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OPTIMIZATION OF FOOD MATRICES ENRICHED WITH BIOACTIVE COMPOUNDS FROM FRUITS AND VEGETABLES

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Abstract

Over the last few decades, topics such as sustainability, environmental impact and waste disposal are widely discussed worldwide. The planet is severely threat by human actions and it is necessary to put in place corrective actions to keep the prosperity of future generations. In particular, the food sector is a priority area where you have to act immediately, given the enormous volumes of recorded waste. Currently, the food system is forced to increase the production to face world population growth, but at the same time it must address the waste problem and the limited natural resources. Every year millions tonnes of food by-products are generated along the whole chain: from industrial production until household consumption, becoming a serious economic and environmental problem. These are commonly managed as waste, therefore sent to landfills, where turned into greenhouse gas by anaerobic digestion. This negatively impacts on the environment, causing climate changes, and provoking economic problems to the producers, being their disposal not free. Thus, in the optic of sustainability, an appropriate strategy of waste management becomes necessary. In this regard, the "zero waste" theory is very interesting. It is a waste management system whose aim is to recycle waste, being considered a resource to be reused in other productions. Zero waste manufacturing involves designing of products and processes in which no trash is sent to landfills or incinerators.

In recent years market needs have changed because of consumers' increasing awareness of diet related health problems. As a result, foods with natural ingredients and a better nutritional quality are increasingly in demand. Therefore, the food by-products, especially fruit and vegetable ones, widely recognized as excellent sources of bioactive compounds, can be used to fortify common foods eaten daily, improving their nutritional value. These can be used as natural colorants or as high-value natural ingredients to produce foods with functional properties, that can have positive effects on human health, such as reducing cholesterol and risk of various chronic diseases, including diabetes, cardiovascular diseases and cancer. However, their incorporation into food products affects the technological and sensory properties, so the challenge is to find a compromise between the nutritional and sensory aspects of enriched foods.

In this context, the present PhD research study has been focused on the enrichment of food matrices with plant by-products and their relative optimization. In particular, cereal products, as bread and pasta, were used as vehicles of beneficial substances from fruit and vegetables by-products, being staple food within human diet. The bread was enriched with

artichoke leaf flour, while spaghetti with red grape marc. In addition, the development of a watermelon-based jelly candy enriched with orange by-products was taken into account, being a product intended for a large group of consumers (from children to adults). Finally, the broccoli by-products extracts were proposed as ingredients to fortify fish-burger. Each case study addressed proves that vegetable by-products from industrial processing can be used as high value food ingredients, allowing to better satisfy consumer demand for healthy food products in a more sustainable perspective.

Riassunto

Negli ultimi decenni, argomenti quali sostenibilità, impatto ambientale e smaltimento degli scarti sono ampiamente discussi in tutto il mondo. Il pianeta è severamente minacciato dalle azioni dell'uomo e se non si interviene tempestivamente con azioni correttive, la prosperità delle future generazioni sarà compromessa. In particolare, il settore alimentare sta suscitando molta attenzione in quanto è responsabile della generazione di enormi volumi di scarti. Attualmente il sistema alimentare è costretto ad aumentare la produzione per far fronte alla crescita della popolazione mondiale, ma allo stesso tempo deve affrontare il problema dei rifiuti e delle risorse naturali limitate. Ogni anno vengono generati milioni di tonnellate di sottoprodotti alimentari lungo l'intera filiera: dalla produzione industriale fino al consumo casalingo, costituendo un serio problema sia economico che ambientale. In genere, i sottoprodotti industriali sono gestiti come scarti, ovvero vengono inviati alle discariche dove vengono trasformati in gas serra mediante digestione anaerobica. Questo impatta negativamente sull'ambiente, causando cambiamenti climatici, e arreca danni economici al produttore, costretto a sostenere spese per il loro smaltimento. Quindi, nell'ottica della sostenibilità, è necessaria un'adeguata strategia di gestione degli scarti. A tal proposito, la teoria dello "zero-waste" risulta molto interessante. Si tratta di un sistema di gestione il cui scopo è di riciclare i sottoprodotti, utilizzandoli come risorse in altri sistemi di produzione. In questo modo nessuno scarto verrà mandato agli inceneritori o alle discariche.

Negli ultimi anni anche i bisogni del mercato sono cambiati in quanto i consumatori sono sempre più consapevoli dei problemi di salute legati all'alimentazione. Di conseguenza, alimenti con ingredienti naturali e una migliore qualità nutrizionale sono sempre più richiesti. Pertanto, i sottoprodotti alimentari, soprattutto quelli provenienti dal settore ortofrutticolo, ampiamente riconosciuti come fonti eccellenti di composti bioattivi, possono essere usati come ingredienti naturali per fortificare i comuni alimenti che vengono consumati quotidianamente. Queste risorse possono essere usate come coloranti naturali o come ingredienti naturali ad alto valore per produrre alimenti con proprietà funzionali, che possono avere effetti positivi sulla salute umana, come riduzione del colesterolo e del rischio di varie malattie croniche, quali diabete, malattie cardiovascolari e cancro. Tuttavia, l'incorporazione delle sostanze vegetali all'interno degli alimenti influenza le proprietà tecnologiche e sensoriali, pertanto la sfida è di trovare un compromesso tra gli aspetti nutrizionali e sensoriali in quanto aumentando la

concentrazione dei prodotti vegetali la qualità nutrizionale del nuovo alimento migliora mentre peggiora quella sensoriale.

In questo contesto, il presente lavoro di dottorato si è focalizzato sull'arricchimento di matrici alimentari con sottoprodotti di origine vegetale e sulla loro relativa ottimizzazione. In particolare, prodotti cerealicoli, quali pane e pasta, sono stati usati come veicoli di sostanze benefiche per la salute essendo alimenti base della dieta umana in tutto il mondo. Il pane è stato arricchito con farina di scarti di carciofo, mentre gli spaghetti con farina di vinaccia rossa. Inoltre, è stato preso in considerazione lo sviluppo di una caramella a base di anguria fortificata con scarti di arancia, essendo un prodotto destinato a un vasto gruppo di consumatori (dai bambini agli anziani). Infine, gli estratti di scarti di broccolo sono stati proposti come ingredienti per fortificare burger di pesce. Ogni caso studio affrontato dimostra come i sottoprodotti vegetali possono essere usati come materia prima di alto valore permettendo di produrre alimenti con proprietà salutari, soddisfando le nuove esigenze dei consumatori e riducendo allo stesso tempo il problema dell'inquinamento ambientale.

