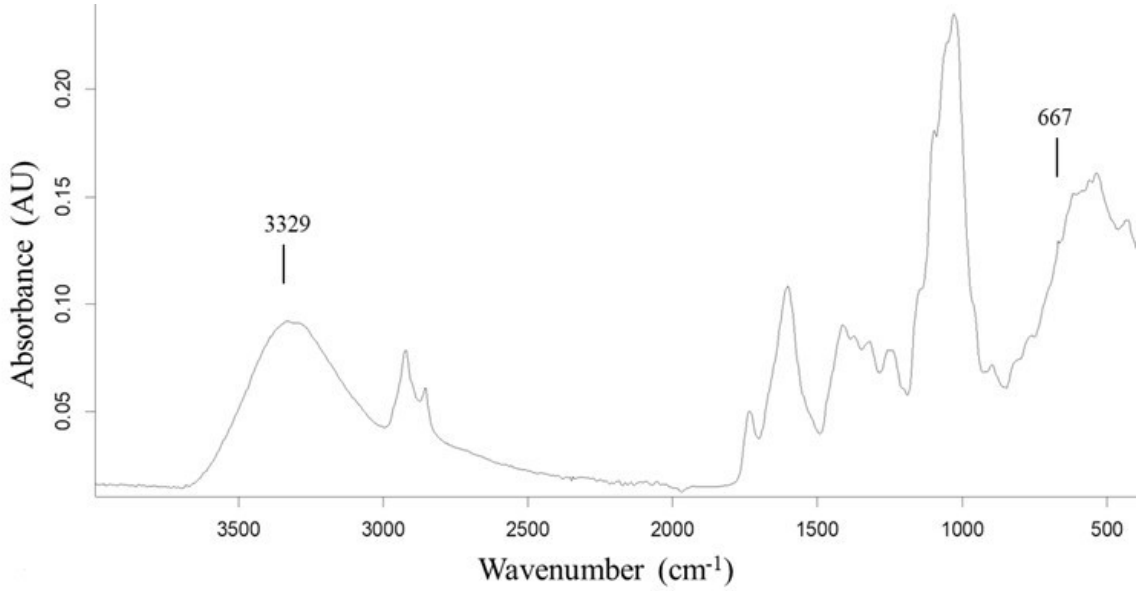


Figure 22. FTIR spectra of supercritical-CO_2-dried lettuce waste. AU = absorbance unit

In particular, the broad band in the IR region from 3700-3000 cm^{-1} and the peak at 667 cm^{-1} have been associated to OH-stretching vibrations arising from hydrogen bonding and to OH out of plane bending, respectively (Abidi, Cabrales, & Haigler, 2014).

As reported for bioareogels, the high porosity of supercritical-CO_2-dried lettuce waste could be exploited to entrap huge amounts of different solvents (Ahmadi et al., 2016; Manzocco et al., 2017). The capacity of supercritical-CO_2-dried sample to load water and oil was thus evaluated by immersing dried leaves in the two solvents (Figure 23). Bioareogel lettuce waste did not lose integrity upon immersion into both water and oil, taking the appearance shown in Figure 24.

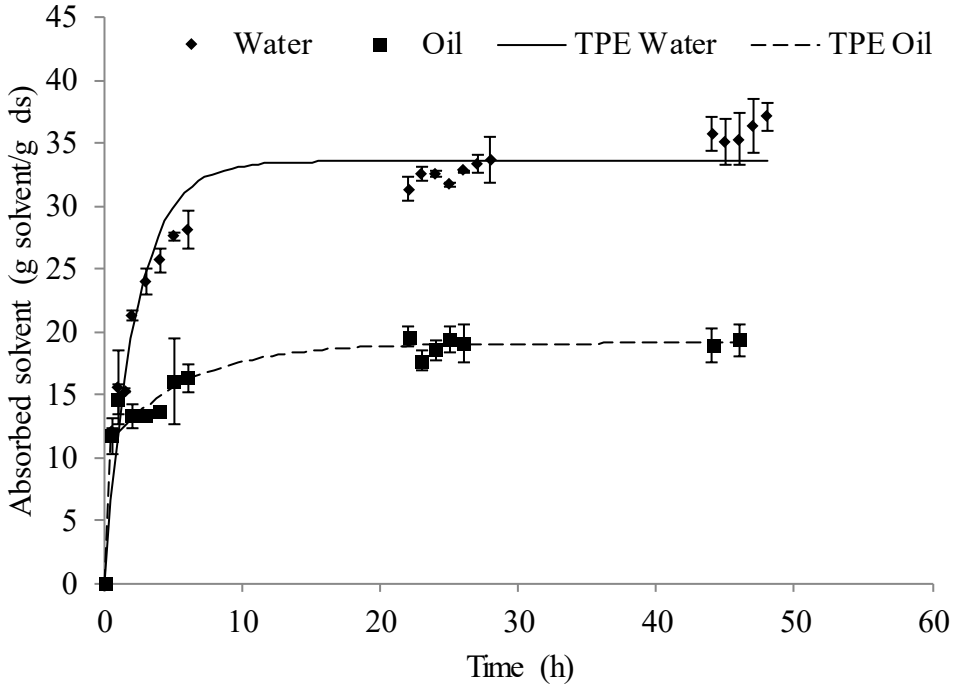


Figure 23. Absorption of water and oil by supercritical-CO_2-dried lettuce waste. Fitting curves of the two-phase exponential model (TPE) are also reported. ds =dry sample

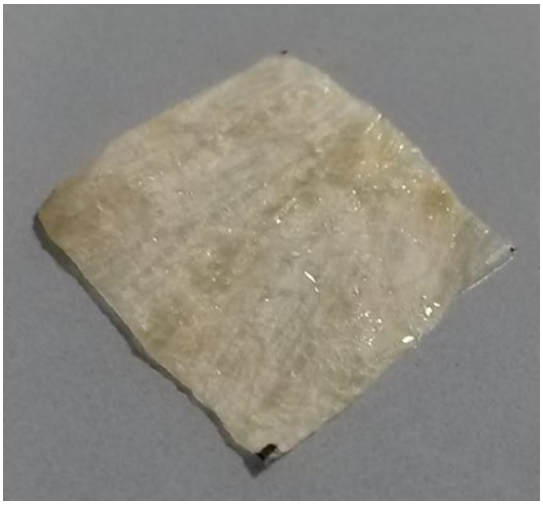


Figure 24. Visual appearance of supercritical-CO_2-dried lettuce waste after immersion in water.

It is noteworthy that different results were observed for other bioaerogels reported in the literature. For instance, k-carrageenan bioaerogels, although maintaining their structure upon oil immersion, quickly dissolved in water (Manzocco et al., 2017).

Water and oil were progressively absorbed by dried lettuce waste during immersion in the solvent, reaching a plateau value after about 40 h immersion (Figure 23). Liquid absorption by a porous material is known to be affected by different factors, such as the capacity of the material to bind the solvent (Blake et al., 2014). To further investigate these aspects, water and oil absorption kinetic of dried lettuce waste were fitted using a two-phase exponential decay model (TPE) (eq. 32) (Blake et al., 2014). The latter can be used to describe solvent absorption kinetics as a result of fast and slow components, which are related to the capacity of the material to bind the solvent. Data were well adapted to the model, leading to parameter estimates reported in Table 31.

Table 31. Experimental regression coefficients estimate, R^2 and adjusted R^2 for the two-phase exponential model (TPE) of water and oil absorption in supercritical-CO_2-dried lettuce waste. Water and oil absorption capacity is also reported.

	Water	**Oil**
k_{fast} (h^{-1})	0.431 ± 0.013	248.2 ± 13.6
k_{slow} (h^{-1})	0.465 ± 0.016	0.175 ± 0.007
y_{fast} ($g_{solvent}/g_{ds}$)	16.8 ± 0.8	10.6 ± 0.2
y_{slow} ($g_{solvent}/g_{ds}$)	16.8 ± 0.9	8.5 ± 0.4
y_{max} ($g_{solvent}/g_{ds}$)	33.5 ± 1.4	19.1 ± 1.3
y_{max} ($mL_{solvent}/g_{ds}$)	37.6 ± 1.6	21.4 ± 1.5
R^2	0.944	0.967
R^2 adj	0.929	0.954
Absorption capacity (g/g)	0.97 ± 0.02	0.95 ± 0.02

ds, dry sample

The TPE model used in the analysis posits that solvent absorption in the material obtained by supercritical-CO_2-drying takes place according to two different phases: the first one in which solvent is loaded relatively quickly (fast phase) and the second one in which solvent is loaded relatively quickly (slow phase), both of which can be differentiated based on the respective rate constants. The fast component refers to unbound or weakly bound (physically-entrapped) solvent while the slow component is relevant to bound solvent. The rate constant for the fast-decaying and slow-decaying components (k_{fast} and k_{slow}) resulted similar ($p \geq 0.05$) for water. This indicates that water absorption was equally distributed between fast and slow components, suggesting water to be both physically entrapped and chemically bound. By contrast, in the case of oil, k_{fast} resulted much higher than k_{slow}, confirming its uptake in the material to be mainly driven by physical interactions. Absorption data were further elaborated to evidence the amount of water and oil absorbed in the fast and slow phases. Data indicate that both y_{fast} and y_{slow} resulted higher for water than oil, indicating that the material presented a higher affinity toward water than oil. Moreover, the maximum amount of absorbed water and oil (y_{max}) was estimated. Given the different density of water and oil, y_{max} was expressed as both weight and volume absorbed by 1 g of dried sample. Results indicate that the water amount absorbed by dried lettuce waste was significantly higher than the oil one ($p<0.05$).

It can be noted that supercritical-CO_2-dried lettuce absorbed an amount of water much higher than that originally entrapped in the fresh lettuce tissue. The latter presented a water content of 941 g/kg, corresponding to about 16 g of water for 1 g of dry sample. The enhanced ability of the material to absorb water could be attributed to its peculiar structure (Table 28). However, it could be inferred

that removal of compounds soluble in ethanol or CO_2 could lead to the exposure of hydrophilic functional groups on the surface of the fibrous network. These features would favour capillary forces and swelling phenomena, leading to both water physical entrapment and hydration of functional groups of polysaccharides. On the contrary, oil absorption would be mainly driven by capillary forces, being hindered by the hydrophilic nature of pore surfaces (Ahmadi et al., 2016). It is noteworthy that the water and oil absorption capacity showed by dried lettuce waste (Table 31) are much higher than those of other bioaerogels reported in the literature. In particular, Ahmadi et al. (2016) and Manzocco et al. (2017) obtained whey protein and k-carrageenan bioaerogels characterized by a maximum oil loading capacity of circa 0.025 and 0.800 g/g respectively. These findings suggest that dried lettuce waste can be resembled to a cloth which can uptake large amounts of water or oil. It could be exploited as template for structuring and delivering both hydrophilic and lipophilic compounds, and thus for the preparation of innovative materials to be used as oil spills absorbers or templates for hydrogels and oleogels.

5.6.4 Conclusions

An expanded, brittle and highly porous material was obtained by supercritical-CO_2-drying of lettuce waste. This material was demonstrated to absorb considerable amounts of water and oil. As other organic aerogels, this dried material is made from renewable sources and is completely biodegradable. Based on these considerations, bioaerogel-like materials derived from supercritical-drying of lettuce waste could fit typical bioaerogel applications, such as thermal insulation, biodegradable packaging development and carriers for lipophilic compounds. In addition, the low firmness of dried lettuce waste and its capacity to withstand water contact without losing integrity suggest further possible applications, including the use as food ingredient, moisture absorber and carrier for hydrophilic molecules.

5.7 Conclusions

The results presented in Chapter 5 highlighted the possibility to valorise the selected waste materials (lettuce waste, peach pomace and okara) by turning them into the different outputs (Chapter 4). In particular, fresh ingredients intended for homogenate and juice production (§ 5.1 and 5.2), antioxidant extracts (§ 5.3 and 5.4) and functional dried derivatives (§ 5.5 and 5.6) were obtained by the application of specific processes. Relevant operative conditions and production capacities are summarised in Table 32. Operative conditions were selected among those tested in Chapter 5 based on time, energy and cost considerations. In particular, only high pressure homogenisation processes based on one single pass of the lettuce waste and okara dispersion through the homogenising valve were considered, since multiple passes would be hardly applicable on an industrial scale (§ 5.1 and 5.2). In the case of peach pomace, only fresh waste was considered, in order to reduce process time, cost and bioactive loss related to preliminary air-drying (§ 5.4). Production capacity was expressed as the process time required for processing a certain amount of waste material, based on laboratory trials.

These encouraging laboratory results suggest that the proposed valorisation strategies could be of interest for exploitation on industrial level. It is noteworthy that the development of food waste valorisation strategies is nowadays absorbing much research effort without demonstrating any possibility of being transferred to real industrial situations. This is because studies are performed just on a laboratory scale, with no awareness of market and scale-up variables that make any valorisation strategy sustainable and economically valuable.

For these reasons, data collected in this Ph.D. thesis, and reported in Table 32 with reference to laboratory scale, only provide a general indication about the potentialities of the valorisation processes. To avoid these data remaining in the laboratory, without ever generating a return in terms of environmental benefit and societal improvement, they should be accurately analysed for feasibility on a real industrial scale (Chapter 6). This is required to fill an evident literature gap and offer the stakeholders involved in food waste management a starting point for practical application.

Table 32. Operative conditions and capacity of processes required for obtaining specific outputs from fresh-cut lettuce waste, peach pomace and okara.

Waste	Output	Process	Operative conditions	Capacity
Fresh-cut lettuce waste	Fresh homogenate	Blanching + High pressure homogenization	90 °C, lettuce waste:water ratio 1:10 (w/v) 40-150 MPa,1 pass	30 s for 100 g of lettuce waste 6 min for 1 kg of lettuce waste
	Bioactive extract	Ultrasound assisted extraction	24 kHz, 400 W, 100 µm, 50 °C, 75% ethanol solution, lettuce waste:solution ratio 1:2.5 (w/v) *	120 s per 25 g of lettuce waste
	Flour	Air-drying	70 °C	24 h for 1 kg of lettuce waste
	Biodegradable material	Ethanol substitution + Supercritical-CO_2-drying	100% ethanol, lettuce waste:ethanol ratio 1:10 (w/v), twice 11 MPa, CO_2 flow 6 L/min, 45 °C	48 h for 5 g of lettuce waste 2.5 h for 5 g of lettuce waste
Peach pomace	Bioactive extract	Microwave assisted extraction	540 W, no temperature control, 70% ethanol solution, peach pomace:solution ratio 1:2 (w/w)	50 s for 60 g of peach pomace
		Ultrasound assisted extraction	24 kHz, 400 W, 14 µm, no temperature control, 70% ethanol solution, peach pomace:solution ratio 1:2 (w/w)	120 s for 60 g of peach pomace
Okara	Fresh homogenate	High pressure homogenization	50-150 MPa, 1 pass okara:water ratio 1:9 (w/w)	6 min for 100 g of okara

* To reduce sample variability, in § 5.3 freeze-dried lettuce waste was used instead of the fresh one. Data here reported is calculated on fresh lettuce basis since preliminary freeze-drying would not be necessary on industrial scale

Chapter 6

Step 4 of the valorisation approach: ***Feasibility study***

The challenge of this chapter was to assess the feasibility of the developed valorisation strategies (Chapter 5), taking into considerations the criteria that should be met by a waste substance to be assigned with the end-of-waste status (specific purpose, technical feasibility, market demand, impact on sustainability) (Table 2). To this aim, the lettuce waste study-case was considered. Lettuce waste provided four different valorisation outputs, possibly exploitable in food and non-food applications: a fresh homogenate intended for blended juice formulation, antioxidant extracts possibly exploitable as food supplements, vegetable flour intended for functional bakery products, and an expanded material possibly applicable as biodegradable packaging. In this chapter, the possibility of selected lettuce waste valorisation outputs to be exploited in specific food applications was validated. Moreover, the possibility for these waste derivatives to have a market demand was estimated by consumer-based methodologies. Finally, a method able to estimate the economic and environmental impact of the proposed valorisation strategies on an industrial scale was set up. In particular, in § 6.1 the case study of flour obtained upon air-drying and grinding of lettuce waste was analysed to assess the technical feasibility of its use for functional bread production. In addition, since a market demand is needed to guarantee a return on investments of FVW valorisation, the potential of lettuce waste bread to be accepted by the consumers was evaluated. In § 6.2 a method for estimating the environmental advantage and the economic and energetic impact of lettuce valorisation outputs was presented.

6.1 Feasibility of lettuce waste flour in functional bread production: technical validation and consumer response

The aim of this study was to evaluate the technical applicability of flour obtained from air-drying of *Iceberg* lettuce waste (§ 5.5) in functional bread production. Bread samples containing increasing amounts of lettuce waste flour were characterised for colour, moisture, phenol and fibre content, antioxidant activity, firmness, specific volume and sensory properties. Bread acceptability was also evaluated and compared to that of commercial bread containing rye bran. Consumer response towards claims associable to bread containing lettuce waste flour was also investigated by conjoint analysis. This part of the Ph.D. project was conducted taking advantage of the scientific collaboration with prof. Sandro Sillani of the Department of Agricultural, Food, Environmental and Animal Sciences of the University of Udine.

6.1.1 Introduction

The market of functional foods has been constantly growing, following consumer awareness of their potential in maintaining a healthy state (Gul, Singh, & Jabeen, 2016; Sikand, Kris-Etherton, & Boulos, 2015). Being a staple food in several countries, bread is an optimal candidate for functionalization (Akhtar, Anjum, & Anjum, 2011). To this aim, wholemeal flour is traditionally used due to its content in antioxidants and fibres from bran and aleurone (Dewettinck et al., 2008; Dziki, Rozylo, Gawlik-Dziki, & Swieca, 2014). This goal could be equally reached using flour from fruit and vegetables or from their wastes, which are rich in nutritional compounds (Mastromatteo, Danza, Guida, & Del Nobile, 2012; Nilnakara et al., 2009). To this regard, functional bread has been produced using flour from tomato, cabbage and pineapple waste (Chareonthaikij, Uan-On, & Prinyawiwatkul, 2016; Nilnakara et al., 2009; Nour, Ionica, & Trandafir, 2015; Wu & Shiau, 2015). As described in § 5.5, fresh-cut lettuce waste can be air-dried and ground to obtain flour with 3.05 mg GAE/g dw polyphenols, similar to that of cabbage and pumpkin functional flour (Nilnakara et al., 2009; Que, Mao, Fang, & Wu, 2008), and fibre content (260 g/kg) comparable to that of rice and oat bran (USDA, 2018). Lettuce waste flour could thus represent a suitable ingredient for functional bread. However, its use is expected to strongly affect product processability as well as physical, sensory and nutritional properties.

Functional bread containing lettuce waste flour could represent a value-added food derived from a cheap and always available source, associated to an eco-friendly image appreciated by consumers (Simoes et al., 2015). Reversely, the presence of a waste derivative in bread could negatively affect consumers' reaction, posing the need for an accurate analysis of these aspects (Pickett-Baker & Ozaki, 2008; Simoes et al., 2015). In this regard, commercial and marketing knowledge regarding consumer response to food waste derivatives is nowadays still limited. Different attitudes can affect consumer acceptance of such innovative products. Innovations in the food industry suffer a high

market failure rate, partly due to a phenomenon known as “neophobia”, which is the rejection that some people express towards new or unfamiliar foods (Barrena & Sánchez, 2013). By contrast, consumers are increasingly concerned about food supply chain sustainability and recent surveys have demonstrated a positive reaction to product labels reporting sustainability claims on them (Simoes et al., 2015).

Based on these considerations, both technical feasibility and consumer response to bread containing lettuce waste flour are evaluated in this study.

6.1.2 Materials and methods

Lettuce waste flour

Air-dried lettuce waste flour was prepared as described in § 5.5.2 and stored at 20 °C in sealed aluminized aseptic bags until use. Flour presented a moisture amount of 42 ± 2 g/kg.

Bread

Bread was obtained substituting Manitoba type “0” wheat flour (Molino Spadoni, Coccolia, Italy; 141 g/kg moisture, 12 g/kg fat, 675 g/kg carbohydrates of which 15 g/kg sugars, 22 g/kg fibres and 135 g/kg proteins) with increasing amounts of lettuce waste flour (0, 10, 25, 70, 225 g/kg of dough, corresponding to a replacement of wheat flour of 2, 4, 12 and 40% w/w), while maintaining a constant ratio among the other ingredients (Table 33). Water (at 30 ± 0.5 °C), sugar and fresh yeast were premixed 3 min (KM285, Kenwood, Milan, Italy), added with flour and salt and mixed 15 min. Subsequently, dough portions (250 g) were manually rounded, leavened on a tray at 37 °C and 80 ERH% (ST500, Pol-Eko-Aparatura S.P.J., Wodzslaw, Poland) for 60 min, baked (170 °C, 20 min) (10GN1/1, Air-O-Steam Touchline, Electrolux, Porcia, Italy) and cooled at room temperature for 1 h (Mastromatteo et al., 2012).