1. Introduction

1.1 Overview of the Current Food System

The food supply chain (FSC), also called food industry or food system, is highly complex and is driven by many economic, cultural and environmental factors. It includes several steps from food production, processing (including transport and distribution), retail, consumption, and end of life (Baldwin, 2009). The role of food industry is to convert raw materials (fruit, vegetables, milk, cereals, etc.) into food products fit for human consumption (Murphy et al., 2014). Food is essential to life. It also forms an important part of our cultural identity and plays an important role in the economy. Nevertheless, different concerns are afflicting the food system.

In the last decades, the industrial food production has increased very fast and is still continuing its increase to face the world population growth, because all people need to be nourished. The world's population has changed from 2.53 billion in 1950 to an approximate amount of 7.32 billion in 2015 (Govindan, 2018). This means greater competition for the natural resources (land, water and energy) from food producers, because the speed with which these are consumed is greater than the speed with which they are generated from nature. Thus, food production will become increasingly difficult and unsustainable in coming years (Godfray et al., 2010).

If it is true that food is essential to our survival, on the other side its production is compromising the environment in which we live. We are witnessing a mass production that damages the environment and erode rapidly the planet's capacity to regenerate the resources and environment services on which our prosperity and growth is based. The current food supply chain has negative impacts on the environment that make it unsustainable, causing also adverse consequences on human well-being. It contributes to climate change due to continuous greenhouse gas emissions (GHG); for example agricultural production alone responsible for 17-32% of global greenhouse gas emissions (Bellarby et al., 2008). Moreover, the food system is responsible for excessive water and energy use, pollution, deforestation, biodiversity loss and waste generation (Baldwin, 2009). Food industry is the main producer of solid and liquid wastes, mainly deriving from fruit processing, cocoa, chocolate, confectionery, brewing, distilling, and meat processing.

This inevitably also means that huge amounts of the resources (land, water, energy, etc.) used in food production are used in vain, and that the greenhouse gas emissions caused by production of food that gets lost or wasted are unnecessary. This represents not only a resource problem but also an environmental and economic one, on top of being an inefficiency symbol of the modern society. Obviously, not all foods make an equal contribution to above-mentioned problems. Numerous assessments of individual food products find that meat and dairy products carry a disproportionately high environmental burden, especially regarding GHG emissions. The problem is that their consumption is high in developed countries and it destined to rise in the next years (Garnett, 2013).

In addition to these problems the food system appears not to be especially able to perform its main function: feeding people effectively. The malnutrition problem, both under- and over- nutrition, is widespread. Some eat too much and suffer the consequences on their health, while others go hungry. In the developing countries food insecurity is also a harsh reality, foods are hardly accessible for many people. Millions of people are undernourished because their diets lack sufficient energy and micronutrients (Garnett, 2013). It is absurd to think that substantial amount of food that should have been eaten ends up as waste along the food chain. Nowadays more than 1 billion people (one-sixth of the world's population) suffer from chronic hunger, though the food system continues to provide enough food to feed the world. One of the reasons for this disconnect is simply waste (Morgan, 2012). On the contrary, in the developed countries the diet is characterized by high intakes of energy, fat, salt and sugar deriving mostly from meat and dairy products, causing individual health risks. The main consequence is a sustained, acute increase in overweight and obesity, in particular in children and adolescents. Others widespread diet-related diseases are type 2 diabetes, hypertension, osteoarthritis, and cancer.

We live in the era of paradoxes, where on the hand there is a starving population and on the other hand a growing number of countries is dealing with over-consumption of food, food related diseases and increasing food waste production (Mirabella et al., 2014). To feed a world of 9 billion people by 2050, to ensure people do not go hungry and to safeguard food security, significant changes need to occur throughout the current food system, from crop management and harvesting, to processing and consumption. These required changes are on a global scale and not confined to a single commodity or region. It is estimated that simply halving the current amount of food waste by 2050 could reduce the projected amount of food required to feed 9 billion people by 25 percent compared to today's production numbers.

This scenario affects the food system, the way food is produced, stored, processed and distributed. Today, the food industry has to face a threefold challenge:

- to be able to meet the demand for foods from a rapidly growing population;
- to make food production more environmental sustainable: the negative effects that occur along the food supply chain must be reduced;
- to ensure that the world's poorest people are no longer hungry (Godfray et al., 2010).

In short, the problem is as follows: on the one hand, we are faced with the urgent need to address the major environmental consequences of our current systems of food production. On the other, and in the context of these environmental constraints, policy makers are tasked with developing food provisioning systems that ensure that world's growing population has access to enough of the right kind of food to meet their nutritional needs (Garnett, 2013). Therefore, the solution to aforementioned problems would be the sustainable use of resources, involving sustainable production and consumption. This would represent the key ingredient of long-term prosperity.
A sustainable food system must be:

- environmentally friendly
- economically convenient
- healthy and safe from the nutritional point of view.

How to make it possible? How can we produce environmental sustainable food and at the same time feed growing global population?

1.2 Food sustainability

There are many different views as to what constitutes a 'sustainable' food system, and what falls within the scope of the term 'sustainability'. Strictly speaking the term "sustainability" implies the use of resources at rates that do not exceed the capacity of the nature to replace them. For food, a sustainable system might be seen as encompassing a range of issues such as security of the supply of food, health, safety, affordability, quality and, at the same time, environmental sustainability, in terms of issues such as climate change, biodiversity, water and soil quality and waste (**http://ec.europa.eu/environment/eussd/food.htm**). Sustainability issues concern the entire food supply chain, from agricultural production and

processing to packing, distribution and final consumption. Each of these steps involves input of resources and generation of wastes and emissions (Pagan and Lake, 1999).

The growing world population has a great impact on the sustainability of supply chains, especially within the food industry. In the past 50 years larger quantities of foods are required to feed the population and this mass production has negative consequence on the environment. We have come up to a point where human consumption is 30% higher than nature's capacity to regenerate resources. Hence, the food supply chains of yesterday can no longer effectively handle demand, so they need to be restructured (Govindan, 2018).

In this context the sustainable development is necessary. People are aware that the food they eat is an important factor affecting their health, but what is less well known is the impact producing and consuming food has on the world's resources.