Commercial wholegrain bread samples (Gilli srl, Laives, Italy) containing 180 and 510 g/kg rye bran (fibre content of 55 and 93 g/kg respectively, as declared on the product label) were also purchased on the local market.

Table 33. Formulation of dough samples containing increasing amounts of lettuce waste flour.

Dough ingredient (g/kg)					
Lettuce waste flour	**Wheat flour**	**Water**	**Sugar**	**Salt**	**Yeast**
0	561	404	9	13	13
10	551	404	9	13	13
25	536	404	9	13	13
70	491	404	9	13	13
225	336	404	9	13	13

Analytical determinations

Colour

Colour determination was carried out as described in § 5.1.2. Samples were positioned on a white cardboard and the colorimeter head was placed perpendicular to sample surface. At least five measures were taken on different points of bread dough samples and on the crust of bread ones.

Image acquisition

Images were acquired as described in § 5.1.2.

Specific volume

Loaf specific volume (cm^3/g) was obtained by rapeseed displacement according to AOAC methods (AOAC, 1997).

Moisture

Moisture content was calculated according to AOAC methods (AOAC, 1997). Around 2 g of sample was dried in a vacuum oven (1.32 kPa) at 75 °C until constant weight (12 h).

Firmness

Firmness was measured by uniaxial compression test using an Instron 4301 (Instron LTD., High Wycombe, UK). Samples were tested by a 12.7 mm diameter cylindrical probe (100 N compression head) at a 5 mm/min crosshead speed. Firmness was taken as the maximum force (N) for 5 mm sample penetration. Dough firmness was evaluated by penetrating the surface of the leavened dough. In the case of bread, six slices (20 mm thick) were cut from the central portion of each loaf. Firmness was evaluated by penetrating the crumb of each slice in 5 different points (Calligaris, Manzocco, Valoppi, & Nicoli, 2013).

Total dietary fibre

Total dietary fibre was determined according to AOAC methods (AOAC, 1997), using TDF-100A kit (Sigma-Aldrich, St. Louis, Missouri, USA). Results were expressed as g of fibres per kg of bread.

Total polyphenolic content

Phenolic extracts were prepared according to Llorach et al. (2004) with some modifications. Bread was extracted by reflux for 60 min in boiling water (dilution 0.2 g/mL). Extracts were then added in methanol (1 mL/g of bread) and centrifuged (9450 g, 15 min, 20 °C) (Mikro 20, Hettich Zentrifugen, Tuttlingen, Germany). The supernatant was used for polyphenols and antioxidant activity analyses. Total polyphenolic content (TPC) was determined as described in § 5.5.2. The reaction mixture contained 100 µL polyphenolic extract, 500 µL Folin-Ciocalteau reagent, 4 mL

water and 2 mL sodium carbonate-water solution (0.15 g/mL). Results were expressed as mg of gallic acid equivalents (GAE) per kg of bread.

Antioxidant activity (DPPH· assay)

Antioxidant activity was determined as described in § 5.5.2. In particular, a volume of 1.80 mL of 6.1×10^{-5} M DPPH· methanol solution was added with 150 µL polyphenolic extract. DPPH· bleaching was followed at 515 nm (UV-2501PC, Shimadzu Corporation, Kyoto, Japan) at 20 °C for 10 min and resulted proportional to extract concentration. The eq. 28 reported in § 5.5.2 was chosen to obtain the reaction rate of DPPH bleaching.

Sensory attributes

A focus group of 10 judges was used to identify sensory attributes of bread containing lettuce flour. Judges were not trained on sensory analysis of bread but were experts in the use of the selected sensory methods. White bread and bread containing 575 g/kg of lettuce flour were evaluated. The focus group decided, through consensus and independently on consumer response, which descriptors better discriminated the samples. Judges were then asked to evaluate the intensity of the selected descriptors in bread containing 0, 170 and 575 g/kg of lettuce waste flour. Descriptors of bread samples, identified with a three-digit random code, were evaluated on a 1-9 point hedonic scale, in which 1 corresponded to "extremely low descriptor intensity", and 9 to "extremely high descriptor intensity". Three bread samples were evaluated in each session and water was used to rinse mouth among samples (Manzocco & Lagazio, 2009).

Consumer acceptability

About 80 bread consumers (37 men and 43 women, age 18-55) were recruited at the University of Udine, Italy. Samples, indicated by a three-digit random code, were served in odourless plastic dishes. Consumers were asked to taste samples and score their acceptability on a 1-9 hedonic scale anchored with ''highly non-acceptable" (score 1) and ''highly acceptable" (score 9) (Peryam & Pilgrim, 1957). Four bread samples containing lettuce flour (170 and 575 g/kg) and rye bran (180 and 510 g/kg) were evaluated in each session. Water was used to rinse mouth among samples.

Consumer response

Conjoint analysis was used to evaluate consumer preference towards bread by decomposing total preference in partial preferences relevant to independent product attributes (De Pelsmaeker, Schouteten, Lagast, Dewettinck, & Gellynck, 2017; Sillani, Miccoli, & Nassivera, 2017). Five attributes of bread containing lettuce flour were selected as experimental variables and named "lettuce flour", "health", "waste recovery", "waste reduction" (discrete variables) and "price" (linear variable). Different levels were associated to each experimental variable (Table 34). For discrete variables, two levels (claim presence or absence) were used. For price, three values were

used. Experimental variables were combined according to an orthogonal experimental design, obtaining 11 product profiles, which represent information available to consumer on a possible bread label. A non-probabilistic sample of 525 bread consumers, equally distributed among men and women (age 18-41), was recruited at the University of Udine, Italy. Consumers were asked to fill up a structured questionnaire, indicating, for each product profile, their preference on a 1-100 scale. No prior information was provided about origin and preparation of lettuce waste flour. In other words, the response of consumers towards a bread label reporting different information was assessed. A total of 370 responses were valid and analysed.

Table 34. Experimental variables defining bread attributes and relevant levels used for conjoint analysis.

Experimental variable	**Levels**
Lettuce flour	Absent; "Containing lettuce flour"
Health	Absent; "Rich in fibre"
Waste recovery	Absent; "Produced recovering lettuce waste"
Waste reduction	Absent; "Produced reducing food waste"
Price (€/kg)	3.00; 4.50; 6.00

Data analysis

Determinations were expressed as the mean ± standard deviation of at least three measurements from three experiment replications. Statistical analysis was performed by using R v.2.15.0 (The R foundation for Statistical Computing). Bartlett's test was used to check the homogeneity of variance. One-way ANOVA was carried out and Tukey-test was used as post-hoc test to determine statistically significant differences among means ($p<0.05$). For conjoint analysis, IBM SPSS Statistics 20 (Armonk, New York) was used to calculate partial preference values, their relative importance and model goodness-of-fit (Pearson's R and Kendall's τ).

6.1.3 Results and discussion

Wheat flour in bread dough was substituted with increasing amounts of lettuce waste flour. Leavened dough was characterised for appearance, colour and firmness (Table 35).

Table 35. Appearance, CIELAB scale colour parameters (L*, a*, b*) and firmness of leavened dough containing increasing amounts of lettuce waste flour.

Lettuce waste flour (g/kg)	Appearance after leavening	Colour			Firmness (N)
		L*	a*	b*	
0		81.8 ± 0.5 [a]	-0.1 ± 0.3 [a]	18.6 ± 0.9 [c]	0.110 ± 0.015 [b]
10		72.6 ± 1.0 [b]	2.4 ± 0.3 [b]	21.7 ± 0.9 [b]	0.139 ± 0.005 [b]
25		60.4 ± 0.7 [c]	7.5 ± 0.4 [c]	28.1 ± 1.6 [a]	0.217 ± 0.023 [b]
70		48.7 ± 0.6 [d]	10.2 ± 0.3 [d]	16.2 ± 0.5 [d]	0.139 ± 0.015 [b]
225		33.3 ± 1.1 [e]	11.0 ± 0.4 [e]	7.3 ± 2.0 [e]	1.803 ± 0.145 [a]

[a-e]: Mean values indicated by different letters are statistically different ($p<0.05$)

The addition of lettuce waste flour decreased luminosity (L*) and yellowness (b*), while increased red-point (a*). This can be attributed to the brownish colour of air-dried lettuce flour. Air-drying has actually been shown to promote oxidation of the main phenolic compounds of *Iceberg* lettuce waste, including 3-O-caffeoylquinic acid, caffeoyltartaric acid, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, caffeic acid derivatives, isochlorogenic acid, chicoric acid, luteolin 7-O-glucuronide and quercetin 3-O-glucuronide (§ 5.5). Images show that lettuce flour hindered dough leavening, leading to progressively firmer and less aerated dough. Lettuce flour reduced gluten concentration in the dough, while increased the presence of water holding fibres. To this regard, as reported in § 5.5, 1 g of lettuce flour can hold up to 9 g of water. Water would thus be less available for gluten-starch network formation, reducing dough elasticity, gas entrapment and leavening capacity. The addition of vegetable fibres was demonstrated to affect dough moisture distribution,

altering rheological properties and leavening (Ameh, Gernah, & Igbabul, 2013; Chareonthaikij et al., 2016).

Increasing amounts of brownish lettuce flour resulted in brown bread samples (Table 36). The changes in luminosity and colorimetric parameters presented a discontinuity point in correspondence of 170 g/kg lettuce flour concentration. This is probably attributable to the counterbalancing colour effects of the increase in brownish flour and the inhibition of Maillard reaction by water holding fibres, which reduce dehydration rate and extent (Kent-Jones & Amos, 1947). As reported for other vegetable flour (Greene & Bovell-Benjamin, 2004; Marpalle, Sonawane, & Arya, 2014), the water holding capacity of lettuce fibres was reflected in progressively increasing bread loaf moisture (Table 36). This might pose stability issues for lettuce flour bread, due to its altered response to both microbial spoilage and staling (Ameh et al., 2013; Rosell & Santos, 2010). Bread containing increasing amounts of lettuce flour resulted progressively firmer and with lower specific volume (Table 36), due to the water absorption capacity of lettuce fibres and reduced dough leavening (Table 36). The latter should not be regarded as a negative feature, since promoting stomach filling and sense of satiety (Ameh et al., 2013; Greene & Bovell-Benjamin, 2004). The addition of lettuce flour, rich in antioxidant polyphenols (§ 5.5), promoted the increase of bread phenolic content and antioxidant activity (Table 36). Phenolic compounds are mostly located in cereal cell wall, linked to hemicelluloses or other wall constituents, with the highest concentration in the aleurone grain layer. Subsequently, white bread is poor in these compounds. *Iceberg* lettuce flour could be exploited to increase its phenolic content, being rich in these compounds (Llorach et al., 2004; Naczk & Shahidi, 2006). An increase in phenolic content and antioxidant activity of bakery products was also obtained by adding mango peel, dried tomato waste, broccoli, carrot and beetroot (Ajila, Leelavathi, & Prasada Rao, 2008; Nour et al., 2015; Ranawana et al., 2016). By contrast, no change in total phenolic was detected when apple and lemon fibre was added to cookies (Bilgiçli, Ibanoğlu, & Herken, 2007). Interactions between phenols and wheat proteins/polysaccharides as well as oxidation, isomerization/epimerization and degradation of bioactive compounds during dough preparation and baking may account for these contrasting results (Wang & Zhou, 2004). As a result, bread antioxidant properties would depend on phenols naturally occurring in wheat and lettuce flour as well as on thermally-induced products and phenol complexes with proteins/polysaccharides (Rupasinghe, Wang, Huber, & Pitts, 2008; Sivam, Sun-Waterhouse, Quek, & Perera, 2010).

As reported for other baked goods containing vegetable derivatives (Ajila et al., 2008; Bilgiçli et al., 2007; Rupasinghe et al., 2008), the addition of lettuce flour also promoted the increase of bread fibre concentration (Table 36). Lettuce mainly contains insoluble dietary fibre, known for its beneficial health effects on intestinal regularity and weight control (Lattimer & Haub, 2010). The fibre content of bread with 170 and 575 g/kg lettuce flour could be associated to the nutritional claims “rich in fibre” and “source of fibres”, respectively (2006/1924/EC).

Since these high levels of fibres and polyphenols may significantly affect bread sensory attributes, these samples were submitted to sensory evaluation, using white bread as control (Mastromatteo et al., 2012). Data reported in Table 37 show that lettuce flour decreased the perceived intensity of yeast odour and flavour while increased silage and herbaceous odour and flavour, dried fruit flavour, acid and sour taste. In accordance with increasing bread firmness data (Table 37), lettuce flour promoted an increase in gumminess and a decrease in bread softness. Sensory attributes of bread containing lettuce flour are those typically associated to wholemeal bread (Ameh et al., 2013; Greene & Bovell-Benjamin, 2004).

Table 36. Visual appearance, crust CIELAB scale colour parameters (L*, a*, b*), crumb firmness, specific volume, humidity, total phenolic content (TPC), chain-breaking activity (CBA) and total dietary fibre (TDF) of bread samples containing increasing amount of lettuce waste flour.

Lettuce waste flour (g/kg)		**Image**	**Colour**			**Firmness (N)**	**Specific volume (cm^3/g)**	**Moisture content (g/kg)**	**TPC (mg GAE/kg)**	**CBA ($DO^{-3}/min/kg$)**	**TDF (g/kg)**
Dough	Bread		L*	a*	b*						
0	0		64.9 ± 2.0 [a]	7.5 ± 1.5 [c]	26.4 ± 0.8 [b]	0.573 ± 0.143 [d]	5.595 ± 0.683 [a]	431 ± 1 [c]	438.5 ± 8.3 [c]	3873.3 ± 505.6 [c]	13.2 ± 1.1 [e]
10	26		52.3 ± 1.1 [c]	11.4 ± 0.4 [a]	24.9 ± 0.9 [cd]	0.523 ± 0.033 [d]	3.580 ± 0.288 [b]	445 ± 1 [c]	585.6 ± 4.2 [c]	4486.7 ± 14.1 [b c]	16.2 ± 1.2 [d]
25	53		50.4 ± 3.3 [c]	12.0 ± 0.9 [a]	23.4 ± 1.8 [c]	0.849 ± 0.056 [c]	3.439 ± 0.259 [b]	462 ± 3 [b]	731.2 ± 2.1 [c]	4644.4 ± 937.5 [b c]	19.3 ± 1.1 [c]
70	170		59.1 ± 2.7 [b]	9.9 ± 0.7 [b]	29.3 ± 1.2 [a]	1.351 ± 0.214 [b]	1.625 ± 0.098 [c]	464 ± 2 [b]	1354.7 ± 6.2 [b]	5602.2 ± 288.8 [b]	31.1 ± 2.0 [b]
225	575		43.9 ± 0.6 [a]	10.9 ± 0.5 [ab]	9.4 ± 1.3 [d]	5.212 ± 0.174 [a]	0.886 ± 0.060 [d]	487 ± 8 [a]	3406.2 ± 78.9 [a]	10290.0 ± 621.6 [a]	71.5 ± 2.2 [a]

[a-c]: Mean values indicated by different letters are statistically different ($p<0.05$)

Table 37. Sensory attribute scores of bread samples containing increasing amounts of lettuce waste flour.

Lettuce waste flour (g/kg)		**Sensory attribute**												
		Odour			**Taste**				**Flavour**				**Texture**	
Dough	Bread	Yeast	Silage	Herbaceous	Acid	Sweet	Salty	Sour	Yeast	Silage	Herbaceous	Dried fruit	Softness	Gumminess
0	0	6.6 ± 1.7 [a]	1.9 ± 1.9 [b]	1.2 ± 0.4 [b]	3.3 ± 1.9 [b]	3.4 ± 1.2 [a]	6.0 ± 1.6 [a]	1.0 ± 0.5 [b]	6.1 ± 1.5 [a]	1.5 ± 1.3 [b]	1.2 ± 0.4 [b]	1.1 ± 0.3 [b]	6.0 ± 1.9 [a]	2.0 ± 0.8 [b]
70	170	1.3 ± 0.5 [b]	5.7 ± 1.4 [a]	6.1 ± 1.4 [a]	3.7 ± 1.9 [ab]	4.0 ± 1.9 [a]	4.6 ± 1.3 [a]	3.1 ± 0.9 [ab]	1.4 ± 0.5 [b]	4.5 ± 1.4 [ab]	5.0 ± 1.3 [ab]	4.2 ± 1.1 [a]	5.2 ± 1.0 [a]	5.5 ± 1.8 [a]
225	575	1.8 ± 1.6 [b]	5.9 ± 1.6 [a]	6.1 ± 1.4 [a]	5.8 ± 1.7 [a]	4.3 ± 1.1 [a]	4.3 ± 1.2 [a]	5.2 ± 1.9 [a]	1.7 ± 0.8 [b]	5.6 ± 1.6 [a]	6.4 ± 1.4 [a]	5.3 ± 1.5 [a]	2.5 ± 1.2 [b]	5.4 ± 1.3 [a]

[a-b]: For each sensory attribute, mean values indicated by different letters are statistically different ($p<0.05$). Sensory attributes were scored by a panel of 10 judges

Consumer acceptability of bread containing lettuce waste flour was thus compared to that of commercial rye bran bread (Table 38). To this aim, two commercial products were selected based on rye bran content (180 and 510 g/kg) comparable to that of lettuce waste flour in the selected samples (170 and 575 g/kg) as well as on similar colour ($p \geq 0.05$) (Table 38). In particular, L*, a* and b* of bread containing 180 and 510 g/kg rye bran were 58.1 ± 0.2, 9.7 ± 1.2, 30.3 ± 0.2 and 41.9 ± 1.1, 11.9 ± 1.6, 10.4 ± 0.9, respectively. Bread containing the highest level of rye bran (510 g/kg) or lettuce waste flour (575 g/kg) resulted less acceptable, probably due to the peculiar sensory attributes of fibre-rich bread (Table 38) (Ameh et al., 2013). Similar concentrations of rye bran or lettuce waste flour were associated to analogous acceptability scores, confirming lettuce flour bread to be just as acceptable as traditional wholemeal bread.

Table 38. Acceptability scores of bread samples containing increasing amounts of lettuce waste flour or rye bran.

Sample	**Acceptability**
Bread with 180 g/kg rye bran	6.4 ± 0.7 [a]
Bread with 170 g/kg lettuce waste flour	6.6 ± 0.4 [a]
Bread with 510 g/kg rye bran	4.4 ± 0.9 [b]
Bread with 575 g/kg lettuce waste flour	4.4 ± 0.4 [b]

[a-b]: Mean values indicated by different letters are statistically different ($p<0.05$); bread acceptability was scored by 80 consumers

Despite consumer acceptability results, the reaction of consumers towards consumption of bread containing an ingredient deriving from waste could be a critical issue. Conjoint analysis was thus applied to assess consumers' response towards a bread label reporting different information relevant to the presence of lettuce waste flour in bread (Table 39). Beside price, claims associated with nutritional value ("containing lettuce flour"; "rich in fibre") or sustainability issues ("produced recovering lettuce waste"; "produced reducing food waste") were considered. The obtained model resulted significant with p-values of both Pearson's R and Kendall's τ <0.0001. Partial preference coefficients and relative importance of each label information in defining consumer preference are reported in Table 39.

Table 39. Partial preference coefficients and relative importance of different label information in defining consumer preference.

Information		Partial preference coefficient	Relative importance
Price (€/kg)	3.00	-16.057 ± 1.133	32.4 ± 1.1
	4.50	-24.086 ± 1.700	
	6.00	-32.114 ± 2.267	
Produced recovering lettuce waste		1.353 ± 0.469	20.9 ± 0.8
Rich in fibre		3.466 ± 0.333	17.2 ± 0.6
Produced reducing food waste		2.668 ± 0.344	15.7 ± 0.6
Containing lettuce flour		1.816 ± 0.328	13.9 ± 0.6

Partial preference was scored by 370 consumers

As expected, the increase in price led to a decrease in consumer preference. Price affected consumer preference by more than 30%, resulting the most important variable among those considered. Partial preference coefficients related to the presence of nutritional claims ("rich in fibre"; "containing lettuce flour") resulted positive, indicating these claims to increase consumer preference. This result is consistent with the increasing consumer awareness of the importance of a diet rich in plant foods containing fibre (Rooney et al., 2017). Sustainability claims ("produced recovering lettuce waste"; "produced reducing food waste") also promoted a positive consumer reaction, probably due to increasing consumer concern about food sustainability (Grunert, Hieke, & Wills, 2014; Simoes et al., 2015). Noteworthy, the use of the word "waste" was not associated with adverse consumer response. By contrast, the claim "produced recovering lettuce waste" was the most important information after price in defining consumer preference.