Sustainable development has first been introduced and defined by the World Commission on Environment and Development (WCED, 1987) in 1987 as "Development which meets the needs of the present without compromising the ability of future generations to meet their own needs" (Govindan, 2018; Murphy et al., 2014). In order to achieve the sustainable development when the world has limited resources, establishing of sustainable consumption and production patterns (SCP) is a necessary requirement. SCP is one aim of the sustainable development; specifically, its goal is having more efficient and profitable production while using fewer raw materials as well as adding value to a product while creating less pollution and waste in the process. Moreover, more consumer needs shall be fulfilled with less energy, water, or waste. Hence, SCP was defined by the UK Department for Environment, Food and Rural Affairs as "a continuous economic and social progress that respects the limits of the earth's ecosystems and meets the needs and aspirations of everyone for a better quality of life, now and for future generations to come" (Govindan, 2018).

The key principles for a sustainable food chain are the following:

- produce safe and healthy foodstuffs in response to market demands and ensure that all consumers have access to foods;
- respect and operate within the biological limits of natural resources;
- achieve consistently high standards of environmental performance by reducing energy consumption, minimization of waste production, minimizing resources inputs and using renewable energy wherever possible;
- achieve high standards of animal health and welfare;

- sustain the resources available for growing food over time.

Food processors and manufactures need to include sustainable actions like waste reduction and recovery, composting, recycling and processing with minimal and energy use (Baldwin, 2009). Pagan and Lake (1999) showed some of the ways the food supply chain – and hence consumption and production – can be made more sustainable through the application of technology, greater resource efficiency, better understanding of consumer demand and consumer education.

Consumers have an important role in achieving of sustainable food chain, because their demand and consumption influence production processes. Therefore, in order to change consumption patterns towards a more sustainable vision, it is necessary to inform consumers about the environmental implications of their consumption and the power they have over production decisions to allow them to make wiser consumption choices. Their purchasing choices will stimulate companies to innovate and to supply more efficient goods and services. Sustainable consumption can drive sustainable production and lead to structural changes in economy, which in turn will form a virtuous circle encouraging ever increasing sustainable consumption. Furthermore, combining sustainable consumption and production conveys a holistic approach to minimize wastes and maximize social benefits (Staniškis, 2012). According to Staniškis (2012) the sustainable food system could be achieved when all stakeholders work together for a common vision of SCP for current and future generations. Multistakeholder cooperation together with new economics approach is challenging, but essential for the planet currently imperiled by overpopulation and overconsumption.

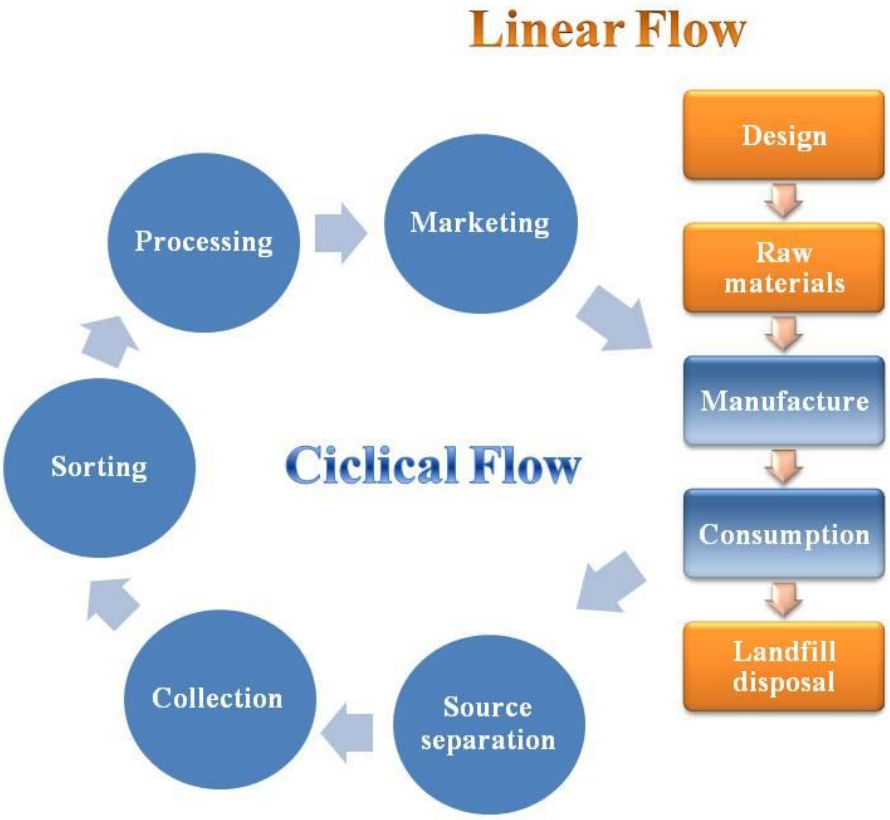
1.3 Waste management

The issue of wastes is highly discussed, because it represents a great concern worldwide, especially in the perspective of developing a sustainable system. The modern society is characterized by an irrational and unbridled consumerism, deriving from a number of economic, social, technological and media-related changes. We moved from a situation, occurred after the World War, where the main question for the majority of the industrialized nations was that of “access” to goods and services, of attaining a decent living standards through consumption, to an “excess” consumption, where more products are required than necessary (Crocker, 2013). This consumers’ new unsustainable behavior,

along with the global population growth, rapid urbanization and the rise in community living standards have greatly accelerated the solid waste generation of any kind, such as industrial waste, electrical and electronic waste (e-waste), food waste and packaging waste. Industrialization has introduced a large number of products which nature cannot, or can only very slowly, decompose or digest. Certain industrial products contain substances which, due to low degradability or even toxic characteristics, may build up in nature to levels representing a threat to humanity's future use of the natural resources and that is, drinking water, agricultural soil, air and so on (Song et al., 2015). It is estimated a total waste production about 11 billion tons per year, and per capita solid waste generation is approximately 1.74 tons/year in the world. In addition, an enormous amount of natural resources is depleted everyday due to the high demand for new products. Globally, 120-130 billion tons of natural resources are consumed every year and produce around 3.4 to 4 billion tons of municipal solid waste (Song et al., 2015). A bad management of these wastes causes both environmental and economic problems. Their increase on the planet means depletion of natural resources, such as land, water, energy, unnecessary CO2 emissions and, finally, creates an additional economic cost for the producer due to their heavy disposal costs. Therefore, a sustainable consumption and waste management strategy are necessary to reduce the resource wastage.