6.1.4 Conclusions

The use of flour obtained by air-drying of lettuce waste represents a promising strategy to improve bread functionality and sustainability. It can be inferred that waste-related claims could contribute in developing an eco-friendly image of bread containing lettuce waste flour and be strategically exploited to steer consumer preference towards more sustainable bread alternatives. Although this valorisation strategy shows the potential for consumer acceptance, additional studies would be required to assess its feasibility.

6.2 Impact of waste valorisation strategies on food supply chain sustainability

A number of different valorisation strategies of lettuce waste were identified in Chapter 5. Some of them also demonstrated a potential for consumer acceptance and thus for market demand (§ 6.1). However, to assess the feasibility of lettuce waste valorisation, its industrial scalability and impact on production system sustainability should be also assessed. This could be carried out by considering a complex system integrating innovative lettuce waste valorisation strategies into the current waste management system.

Based on these considerations, the aim of this chapter was to develop an approach able to estimate the potential impact of innovative lettuce waste valorisation strategies on environmental and economic sustainability. To this aim, an industrial park integrating traditional and innovative lettuce waste valorisation strategies was hypothesized. Investment and management costs as well as the energetic demand of such industrial reality were collected based on laboratory data (Chapter 5) and industrial surveys. Such data were then elaborated in a multi-objective study, aiming at understanding the environmental and economic impact of lettuce waste valorisation strategies.

This part of the Ph.D. project was conducted taking advantage of the scientific collaboration with prof. Patrizia Simeoni of the Polytechnic Department of Engineering and Architecture of the University of Udine.

6.2.1 Introduction

Fruit and vegetable waste valorisation has been extensively and increasingly studied in the last years, as evidenced by the enormous number of relevant publications (Figure 25). The general aim of these studies was to maximally exploit the potentialities of FVW, by producing value-added derivatives (Matharu, Melo, & Houghton, 2016; Ong, Kaur, Pensupa, Uisan, & Lin, 2018). Despite this intense research activity, the current destination of FVW is mainly represented by landfilling, or, in the best case, by the production of compost or energy through anaerobic digestion and carbonisation (Cristóbal, Caldeira, Corrado, & Sala, 2018).

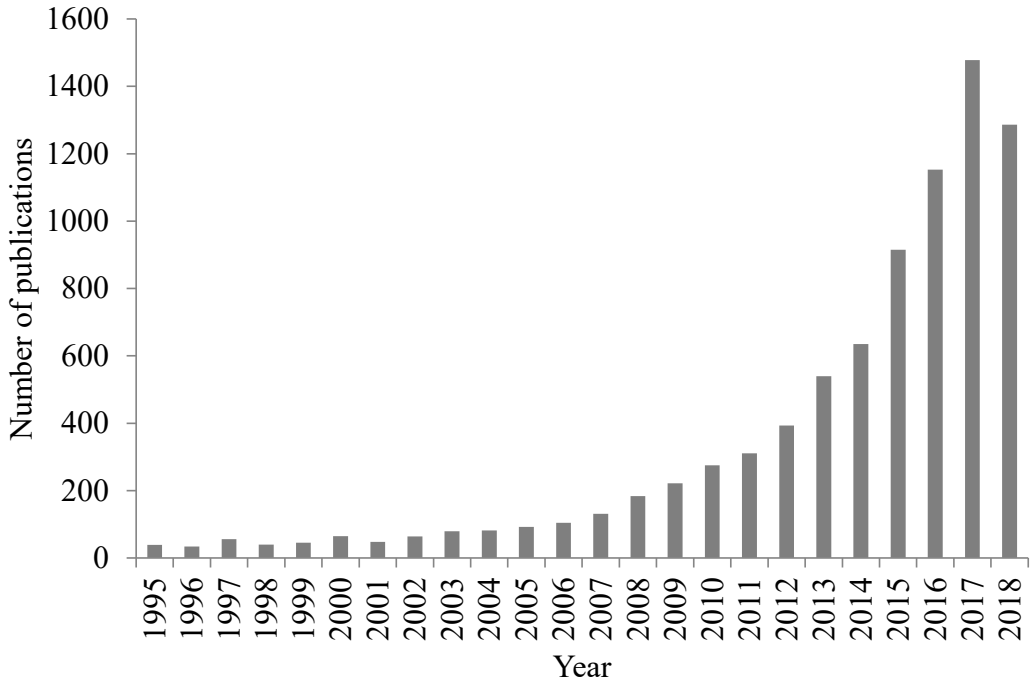


Figure 25. Number of publications relevant to fruit and vegetable waste valorisation from 1995 up to 2018. (Data collected from Web of Scince databases, Clarivate Analytics, using as key-words "Fruit and vegetable waste" or "FVW" and "valorisation" or "valorization").

In this regard, it must be noted that the valorisation of FVW is at an early stage of development and that essential elements must be still clarified to assess its viability. In particular, they include (Cristóbal et al., 2018; Heck & Rogers, 2014):

(1) the amount of FVW available for valorisation: as highlighted by Pfaltzgraff et al. (2013), data on the exact amount of waste produced from food processing is very limited;

(2) the resource demand of valorisation strategies as compared to the traditional ones: the implementation of an innovative valorisation strategy is viable only if bringing economic and environmental advantages as compared to traditional strategies such as composting, anaerobic digestion or carbonisation. Although not discussing at all these crucial aspects, most of literature studies dealing with FVW valorisation generally assume that FVW valorisation would lead to economic and environmental advantages. However, most of them exploit innovative technologies which are well-known to present huge investment and maintenance costs and different requirements in terms of resources, know-how and plants as compared to those used in common industrial practices;

(3) the possibility to scale-up valorisation strategies from laboratory to industry: only few recent works have addressed the issue of FVW scaling-up at industrial level (Meullemiestre et al., 2016; Sicaire et al., 2016), sometimes also obtaining daunting results, due to the different performances of new technologies on a laboratory and industrial scale (Preece, Hooshyar, Krijgsman, Fryer, & Zuidam, 2017b);

(4) the investigation of the integration of innovative valorisation pathways in existing ones, towards a multi FVW biorefinery concept (Cristóbal et al., 2018). Most studies analyse the valorisation process of a certain waste without considering the potential interactions with other possible valorisation pathways or existing processes.

Based on these considerations, there is the need for a method allowing to take into considerations all these aspects related to the actual feasibility of FVW valorisation. In this regard, the multi-objective method described by Simeoni, Nardin and Ciotti (2018) represents a valuable tool for estimating the environmental and economic impact of an industrial-scale reality integrating different production activities. Thus, if applied to FVW valorisation, such a method should be able to estimate the environmental and economic implications related to the integration of FVW valorisation strategies in the traditional waste management system, on an industrial scale. The application of this method should ultimately allow the development of a decision support system for the stakeholders involved in FVW management. In fact, the main objective of this multi-objective method is to help the decision-makers comparing possible scenarios deriving from the combination of different variables. In the case of FVW valorisation, such variables could include available FVW amount, amount of waste subjected to traditional and innovative valorisation strategies, economic investment for the implementation of innovative valorisation technologies, and market value of valorisation outputs. Eventually, this would lead to the identification of the solutions allowing to concomitantly maximize environmental advantage and minimize economic impact, taking into account the benefits of synergies offered by collaboration among companies engaged in FVW valorisation (Simeoni et al., 2018).

Based on these considerations, in the present study a multi-objective study was applied to the case of lettuce waste, in order to get a first insight in the potential impact on environmental and economic sustainability of the innovative strategies developed for its valorisation. In particular, as reported in Chapter 5, lettuce waste can be successfully valorised on a laboratory scale by turning it into:

- a fresh homogenate, by using high pressure homogenisation (HPH) (§ 5.1);
- an antioxidant extract, by exploiting ultrasounds (US) (§ 5.3);
- functional flour, by using air-drying (§ 5.5);
- an innovative biodegradable material, by applying supercritical-CO_2-drying (§ 5.6).

Moreover, the study-case relevant to the use of vegetable flour deriving from lettuce waste into the production of functional bread has demonstrated the potential for consumer acceptance of food items containing lettuce waste derivatives (§ 6.1). An industrial park integrating these innovative

valorisation processes with those commonly applied for FVW management (anaerobic digestion, composting, carbonisation) was thus hypothesized. Different possible scenarios were then identified and discussed based on economic and environmental indexes related to lettuce waste valorisation activities.

6.2.2 Materials and methods

The study consisted of three parts: investigative phase, design phase and analysis phase (Simeoni et al., 2018).

Investigative phase: data collection

Lettuce waste quantification

Data about fresh-cut lettuce market (M_L) were retrieved from official data and dedicated literature (Casati & Baldi, 2012; Confcoperative, 2016). Data relevant to the percentage amount of waste generated during a typical fresh-cut processing of whole-head lettuce ($\%_{WL}$) were collected in a large Italian company, as described in Chapter 3. Based on this information, the total waste amount generated in Italy from fresh-cut processing of lettuce heads (W_L), was quantified using eq. 34:

$$W_L = M_L \times \%_{WL} \qquad \text{eq. 34}$$

Industrial park layout

An industrial park integrating traditional valorisation strategies applied to lettuce waste (i.e. anaerobic digestion, composting and carbonization) with the innovative ones was hypothesized. To this aim, possible interactions among the different processes were identified along with mass flows of raw materials, wastes and utilities (energy, water).

Techno-economic and profitability assessment

Total capital investment (C_I)

The estimation of process cost of traditional valorisation strategies (anaerobic digestion, composting and carbonization) was based on data collected on local industrial activities. By contrast, innovative valorisation strategies based on the production of value-added derivatives such as functional beverages, antioxidant extracts, vegetable flour and biodegradable materials, present a low Technology Readiness Levels (TRL), being relatively new and mainly tested only on a lab scale. For these reasons, the estimation of process cost of these strategies was based on escalation factors based on existing similar plants and equipment (Cristóbal et al., 2018).

The principal unit operations involved in the different valorisation strategies were identified and data relevant to total equipment investment (C_E) and nominal energetic demand of equipment and plants required for their implementation were collected. To this aim, laboratory-scale data were directly derived from experimental activity (Chapter 5, Table 32) while data relevant to industrial scale were obtained from company survey. Collected data were thus elaborated to obtain cost and energy functions, describing all the possibilities from a small laboratory scale up to large industrial ones. To this aim, regression equations describing the variation of absorbed nominal power as a function of maximum capacity and cost as a function of nominal power were obtained and compared based on the R^2 (Microsoft® Excel 2016). The equation presenting the higher R^2 was selected.

Additional costs for plant design (C_{PD}) were calculated as 2% of equipment and plant investment. The latter was set as 1/3 of the total capital investment (C_I), while the remaining 2/3 was attributed to civil work (C_{CW}) (Cristóbal et al., 2018). Thus, C_I was calculated as reported in eq. 35 (Table 40).

Manufacturing costs

The cost of manufacturing (C_M) associated with daily operation of the considered industrial park was calculated as the sum of:

- cost of derived from C_I: costs for unscheduled and regular maintenance, and interest rate per year, were calculated as 7.5 and 15% of equipment and plant investment, respectively (Cristóbal et al., 2018);
- cost of workforce required for plant operation (C_W). The latter was quantified based on common requirements of local waste management installations and food industries. Basic salary was obtained from tables of national collective labour agreements work in the food sector (CCNL, 2018). The workforce requirement was maintained constant, i.e. independent on the lettuce waste amount processed in the industrial park. This simplification was based on the high level of automation of most of the unit operations involved in the different processes, allowing to maintain the number of labourers independent on the amount of processed raw material;
- cost of utilities (C_U). The latter is directly influenced by the cost of fuels. Only the most relevant utilities were here considered, namely electric power and water. The cost of these utilities was retrieved from average European prices from EUROSTAT (2018);
- cost of raw materials (C_{RM}). It includes (i) the cost of lettuce waste, that was considered negligible, since it has not (yet) a market value; (ii) the cost of chemicals and reactants (i.e. CO_2 and ethanol), that was obtained by a survey on producers (Sigma Aldrich. Milan, Italy); (iii) cost of waste transport, that was considered negligible, due to the geographic proximity of companies in the considered industrial park;

- cost of waste streams (C_{WS}). The cost of ethanol, CO_2 and wastewater streams was considered negligible, since they can be purified and recycled in the industrial process or used as fuels in cogeneration systems or incorporated back in the soil for nutrient uptake (Attard, Mcelroy, & Hunt, 2015).

Revenues and profitability ratios

The last section of the techno-economic and profitability assessment is the calculation of the revenues obtained from selling the valorisation outputs in the market. The outputs of both innovative (Chapter 4) and traditional valorisation strategies and their intended use was thus individuated, along with their unit price range, that was set based on that of corresponding market products.

To calculate the quantity of products deriving from the different valorisation processes, industrial yields already known for traditional strategies were used (Keeling, McCallum, & Beckwith, 2003; Rossi & Bientinesi, 2016). By contrast, in the case of innovative valorisation strategies, laboratory results (Table 32) were scaled up under the assumption that the same yields and performances would be obtained, given the same processing conditions (Albarelli, Santos, Cocero, & Meireles, 2016). Yields were defined as % ratio of final product as compared to the initial amount of raw materials entering the specific valorisation process.

Beside economic incomes from selling the value-added derivatives obtained by lettuce waste valorisation, also possible incentives for sustainable development were identified as possible economic incomes of the considered system. In particular, "White Certificates" were considered as possible incentives for energy saving deriving from the production of renewable energy from lettuce waste-derived biogas (Oikonomou, Patel, Gaast, & Rietbergen, 2009). The range for incentives deriving from "White Certificates" was set based on most recent updates (GME-GSE, 2018). Saved energy was calculated as ton of oil equivalent per year, using eq. 37, where $1.87 \cdot 10^{-4}$ is the standard coefficient for natural gas conversion into oil equivalent (Simeoni et al., 2018).

Design phase

Formative Scenario Analysis (FSA) was used, allowing to identify, using a factorial analysis, different scenarios combining all the following variables:

- the initial amount of lettuce waste available for valorisation;
- the fractions of lettuce waste subjected to different valorisation processes;
- the price of valorisation outputs;
- the value of incentives for sustainable development.

Some scenarios were eliminated in advance based on defined constraints. The latter, in turn, were based on technical or legal issues:

- scenarios in which the pay-back time of the investment was higher than 10 years were not considered, since not economically advantageous (Heck & Rogers, 2014);
- at least 10% of total lettuce waste was allocated to traditional valorisation strategies, which represent an important source of biogas and fertilizers;
- selected lettuce waste deriving from removal of bruised parts and washing of waste was set at a value lower than 50% of the initial lettuce waste weight, due to the possible poor conditions of waste.

Simulation was carried out using ModeFRONTIER® software (Esteco, Trieste, Italy).

Scenario analysis

Obtained scenarios were analysed in the light of different objectives. In particular, the objectives of this multi-objective study applied to lettuce waste valorisation are described in Table 40. The economic feasibility was measured by calculating the total investment cost and pay-back time of the investment, as described in eq. 35 and eq. 36. The environmental impact was calculated based on saved energy (eq. 37) and on the consequent reduction of greenhouse gas emissions (eq. 38), where carbon dioxide emissions were calculated through the proper emission conversion factor of electricity for the Italian electricity production system (Simeoni et al., 2018).

Table 40. Objectives of the lettuce waste valorisation multi-objective study.

Objective	**Definition**	**Equation**	
Minimization of investment cost (C_I)	Sum of costs for equipment (C_E), plant design (C_{PD}) and civil work (C_W) (€)	$C_I = C_E + C_{PD} + C_{CW}$	eq. 35
Minimization of pay-back time	Ratio between total investment cost (C_I) and the annual net profit (years)	$Payback\ time = \frac{C_I}{annual\ net\ profit}$	eq. 36
Minimization of environmental impact	Energy produced in one year from the biogas deriving from anaerobic digestion (tons of oil equivalent);	$Oil\ equivalent = Saved\ energy\ (kWh) \times 1.87 \cdot 10^{-4}$	eq. 37
	Greenhouse gases emission (tons of CO_2 equivalents)	$CO_2 equivalent = Saved\ energy\ (kWh) \times 4.22 \cdot 10^{-4}$	eq. 38

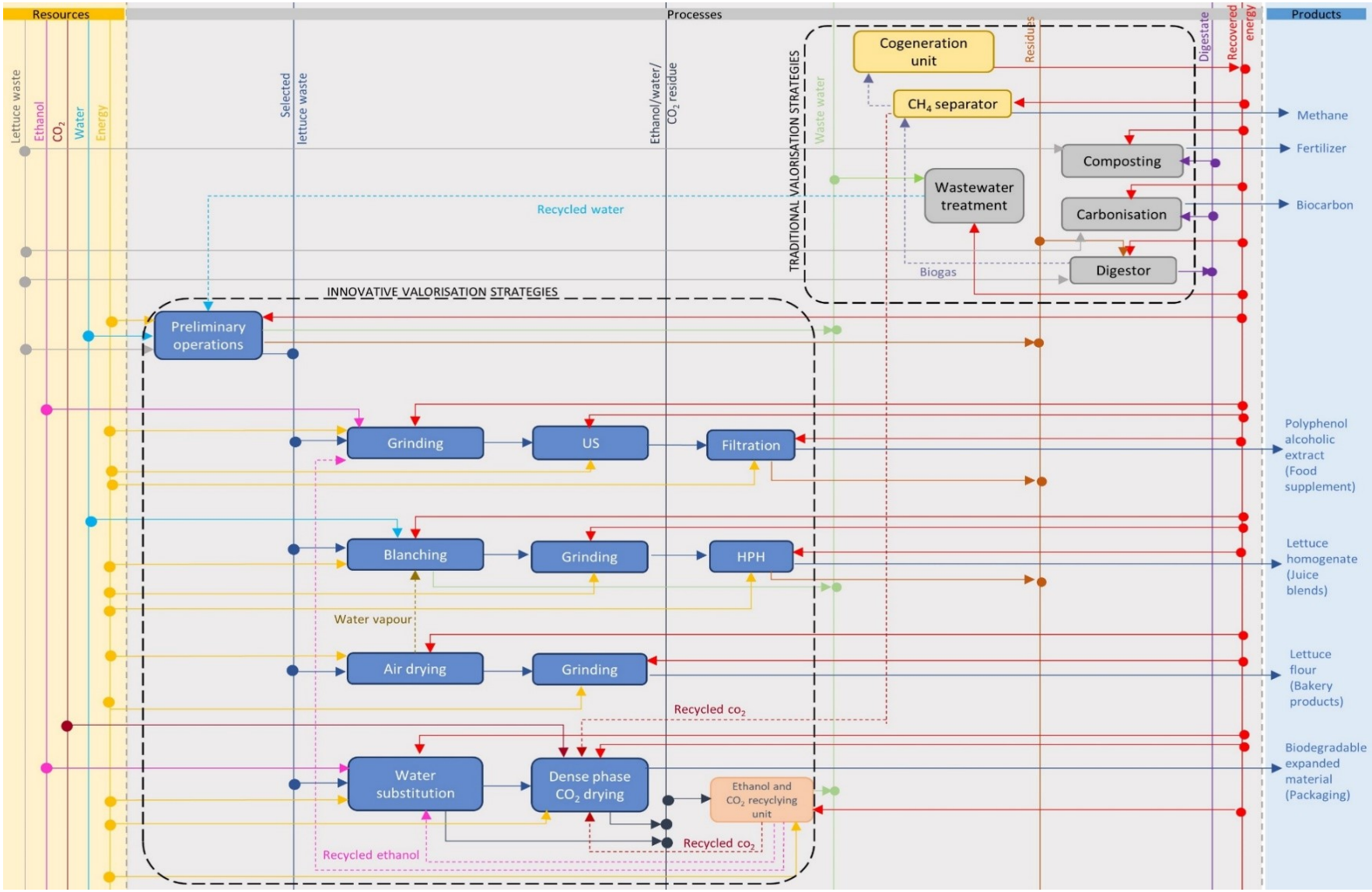


Figure 26. Flow diagram of resources (lettuce waste, ethanol, carbon dioxide, water and energy) in an industrial park integrating innovative and traditional lettuce waste valorisation strategies.

6.2.3 Results and discussion

In this study, a multi-objective study aiming at the estimation of economic and environmental impact of lettuce waste valorisation was conducted.

Investigative phase

Lettuce waste quantification

To produce value-added derivatives intended for food use, lettuce waste is required to present a high homogeneity level. In addition, waste generation sites should not be very scattered to facilitate the collection and thus cut both collection and transport costs (Galanakis, 2012). For these reasons, this work was focused on lettuce waste generated in the food processing stage, that can ensure both a high compositional homogeneity and large quantities in a reduced number of locations (i.e. the industrial plants).