Increasingly, industrial ecology concepts, such as *cradle to cradle,* and circular economy are considered leading principle for eco-innovation, that is the development of products and processes which contribute to the sustainable development. Historically, the waste management was based on the linear economy, but today is no longer accepted since unsustainable.

According to linear thought the resources are taken, used and dumped and this is not justifiable in a world where resources become increasingly scarce with considerable environmental impacts. Instead, circular economy is a closed system, it is the basis of so-called industrial symbiosis, in which the goal is to use wastes from one sector as an input for other sectors (Burlakovs et al., 2018; Mirabella et al., 2014). It is necessary to shift from the concept of a production process focused on a linear economy to the concept of a production process focused on a circular economy (Curran and Williams, 2012; Lehmann, 2011; Zaman, 2015). Circular economy aims to increase the efficiency of natural resource usage, especially on urban and industrial wastes. The following figure shows the difference between linear and cyclical flows.



Recently, with the aim to guarantee sustainable growth, the European Union introduced new directive concentrating efforts on this approach (EU 416/2015). Specifically, in order to facilitate the transition to a more circular economy, several initiatives have been proposed to increase recycling and reduce landfill disposal. This means that the life cycle of the products should not finish to the point of disposal, but solid wastes should be reintegrated into new production processes (Pietzsch et al., 2017). Lately, waste management is highly inspired in the "solid waste hierarchy", a philosophy that prioritizes practices from waste prevention to the landfill. The waste management hierarchy is a nationally and internationally accepted guide whose aim is achieving optimal environmental outcomes and resource utilization. It sets out the preferred order of waste management practices, from most to least preferred: waste prevention is the best option, followed by reuse, recycling and recovery. The disposal, such as composting, incineration and landfill, is the worst environmental option (Murphy et al., 2014; Song et al., 2015). Nevertheless, the solid waste hierarchy does not provide a sufficient background for waste and resource policy regarding the absolute reduction in material production, that is, zero

waste (Ewijk and Stegemann, 2016). In fact, current environmental, social and economic demands focus on the identification of more efficient materials to be used in the transformation industry and, besides that, it focuses on the adoption of a concept based on the waste's value, which should be converted into resources without, necessarily, reprocessing (Pietzsch et al., 2017). Thus, considering the need for a holistic view for solid waste management, modern waste management is on the way to implement the concept of "zero-waste", which is a broader approach when compared to that described in the "solid waste hierarchy". It was defined by the Zero Waste International Alliance in 2004 as follows: "*Zero waste"is a goal that is ethical, economical, efficient and visionary, to guide people in changing their lifestyles and practices to emulate sustainable natural cycles, where all discarded materials are designed to become resources for others to use* (Burlakovs et al., 2018).

"ZeroWaste" is a new waste management strategy, where wastes are used as raw material for new products and applications. In recent years this approach has been suggested as a means of addressing above mentioned concerns that affect the whole world (Song et al., 2015). Many developed countries are using the zero-waste concept to change current waste management practices to more sustainable methods of managing waste, including household waste. This theory includes waste prevention; high levels of recycling and recovery of all resources from waste; and behavioral change (Cole et al., 2014). Thus, it implies the redesign of resource's life cycles so that all products are recycled (Lehmann, 2011; Zaman, 2014). According to zero waste system no trash is sent to landfills and incinerators. Material flow is circular, which means the same materials are used again and again until the optimum level of consumption. No materials are wasted or underused in circular system. Therefore, at the end of their lives products are reused, repaired, sold or redistributed within the system. If reuse or repairs are not possible, they can be recycled or recovered from the waste stream and used as inputs, substituting the demand for the extraction of natural resources. Zero waste represents a shift from the traditional industrial model in which wastes are considered the norm, to integrated systems in which everything has its use. It includes the "3R rule": "Reduce, Reuse, Recycling", which claims to do more with the Earth's resources, promoting environmental awareness. It is generally accepted that these behavior patterns and consumer choices will lead to savings in materials and energy which will benefit the environment (Song et al., 2015). In a world with finite resources, achieving a state of Zero-waste may eventually become an imperative due to the great environmental pressures in future. However, it is still difficult

to answer how to transform our existing situation into zero waste, and how to measure the performance of a zero waste city.

1.3.1 Food Waste

According to the United Nations Environment Program (UNEP), one of the most striking examples of dysfunction with regard to consumption and production is the issue of food loss and waste (Govindan, 2018). A division is made into food loss and food waste: food losses refer to the decrease in edible food mass throughout the supply chain and specifically they take place at production, postharvest and processing stages in the food supply chain. Food losses occurring at the end of the food chain (distribution, retail and final consumption) are rather called "food waste", which relates to retailers' and consumers' behavior (Parfitt et al., 2010). Instead, the term "food by-products" has been increasingly used, indicating that biomass and waste can be properly treated and converted into valuable marketable products (Plazzotta et al., 2017).

Around 89 million tons of food are wasted annually in the European Union and this value is expected to further increase by 40% in the next 4 years. Meanwhile, according to the World and Agriculture Organization one-third of all food produced for human consumption on the planet, about 1.3 billion tons, is lost or wasted each year (Gustavsson et al., 2013).