The first step was thus the collection of data relevant to the amount of available lettuce waste generated during fresh-cut processing. Official data report that in Italy the fresh-cut lettuce market amounts up to about 105,300 tons/year (Confcoperative, 2016). Of that, 60% is represented by whole-head lettuces, mainly *Iceberg* lettuce (Casati & Baldi, 2012). A survey conducted in a large Italian fresh-cut company revealed that at least 35% of lettuce head weight is wasted, mainly due to initial operations of external leaves and core removal (§ 3.2). Based on these data, the total amount of waste generated in 1 year in Italy by the fresh-cut processing of whole-head lettuce was quantified in about 36,855 tons. Similarly, the total amount of whole-head lettuce waste generated by the large fresh cut company considered in the survey was evaluated. In this company, about 20,000 tons of lettuce are processed into fresh-cut derivatives. Considering 60% of this value to be represented by whole-head lettuces and 35% waste production, the company would manage every year about 4,200 tons of whole-head lettuce waste.

Industrial park layout

The second step was the design of an industrial park integrating the innovative valorisation strategies of lettuce waste proposed in the previous chapters (§ 5.1, 5.3, 5.5 and 5.6) in the current waste management system. The hypothesized industrial park is represented in Figure 26, where the flow diagram of the different processes involved in the implementation of both traditional and innovative valorisation strategies of lettuce waste, as well as their interactions is reported.

Traditionally, lettuce waste is subjected to (Figure 26):

- anaerobic digestion to produce digestate (fertilizer), biogas and, in turn, energy (by means of the cogeneration unit);
- composting to produce fertilizers;
- carbonisation to produce biocarbon.

In this case, lettuce waste is directly directed to the proper industrial facility. By contrast, the implementation of the innovative valorisation strategies described in Chapter 5, would require a preliminary selection of lettuce waste, to remove spoiled and bruised parts. The latter would be managed by means of composting, anaerobic digestion or carbonisation. On the contrary, the selected lettuce waste could be exploited as raw material to produce different valorisation outputs. It must be noted that the need for lettuce waste selection introduce a high uncertainty in the amount of lettuce waste available for innovative valorisation strategies, since the initial condition of lettuce waste depends on unpredictable factors, such as weather and cultivation conditions. Selected lettuce waste could be subjected to (Figure 26):

- blanching and high pressure homogenisation to produce fresh juices;
- ultrasound-assisted extraction to produce alcoholic antioxidant extracts;
- air-drying and grinding to produce functional flour intended for functional bakery products;
- water substitution with ethanol and supercritical-CO_2-drying to produce biodegradable expanded materials for packaging applications.

In addition, side activities for the purification and recycling of resources such as spent ethanol and wastewater were hypothesized.

Possible interactions among the different processing steps involved in traditional and innovative valorisation strategies were also identified. The integration of innovative strategies in the existing waste management framework is surely most likely to represent the real scenario of lettuce waste valorisation, differently from most available literature studies in which waste valorisation processes are described and analysed without considering their integration in the existing waste management system (Cristóbal et al., 2018). In particular, the attention was focused on the possibility to satisfy energy, water and raw material requirements of a valorisation process with the waste streams of other processes integrated in the industrial park. As an example, the carbon dioxide deriving from the co-generation unit involved in the conversion of biogas from anaerobic digestion in methane, could be used in the supercritical-CO_2-drying of lettuce waste. Moreover, the digestate and the biogas-based energy deriving from anaerobic digestion could be entirely recycled for lettuce cultivation and electrical supply of plants and equipment present in the industrial park, respectively. This strategy integration would not only lead to a higher energy self-sufficiency and independence on primary energy sources (fossil fuels), but also to a negligible cost for waste stream management.

Techno-economic and profitability assessment

The processes involved in lettuce waste valorisation are reported in Table 41. Among them, traditional valorisation strategies (i.e. composting, anaerobic digestion and carbonisation) and side activities (i.e. wastewater treatment and ethanol recycling) usually present high TRL, being already applied at industrial level. Real industrial plants were thus considered for cost and energy estimation. By contrast, innovative valorisation strategies based on the application of new technologies to produce value-added derivatives such as functional beverages, antioxidant extracts, vegetable flour and biodegradable materials, present a low TRL, being relatively new and mainly tested only on a lab scale. For this reason, similar plants present on the market were taken into considerations for cost and energy calculations.

Cost and energy functions of equipment and plants required for the various unit operations of the processes involved in the implementation of traditional and innovative lettuce waste management strategies are reported in Table 41. Such functions allow the estimation of investment cost and absorbed nominal power of specific plants and equipment as a function of their maximum capacity (tons of processed raw material or semi-finished product). Thus, they represent a flexible tool for describing a wide-range of possible scenarios, according to the available amount of lettuce waste. This is of extreme importance, considering the overmentioned high uncertainty about the actual amount of lettuce waste possibly exploitable for valorisation.

The yields of valorisation processes are reported in Table 42. As explained in the Materials and Methods, real industrial data were used for traditional strategies, while yields of innovative processes were based on laboratory data. For example, the yield of air-drying and supercritical-CO_2-drying resulted of 5%, due to 95% moisture content of lettuce waste (§ 5.5). Similarly, in the ultrasound assisted extraction of lettuce polyphenols, about 20% of solid residue was retained in the filtration step, leading to 80% yield (§ 5.3). Table 42 reports the identified outputs of both traditional and innovative (Chapter 4) valorisation strategies, along with their intended use, and the unit price range of corresponding market products.

The possibility to increase economic profitability of valorisation activities was also considered. In this regard, White Certificates are an energy efficiency market-based instrument, based on the idea that producers can fulfil specific energy saving targets by implementing energy efficiency measures. Such fulfilment is acknowledged by means of White Certificates. Producers that save more energy than their targets can sell these surplus energy efficiency equivalents in the form of White Certificates (Oikonomou et al., 2009). In this study, a variable value, ranging from 0 to 300 € was set for White Certificates.

The choice to use a price and White Certificate range rather than a medium price was based on the extreme variability and uncertainty of their values over time (Cristóbal et al., 2018; Giraudet, Bodineau, & Finon, 2011).

Table 41. Cost and energy functions of equipment and plants required for the various unit operations of processes involved in the implementation of traditional and innovative lettuce waste valorisation strategies.

Strategy	Process	Unit operation	Energy function	Cost function
Traditional valorisation	Anaerobic digestion	Digestion	P = 0.006 LW + 562.5	C = 4,012 P
	Cogeneration	CH_4 purification		C = -0.09 P + 735.4
	Composting		P = 4.16 LW + 3.19	C = 5,400 P + 401.4
	Carbonization		P = 0.02 LW + 1.59	C = 562 P + 13,108
Innovative valorisation	Preliminary operations	Selection, washing, centrifugation	P = 4.19 ln (LW)	C = 21,557 P
	Bioactive extraction	Grinding	P = 0.0003 LW + 1.37	C = 10,544 P
		Ultrasonication, filtration	P = 0.01 LW + 8.4	C = 4,466 ln (P) + 4,670
		Packaging	P = 3.00 ln (LW)	C = 7,467 P
	Homogenisation	Blanching	P = 0.002 LW + 0.954	C = 17,717 ln (P) + 1,151
		Grinding	P = 0.0003 LW + 1.37	C = 10,544 P
		High pressure homogenisation	P = 0.01 LW + 8.4	C = 4,466 ln (P) + 4,670
		Packaging	P = 3.00 ln (LW)	C = 7,467 P
	Flour production	Air drying	P = 0.43 LW + 5.71	C = 235,048 ln (P)
		Grinding	P = 0.0003 LW + 1.37	C = 10,544 P
		Packaging	P = 3.00 ln (LW)	C = 7,467 P
	Supercritical-CO_2-drying	Water substitution with ethanol	P = 0.0003 LW + 1.37	C = 10,544 P
		Supercritical-CO_2 drying	P = 0.03 LW + 3.05	C = 9,999 P
		Packaging	P = 3.00 ln (LW)	C = 7,467 P
Side activities	Ethanol recycling	Distillation	P = 3.95 ln (LW)	C = 979 P + 4,462
	Wastewater treatment	Filtration	P = 0.02 LW + 0.43	C = 7,852 P

LW = lettuce waste (kg), P = Power (kW), C = cost (€)

Table 42. Intended use and unit price of outputs obtained by the applciation of traditional and innovative valorisation strategies of lettuce waste.

Strategy	Process	Yield (%)	Output	Intended use	Price per unit (€/kg)
Traditional valorisation	Anaerobic digestion	3	Biogas	Energy *	-
	Cogeneration	60	Pure CH_4	Energy *	-
	Composting	30	Fertilizer	Fertilizer *	-
	Carbonization	10	Biocarbon	Fuel	0.25-0.90
Innovative valorisation	Preliminary operations	<50	Selected lettuce waste	Raw material **	-
	Bioactive extraction	80	Polyphenol extract	Food supplement	4.00-18.00
	Homogenisation	85	Lettuce homogenate	Ready to eat soup, juice blends	1.15-6.15
	Flour production	5	Lettuce flour	Functional bakery products	0.16-1.62
	Supercritical-CO_2-drying	5	Lettuce material	Biodegradable expanded material for packaging applications	0.03-0.18
Side activities	Ethanol recycling	80	Ethanol	Resource for industrial facilities *	-
	Wastewater treatment	60	Water	Resource for industrial facilities *	-

* Recycle within the industrial park; ** Use for innovative valorisation

Table 43 and Table 44 show workforce, raw material and utility costs, calculated as detailed in the Material and Method section. Although these costs are likely to variate in a real context, they were maintained fixed. Even if possibly reducing result robustness, this choice allowed to reduce the number of variables in the computing system, making the discussion about possible scenarios less complex.

Table 43. Price per unit of the different raw materials and utilities entering the processes involved in innovative and traditional lettuce waste management strategies.

Resource	Price per unit (€)
Ethanol (kg)	0.20
CO_2 (L)	0.0015
Water (L)	0.0015
Energy (kW)	0.17
Biogas (m^3) *	0.13

* Referred to the cost of biogas conversion into methane

Table 44. Quantification and corresponding salary of workforce required for the various unit operations of processes involved in the implementation of innovative and traditional lettuce waste management strategies.