Food is wasted throughout the food supply chain, from agriculture phase, up to industrial manufacturing and processing, distribution, retail and household consumption (Mirabella et al., 2014). These losses occur in industrialized countries as much as in developing countries, but for different reasons. Overall, on a per-capita basis, much more food is wasted in the industrialized world than in developing countries. In low-income countries food is lost mostly during the early and middle stages of the food supply chain due to financial, managerial and technical limitations in harvesting techniques, storage and cooling facilities, infrastructure, packaging and marketing systems. The food supply chains in developing countries need to be strengthened by encouraging small farmers to organize, diversify and enhance their production and marketing. Investments in infrastructure, transportation, food industries and packaging industries are also required. Meanwhile, in medium- and high-income countries food is wasted at the consumption level, meaning that it is thrown away even if it is still suitable for human consumption. The causes of these losses and wastes mainly relate to consumer behavior as well as to a lack of coordination

between different actors in the supply chain. In general, food products are wasted because they do not respect the required quality standards or because of the careless attitude of consumers and of bad purchase planning. In industrialized countries food waste could be reduced by raising awareness among food industries, retailers and consumers (Gustavsson et al., 2011; Kosseva, 2013). The wastage issue involves concerns of great interest worldwide, because wasted food means water losses and also wasted energy, because the calories in food thrown away are never consumed and the energy used to grow the food, process it, package it, and transport it is also wasted. Furthermore, when the excess food is sent to landfills for disposal, it decomposes and turns into greenhouse gas, in particular methane gas, which is 20 times more effective at trapping heat in the atmosphere than carbon dioxide. At the European level, the overall environmental impact is at least 170 metric tons of CO_2 equivalent emitted per year. Traditionally, food wastes are sent to landfill, but this is not now deemed appropriate as it poses a serious environmental concern (Murphy et al., 2014). Tackling food waste problem requires increasingly smart management approaches across the food system to avoid needless losses while also preserving food quality and maintaining food safety levels. Moreover, consumers' behavioral changes are necessary, though it will be a hard challenge (Morgan, 2012). In this context the development of a sustainable production and consumption is substantial, so cooperation between supply chain members is required. Environmental sustainability can be achieved by developing and implementing alternative technologies and products that maximize the efficient use of resources and achieve cost savings, while minimizing negative human and environmental impacts (Murphy et al., 2014). As already mentioned above, the food industry is the largest generator of both solid wastes and liquid effluents throughout the process chain and these are subdivided into animal-derived and vegetable products (Edjabou et al., 2016). Clearly the composition of the bio-waste stream is dependent on the process that generates it, and its composition will, in turn, determine how the waste must be processed.

Especially the fruit and vegetable sector generates amounts of waste, called Fruit and Vegetable Waste (FVW). According to FAO estimation (FAO, 2011) pre-consumer phases are particularly critical in terms of FVW generation, in fact in Italy about 87% of fruit, vegetable and cereals are discarded before reaching consumer. This phenomenon occurs in developing, because of lack of proper conservation strategies for crops and in developed countries due to programmed overproduction and to unfulfillment of retailer quality standards. FVW poses environmental problems due to its high biodegradability and

represents an economic cost for companies. In the past, FVW was sent to landfills or incinerators (without energy recovery) for final disposal. However, this is not a good option for FVW, due to its high water content which is, in turn, responsible for microbiological instability and formation of off-odors. On the other hand, FVW has the potential to be reused into other production systems and also it represents substances of commercial interest because containing valuable components (fiber, vitamins, minerals, phytochemicals). Thus, the fruit and vegetable waste can be an environmental and economic problem if not managed properly, while it can meet various demands of a country if it is considered as a resource. Currently, different reduction, reuse and recycle strategies to tackle FVW have been proposed. Reduction depends on production practices that have to be managed in optimal way to reduce the overproduction and to ensure fulfilment of quality standard set by retailers or consumers. Instead, reuse indicates the use of waste materials for other purposes without or with minor modification. In the FVW case, it is applied to formulate animal feeds with increased nutritional value and for soil amendment, thanks to its ability to increase properties of polluted soil by immobilizing trace metals and metalloids, preventing their transfer to ground water and living organism, and promoting the establishment of plants. However, this reuse 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. As a result, recycle strategies are preferred. These are based on the recovery of waste materials after a major modification of their characteristics. Recycle strategies for FVW can be divided in two categories: in the first the whole waste mass is recycled (composting, processing to flour, conversion into water), while in the second extraction techniques are used to recover specific compounds (oils, bioactive extracts, fiber extracts). Recently many researchers have tried to use FVW flour as ingredient for the formulation of products rich in bioactive compounds (Ferreira et al., 2015b; García et al., 2009; Larrauri, 1999; Özvural and Vural, 2011). The main advantage of this recycle strategy is that valuable products such are obtained from low-cost raw materials. Moreover, after processing to flour, no residual waste has to be disposed of. However, the main issue is the high cost required for FVW drying, due to the high water content. As a consequence, the production of FVW flour is affordable only if high value-added ingredients and products are developed. Also the extraction of bioactive compounds from FVW and their application in food field has been largely studied (Ayala-Zavala et al., 2010; Choi et al., 2015).

New and more efficient technologies (ultrasounds, supercritical carbon dioxide, microwaves and pulsed electric fields) have been developed. However, it should be considered that novel technologies often require high initial investment and their industrial application is still limited. Moreover, after the extraction process, relatively high amounts of residual waste have to be still disposed of. Another way to use this kind of wastes is to recover the energy contained in the waste material. Energy from waste materials can be recovered by several strategies, including thermochemical conversions, like incineration, pyrolysis and gasification or biochemical strategies, such as anaerobic digestion and fermentation. Only some of these strategies can be used for FVW, for instance thermochemical conversation is not suitable due to high moisture in these wastes. Today, alternatively, other strategies are being studied to formulate new products with zero waste final, in which every component is recycled.

An interesting initiative on this road have been made by Plazzotta et al. (2017) with fresh-cut salad, to recycle every part of whole-head salads, that produce a large amount of waste due to preliminary removal of external leaves and core. These authors proposed production of fruit juices, bitter and gelling compounds from salad waste. Obviously, these new management strategies require investment costs by companies for their implementing and this could represent an issue. However, on the other hand this new philosophy based on zero waste brings some benefits on behalf of the community, environmental and industries and their stakeholders, besides economic and financial benefits (Pietzsch et al., 2017). In addition, new high value-added ingredients are produced allowing developing foods enriched with natural compounds. However, it is need to produce sensory and visually good product in order to minimize differences with traditional products and to make them acceptable by the consumer.

1.3.2 Food waste or by-products?

The healthy trend of food industry is to convert food wastes or by-product to the valuable food ingredients, since food wastes or by-products are considered an excellent source of nutraceuticals, bioactives, inherently functional and possess many components that are good for human health. In addition, food industries benefit economic advantages because food by-products are low-cost raw material and always available and besides the disposal problem is solved. Consumer attitude towards health foods is strongly increasing, because today foods are not only used to satisfy our hunger but also to provide essential nutrients

for humans and these nutrients having the health benefits, protecting and controlling from the diseases.

Every kind of food processing generates specific by-products characterized by a determined composition. They can be subdivided as follow:

- ✓ animal-derived processing food wastes: carcasses, hides, hoofs, heads, feathers, manure, offal, viscera, bones, fat and meat trimmings, blood;
- ✓ wastes from seafood: skins, bones, oils, blood;
- ✓ wastes from dairy processing industry: whey, curd, and milk sludge from the separation process;
- ✓ vegetable-derived processing food wastes: peelings, stems, seeds, shells, bran, trimmings residues after extraction of oil, starch, juice and sugars.

The disposal of these food industry wastes in the environment it is inconvenience to the ecosystem, because of poor biological stability, significant nutritional value, high concentration of organic compounds, high water activity, poor oxidative stability and optimum enzymatic activity (Helkar et al., 2016). However, many of these residues, especially those of vegetable origin, have the potential to be reused into other production systems as raw materials to obtain value added ingredients. They represent sources of valuable compounds such as vitamins, minerals, polysaccharides, unsaturated fatty acids, fibres, phytochemicals and bioactive compounds which are beneficial to health (Helkar et al., 2016; Mirabella et al., 2014).

Considerable number of by-products arises from fruit and vegetable sector. So, many scientists have qualitatively and quantitatively studied the chemical composition of different fruits and vegetables and their processing by-products to understand how to reuse them, instead of being discarded. Processing of fruit and vegetables produces various types of by-products such as solid residue of peel/skin, seeds, stones, stem and pulp. Some fruit and vegetable products and respective by-products, those most studied, and some possible applications are described below.

Apple (*Malus* sp., Rosaceae) is the most favoured fruit of millions of people and is widely grown in temperate regions of the globe. The world production of apple is about 58 million tons, of which about 71% of apple is consumed as fresh fruit while the remaining is used by the industries to produce apple-based foods, as juice, cider, vermouth, purees, jams and dried apple product. The industrial processing leads to large quantities of by-products

known as “apple pomace”, represented by fruit pulp, peels, seeds; besides fruits discarded into sorting belt due to the failure to meet the quality standards. Apple pomace, though traditionally utilized as cattle feed, may be exploited as source of nutrients, since it contains different functional components such as polysaccharides, pectin, vitamins, minerals, fibres and polyphenols (Grigoras et al., 2012; Shalini and Gupta, 2010). Production of pectin is considered the most reasonable way of utilizing apple pomace both from an economical and from an ecological point of view. Apple pectins are characterized by superior gelling properties compared to citrus pectins. However, the slightly brown hue of apple pectins caused by enzymatic browning may lead to limitations with respect to their use in very light-coloured foods (Schieber et al., 2001). In the literature, different extraction methods to recover pectin from apple pomace have been proposed (Shalini and Gupta, 2010). Furthermore, many authors showed that apple pomace represents a good source of polyphenols, predominantly localized in the peel. Major compounds identified include chlorogenic, caffeic and cinnamic acids, catechins, hydroxycinnamates, quercetin glycosides and procyanidins (Foo and Lu, 1999) and experimental tests demonstrated that same phenolic constituents have a strong antioxidant activity in vitro (Lu and Foo, 2000). Different efforts have been made to utilize apple pomace in the preparation of edible products like apple pomace jam and sauce. Using apple pomace as value-added food ingredients could be a valuable and attractive solution to healthy food products, since a small amount could greatly increase the phytochemical content and antioxidant activity of foods (Shalini and Gupta, 2010). This kind of by-product is also interesting for its high amount of dietary fiber, which has an important role in management and regulation of gastrointestinal system. Cellulose and hemicelluloses are the most important fractions, constituting respectively 43% and 20-32% of apple pomace. Exploiting the apple pomace as powder ingredient, the fiber content of many foods could be increased (Kowalska et al., 2017).

Grapes (*Vitis* sp., Vitaceae) are the world’s largest fruit crop with more than 60 million tons produced annually. About 80% of the total crop is used by industries for wine production, generating large amount of solid wastes, known as grape pomaces (skins and seeds) and stems, which account for approximately 20% of the weight of grapes processed. Winery residues composition varies considerably, depending on grape variety, vineyard location and vinification process (González-Centeno et al., 2012; Schieber et al., 2001). In general, winemaking by-products are outstanding sources of oil, phenolic compounds with high antioxidant activity, dietary fibre and possess numerous health benefits and

multifunctional characteristics, such as antioxidant, colouring, antimicrobial and texturizing properties (Lavelli et al., 2016). Owed to their chemical composition, they could be exploited in different process, instead of being discarded. The grape stems are mainly characterized by proanthocyanidins, instead a great range of products such as ethanol, tartrates, citric acid, grape seed oil, hydrocolloids and dietary fiber are recovered from grape pomace. This is very well known for its phenolic composition, which has been widely studied by many authors (Ferrari et al., 2019; González-Centeno et al., 2012; Teixeira et al., 2018). Anthocyanins, catechins, flavonol glycosides, phenolic acids and alcohols and stilbenes are the principal phenolic constituents of grape pomace, in particular anthocyanins have been considered the most valuable components. Catechin, epicatechin, epicatechin gallate and epigallocatechin were the major constitutive units of grape skin tannins (Ferrari et al., 2019; Schieber et al., 2001). Conventionally, winemaking by-products were used for agronomic purposes, animal feed production and compost and distillates production; but lately they are used for new applications, such as natural colorants, nutritional supplements and value added ingredients. If properly recovered, winemaking by-products show a wide range of potential and remunerative applications in many industrial sectors, including cosmetics, pharmaceuticals and food, being a rich source of both dietary fibre and various phenolic compounds. Tartaric acid, enocyanine (E163) and grape seed oil are classical examples of successful commercial products obtained from winemaking by-products. Additionally, in the last several years, grape seed and grape skin powders have been promoted as highly nutritional ingredients to enrich conventional cereal flours and baked products with fibre, minerals, antioxidants, colour and aroma (Lavelli et al., 2016).