Strategy	Process	Unit operation	Laborers	Administration employees	Managers	Maintenance workers
			Number			
Traditional valorisation	Anaerobic digestion	Digestion	4	1	1	2
	Cogeneration	CH_4 purification				
	Composting		4			2
	Carbonization		1	1	1	2
Innovative valorisation	Preliminary operations	Selection, washing, centrifugation	6	1	1	2
	Bioactive extraction	Grinding	1	1	1	2
		Ultrasonication, filtration	2			
		Packaging	1			
	Homogenisation	Blanching	1	1	1	2
		Grinding	1			
		High pressure homogenisation	1			
		Packaging	1			
	Flour production	Air drying	1	1	1	2
		Grinding	1			
		Packaging	1			
	Supercritical-CO_2-drying	Water substitution with ethanol	1	1	1	2
		Supercritical-CO_2 drying	1			
		Packaging	1			
Recycling/ purification	Ethanol recycling	Distillation	1	1	1	2
	Wastewater treatment	Filtration	1	1	1	2
			Basic salary (€)			
			1446.26	1645.75	2294.06	1446.26

Design phase and Scenario analysis

In the Design phase, possible alternative scenarios, generated by the variation of system variables under specific constraints (as described in the Materials and Method section) were individuated. The simulation resulted in a total of 121,560 possible scenarios. The latter were then compared in order to identify those in which the different targets of the multi-objective study could be reached (Scenario analysis phase). In this study, these objectives were chosen: (i) minimization of investment cost, (ii) minimization of payback time, (iii) minimization of environmental impact (Table 40). Table 45 reports possible scenarios, that were selected based on the achievement of each one of the study objectives. These scenarios took into considerations the amount of whole-head lettuce waste processed during 1 year from a large fresh-cut company (about 4,200 tons, as previously discussed). Based on the collected data and on the set constraints (Materials and Method section), the scenario allowing to minimise the investment cost resulted the one valorising 4 and 36% of lettuce waste into functional flour (§ 5.5) and fresh homogenate (§ 5.1), respectively. Among those considered, flour production would present the economic advantage of using relatively low-cost equipment (air-driers); similarly, the application of high pressure homogenisation to produce a lettuce juice would require no further raw materials or reactants. However, this scenario was also characterised by a very long pay-back time (Table 45). In order to obtain the shortest pay-back time, composting, carbonisation and anaerobic digestion should be properly combined, as shown in the corresponding scenario (Table 45), which did not include innovative valorisation strategies. Finally, in order to minimize the environmental impact of fresh-cut lettuce process, all generated waste should be anaerobically digested to produce biogas, not allowing a proper valorisation of its rich composition. Thus, all the scenarios reaching one of the study objectives (Table 40), presented some drawbacks. In this regard, the selection of a specific scenario should be driven by a compromise among the defined economic and environmental objectives. For this reason, a further scenario, deriving from a compromise solution is presented in Table 45. In this scenario, 40% of lettuce waste was valorised by the application of innovative valorisation strategies, having an investment cost lower than 20 million € and a pay-back time of only 2 months. The remaining 60% lettuce waste would be subjected to anaerobic digestion, allowing the production of at least 3 million kWh of energy and accounting for a reduction of carbon dioxide emissions up to 10,000 tons.

Table 45. Possible scenarios of lettuce waste valorisation, according to the main objective.

Objective	Processed waste (%, w/w)							Investment (€)	Payback time	Reduced CO_2 emission (ton/year)	Saved energy (kWh/year)
	Traditional valorisation			Innovative valorisation							
	Carbonisa-tion	Compo-sting	Anaerobic digestion	Flour production	Homogeni-sation	Bioactive extraction	Supercritical-CO_2-drying				
Minimization of investment cost	30	30		4	36			16,571,985	7 years	2,074	539,294
Minimization of pay-back time	20	10	70					21,567,712	18 days	13,386	3,480,467
Minimization of environmental impact			100					21,746,027	2 months	18,110	4,708,727
Compromise			60		16	16	8	19,674,589	2 months	11,677.25	3,036,202

Sources of uncertainty

Although representing a valuable support to decision makers, the conducted study entails a high uncertainty, leading to the need for an accurate validation of obtained results before application in a real context. The main sources of uncertainty of this study are those commonly found in similar estimation approaches, as reported by Cristóbal et al. (2018), and include:

- cost estimation: for low TRL technologies, cost estimation presents a -30/+30% accuracy, due to possible failures in inflation projection and cost growth due to unpredictable events related to the high complex process and unproven technology (Tsagkari, Couturier, Kokossis, & Dubois, 2016);
- cost of utilities: the electricity and natural gas prices for industrial users in the European Union depend on a range of different supply and demand conditions, including the geopolitical situation, import diversification, network costs, environmental protection costs, weather conditions, and levels of taxation (EUROSTAT, 2018);
- scaling-up variables: laboratory results were used to scale-up the industrial process considering that the same performance would be obtained. However, this should be carefully evaluated in pilot plants and corrected if necessary;
- start-up issues: in this study, the maximum productivity of processes was hypothesized, without considering possible economic issues of the start-up phase;
- wastes: in the present study, wastes were considered to be fully recycled in the industrial park economy. However, if they cannot be fully or partially used within the system, additional waste management costs should be considered;
- lettuce waste amount: although based on data collected in a real company, the estimation of lettuce waste quantity available for the valorisation is uncertain. Waste amount and quality, in fact, can vary according to unpredictable conditions, including weather, cultivation yield, pests;
- transport cost: in the present study, transport cost was considered negligible, due to geographical proximity of companies in the industrial park. However, a wider industrial park could be imagined, possibly collecting wastes from the entire country. In that case, transport cost and environmental impact should be computed in the system sustainability.

6.2.4 Conclusions

In this study, a method for estimating the impact of FVW valorisation on economic and environmental sustainability was presented. The proposed method aims at solving, or at least taking into consideration, the pitfalls commonly found in similar studies. In this regard, the main gains of the proposed method are based on the design of a system that:

- is flexible, since considering a variable range of waste amount, equipment cost, energy demand, and plant productive capacity;
- includes a cascade use of wastes generated by valorisation processes, such as their final exploitation in biofuel and energy production aiming at “zero wastes”;
- integrates multiple innovative valorisation strategies;
- investigates the integration of innovative valorisation pathways in existing FVW management system towards a multi FVW feedstock biorefinery concept.

Although further research is needed for a robust validation of data obtained on the economic and environmental sustainability of lettuce waste valorisation, the application of the proposed method led to the identification of different rational solutions. The latter could lead either to the maximisation of a specific economic or environmental objective, or to the identification of a compromise among the different sustainability objectives.

Conclusions

In this Ph.D. project a rational approach to fruit and vegetable waste valorisation was developed and validated on different vegetable discards deriving from fresh-cut lettuce, peach juice and soy milk production. The pillar of the developed approach is the collection of accurate quantitative data relevant to waste material (composition, amount), valorisation outputs (composition, amount, final application, consumer acceptance, possible price) and valorisation processes (performances, costs, energy requirements, scale-up factors). Based on these data, technical, economic and environmental feasibility of a valorisation strategy could be assessed.

The acquired results allowed the generation of a flexible decision support tool to guide stakeholders' aware choice and investment in the most sustainable valorisation strategies. This tool could be also exploited for promoting advantageous industrial symbiosis opportunities in the waste management sector. In particular, the optimal amount of waste to be diverted from landfilling, anaerobic digestion, carbonisation and composting plants to food industries could be identified, allowing its valorisation into derivatives for food, cosmetic and packaging applications. This, in turn, represents a fundamental advancement for the entire manufacturing compartment, since reducing carbon emissions, while promoting circular economy and business growth by showing opportunities for a real increase of the value chain. In addition, it would support decision and policy makers in boosting development of integrated industrial areas. The latter have a high probability to promote an overall improvement of societal and economic system by providing products with high functionality or technological content, manufactured in the respect of future generation needs.

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Awards

Premio What for 2018 for the most effective presentation of the Ph.D. Thesis results. September 2018. 23rd Workshop on the developments in the Italian Ph.D.: research on food science, technology and biotechnology.

Grant from SISSG for the participation at the *EUROFED* 2018, Belfast, UK.

Grant from INRA for the participation at *Fruit & Vegetable Processing Symposium* 2016; Avignon, France.

Premio Marco Riva per l'Innovazione nelle Tecnologie Alimentari. September 2015. National award for the Master Thesis. Effetto di trattamenti combinati di omogeneizzazione ad alta pressione e ultrasuoni su alcune proprietà fisiche e sulla stabilità di emulsioni O/W.

About the Author

Stella Plazzotta was born in Tolmezzo (Italy) on 7th February 1989. After the high school diploma (Maturità Classica), she started studying Food Science and Technology at the University of Udine. She took the Bachelor's and Master's studies, and she graduated in Food Science and Technology in 2014 with a Thesis dealing with the preparation of nanoemulsions exploiting innovative emulsifying technologies. Thereafter, she worked as a research fellow at the University of Udine in the Food Science group. In November 2015 she started the Ph.D. in Food and Human Health, which ended in the present Ph.D. Thesis.

Acknowledgments

First of all, I would like to thank prof. Lara Manzocco for giving me the opportunity to conduct this Ph.D. and for her teachings, both educational and professional. Warm thanks to all the Food Science group: prof. Monica Anese, Sonia Calligaris and Maria Cristina Nicoli for the scientific and personal support during these three years. I learned really more than what I expected, not only from a scientific point of view. Thanks to prof. Olga Martín-Belloso for giving me the opportunity to spend a period abroad at Lleida University where I have lived a great experience. Thanks to prof. Sandro Sillani, Patrizia Simeoni and Mattia Cottes for the support in economic and engineering parts of my project.

Thanks to all the students (the *Iceberg* gang: Andrea, Valentina, Aurora, Matías; Marina; the okara one: Deborah, Anna, Martina) that participated at the laboratory activities linked to my project development.

Thanks to all the colleagues but first of all friends that I met in these years: in particular, to Marilisa, Sofia, Goly, Giulia R., Giulia D., Francesco, Francesca and Fabio, who contributed to make this experience unforgettable and made me felt home every day in the office and in the lab! This experience would not have been the same without you.

A special thanks goes to my family, especially to my parents, my sister Serena and Gabriele, who have always been there for me. Thanks for the support, for always believed in me, and for teaching me to be a positive person. Last but not least, thanks to all my friends, especially to Giulia C., Giulia V., Sara and Fernanda who played a key role in these years. You have made all the times best. Thanks to everyone who played a role in my professional and personal training.

This is only the beginning!