Citrus fruits, including oranges, grapefruits, lemons, limes, tangerines and mandarins, are among the most widely cultivated fruits around the globe. In particular, sweet orange (*Citrus sinensis*) is the major fruit in this group constituting about 70% of the total citrus production and consumption (Sharma et al., 2017). In contrast with other types of fruits, citrus fruits have a small edible portion and so large amounts of waste material are discarded during juice and food processing. Citrus by-products consist of seeds and peels and the latter are subdivided into the epicarp or flavedo (coloured peripheral surface) and mesocarp or albedo (white soft middle layer). These by-products can also be useful to the food industry, because a number of studies have recognized the presence of important compounds, such as polyphenols, vitamins, minerals, dietary fibres, essential oils and carotenoids, which make waste material an useful ingredient when designing healthy foods

(Rafiq et al., 2018). Fernández-López et al. (2004) found promising results and potential applications to produce healthier products exploiting citrus by-products. The chemical composition varies depending on the fraction of the fruit (juice, albedo, flavedo, pulp and seeds), and therefore proportion of these components in citrus juice residues depends on the juice extraction system used (Fernández-López et al., 2004; Mirabella et al., 2014). As reported in the literature, these by-products are mainly known for their high fiber and phenolic compounds contents. The content of all dietary fiber fractions (total, soluble and insoluble) is higher in peels (65% aprox.) than in peeled citrus fruits, as well as the total polyphenolic amount (Gorinstein et al., 2001). Flavonoids, present as flavanones, are the major polyphenols in citrus fruit and the main ones are hesperidine, narirutin, naringin and eriocitrin. These compounds have shown antioxidant, antiatherogenic, hypocholesterolaemic, hypoglycaemic, anti-inflammatory, anticancer, antiviral, antimicrobial and antiallergenic activities. Both *in vitro* and *in vivo* studies have recently demonstrated health-protecting effects of certain citrus flavonoids (Escobedo-Avellaneda et al., 2014; Manthey John et al., 2001; Schieber et al., 2001). Even the ascorbic acid (vitamin C) content in citrus fruits is of considerable importance. Gorinstein et al. (2001) reported that the content of ascorbic acid is higher in peels than in peeled fruits and it is also higher in peeled lemons and oranges and their peels than in peeled grapefruits and their peels. Citrus fruit are characterized by presence of carotenoids, especially β-carotene, β-criptoxanthin, zeaxanthin and lutein, and they also exhibit beneficial effects on human health (Escobedo-Avellaneda et al., 2014). Definitely, the exploitation of by-products of citrus fruit processing as a source of functional compounds and their application to foods is promising. The presence of dietary fiber and antioxidants in citrus by-products allow to obtain healthy products. Overall it could be interesting their incorporation in frequently consumed foods which could help to overcome the fiber deficit in actual human diet and to prevent the development of cancer and other diseases.

Pomegranate (*Punica granatum L.*) is one of the oldest edible fruits widely grown in many tropical and subtropical countries. There is growing interest in this fruit because it is considered to be a functional product of great benefit in the human diet. The edible part of the fruit can be consumed fresh, although in the last years a large proportion of worldwide fruit production is processed in several pomegranate foods, such as jams, jelly, beverages or juice.

Food production processes generate great amount of by-products, which are divided into two categories: peels and seeds. However, they are valuable sources of bioactive

phytochemicals, thus they have a great potential to be converted into value added products (Charalampia and Koutelidakis, 2017; Gullon et al., 2016). Pomegranate peel accounts about 50% of the total fruit weight and is comprised mainly of several bioactive compounds such as hydrolysable tannins at variant concentrations (pedunculagin, punicalin, punicalagin and ellagic and gallic acids), flavonoids (catechins, anthocyanins, and other complex flavonoids), complex polysaccharides and minerals (phosphorus, magnesium, calcium, potassium and nitrogen). Its primary phenol acids are gallic acid, protocatechuic acid, chlorogenic acid, vanillic acid, coumarin and caffeic acid (Charalampia and Koutelidakis, 2017; Gullon et al., 2016). The concentration of these compounds depends on the cultivar type and on the various developmental phases of the fruit, and is responsible for the variations in pomegranate peel color. By its valuable chemical composition, it was shown that pomegranate peel posses antioxidant and antimicrobial activities (Hasnaoui et al., 2014). As concern pomegranate seeds, these comprise about 10% of the edible part of pomegranate. They are important for the high content of polyunsaturated fatty acids. More specifically, the seed oil consists up to 80% of conjugated octadecatrienoic fatty, while sterols, steroids, tocopherols and cerebroside are present in minor quantities (Akhtar et al., 2015; Charalampia and Koutelidakis, 2017). Many scientific works confirm the potentiality of pomegranate residues to be used in food sector as natural additives or value added ingredients.

Tomato (*Lycopersicon esculentum L.)* is the second most important vegetable crop worldwide, with annual production of 100 million tons fresh fruit produced in 144 countries. Significant amounts are consumed either as fresh fruit or as processed products. Food industry produces several tomato foods: juice, sauce and concentrate, losing a high quantity of raw material during transformation. Tomato by-products consist of peel and seeds, which constitute about 3–5% of total weight of processed tomatoes, leading to annual production volumes of over one million ton worldwide (Zuorro et al., 2011). However, these are important sources rich in nutrients and bioactive compounds (as sugars, organic acids, carotenoids, mainly lycopene and β-carotene, fiber, proteins, oils, phenol compounds and vitamins) (Mirabella et al., 2014). According to different authors, peels present high fiber values (41%) and significant amount of proteins (14%), being fat only 3%. This profile is inverted in the case of the seeds, in which proteins are the major component (32%) followed by total fat (27%) and fiber (18%). Herrera et al. (2010), after having characterized tomato peels in terms of fiber and macronutrients (proteins, ash, total available carbohydrates and soluble sugars), stated that they may have a great nutritional

and technological interest. Instead, Kalogeropoulos et al. (2012) showed that bioactive phytochemicals contained in tomato processing by-products exert powerful antioxidant activities, therefore, these constituents could be either isolated from the wastes to be used as natural antioxidants for the formulation of functional foods, or to serve as additives in food systems to elongate their shelf-life. The majority of the studies published in literature focus on characterization and recovery of carotenoids, especially lycopene and β -carotene, for their possible role in disease prevention; also suggesting different extraction methods (Baysal et al., 2000; Topal et al., 2006; Zuorro et al., 2011).

Artichoke (*Cynara scolymus L*) is vegetable originating from the Mediterranean area and today it is widely cultivated all over the world. The edible parts of the artichoke plants are the large immature flowers (more known as capitula or heads), harvested in the early stages of their development, which represent about the 30–40% of its fresh weight, depending on the variety and the harvesting time. Since only the central portion of the capitula is consumed, every year artichoke by-products (stems, external bracts and leaves) discarded at harvesting or after industrial processing are very abundant. However, artichoke and then its by-products are characterized by a high concentration of bioactive phenol compounds, besides inulin, fibres and minerals. In fact, its beneficial properties on human health are linked to its valuable chemical composition. This has been highly studied and according to the data in the literature, caffeic acid derivatives are the main phenolic compounds in artichoke, including chlorogenic acid (5-O-caffeoylquinic acid) as the most important. Other phenolics are the flavonoids (apigenin and luteolin) as well as different cyanidin caffeoylglucoside derivatives (Lattanzio et al., 2009; Ruiz-Cano et al., 2014). In various pharmacological tests, artichoke leaf extracts have displayed hepatoprotective, anticarcinogenic, antibacterial, antioxidative activities as well as the ability to inhibit cholesterol biosynthesis and LDL oxidation (Helkar et al., 2016). Furthermore, artichoke by-products contein inulin, a soluble fibre belonging to a group of fructose-based polysaccharides called fructans, which are not processed in the small digestive system because of humans lack the enzymes required for hydrolysis of fructans. The recent interest in inulin in nutraceutical has been due to its great influence on the composition of the gut microflora: it can increase the number of bifidobacteria, lactobacilli and certain butyrate-producing bacteria in the colon, as well as decrease the population of harmful *Clostridium perfringens*. Moreover, inulin is a low-calorie fibre that has potential for use in the production of fat-reduced foods (Helkar et al., 2016; Lattanzio et al., 2009; Yu et al., 2018).

Broccoli (*Brassica oleracea* var *italica)* is vegetable originating from the Mediterranean area, which has acquired a considerable relevance in the last few years as a health-promoting food, due to the recognized beneficial properties (Arnáiz et al., 2012). Specifically, in this vegetable high contents of bioactive compounds such as glucosinates, isothiocyanates, mostly sulforaphane, phenolic compounds with high antioxidant activity (chlorogenic and sinapic acid derivates and flavonoids), vitamin A, C, E, K and several important minerals have been found. Therefore, its consumption allow to reduce the risk of several diseases associated with oxidative stress, such as cardiovascular and neurodegenerative diseases as well as cancer (Domínguez-Perles et al., 2010; Drabińska et al., 2018). Since most people and during the manufacturing process of broccoli, only broccoli florets are used, accounting for around 30% of the biomass of vegetable, high amounts of waste (mainly leaves) are generated (Arnáiz et al., 2012). Several authors focused on broccoli by-products and studied their chemical composition and antioxidant activity that resulted to be similar to those of broccoli florets. Consequently, literature data suggest that broccoli leaves may be used in food sector as natural ingredients or additives because of their high nutritional value and functional capacities, such as gelling and water binding properties, giving the opportunity to obtain added value products. The incorporation of broccoli leaves into common foods could improve the nutritional quality and facilitate the management of vegetable processing wastes (Domínguez-Perles et al., 2010; Drabińska et al., 2018).

1.4 Valorisation of food by-products

The interest in searching for new natural ingredients to be used in industrial production to develop foodstuff with healthy properties deriving from the presence of bioactive compounds is increasingly growing, because of new consumers' demands. Therefore, researchers are trying to exploit vegetable food wastes, better called as "food by-products", as high-quality ingredients in food production. This reuse strategy has aroused considerable attention in order to reduce the above problems (Kowalska et al., 2017). In recent times there is a rapidly growing attention concerning the role of plant secondary metabolites in food and their potential effects on human health (Schieber et al., 2001). It is widely known that fruit and vegetables are important elements of a healthy and balanced diet, being rich in beneficial substances to human health, as vitamins, minerals, fiber and phytochemicals (polyphenols, tocopherols, organic acids, flavonoids, ect.). These latter are

powerful antioxidants and free radical scavengers that protect the organism from many diseases by means of various mechanisms (Dziki et al., 2014; Gawlik-Dziki et al., 2015). Nevertheless, though the public institutions advise to increase their level of intake, the consumers fail to assume the recommended daily amount by the World Health Organization (400g per day). Thus, the incorporation of the fruit and vegetable products into foods eaten daily is a way to increase the consumption of beneficial substances and then to reduce the risk of chronic diseases.

Epidemiological studies strongly suggest that diet plays a significant role in the prevention of many diseases. In particular, a diet rich in natural antioxidants can have a positive effect on reducing cardiovascular diseases, neurodegenerative disorders, cancer and diabetes (Dziki et al., 2014; Gawlik-Dziki et al., 2015). Nowadays consumers are increasingly aware of diet related health problems, therefore requiring foods with natural ingredients, which are expected to be safe and health-promoting (Schieber et al., 2001). They look forward not only safe or nutritious food products, but also natural and healthy food. The increasing interest of consumers in functional foods has brought about a rise in demand of natural food. The search for new functional food ingredients from natural sources is one of the most important challenges in food science and technology. By using science and innovation it is possible to transform food by-products in valuable ingredients and increase profitability (Helkar et al., 2016). Thus, in the response to the market needs industry and researchers are involved in optimizing production technology to improve the quality, taste, functionality and bioavailability of food matrices. Recently, there has been a global trend in developing novel products supplemented with natural antioxidants, which are derived from vegetable world (Hao and Beta, 2012). The interest in food by-products as promising sources of bioactive compounds is due to the fact that the agri-food industries generate substantial quantities of wastes, which could be valuable natural sources of antioxidants to be employed as ingredients (Gawlik-Dziki et al., 2013), as well as being low-cost raw materials. These can be used as natural colorants, replacing synthetic additives that are rejected by consumers, or as high-value natural ingredients to produce foods with functional properties. Functional foods are regarded as innovative and promising products, which can provide additional health benefits beyond the basic nutrition. Although the functional foods have no formal definition, some groups define the primary category of functional foods as modified foods that claim to have been fortified with nutrients or enhanced with phytochemicals or botanicals to provide specific health benefits, when they are consumed at efficacious levels as part of a varied diet on a regular basis (Hao and Beta,

2012; Hasler, 2002). In this way, is it possible both to satisfy consumer demands for food products with functional properties, both of solving economic and environmental problems linked to food wastes production. Obviously, the incorporation of vegetable material into food eaten daily is a great challenge because it improves the nutritional composition, increasing antioxidant activity level, the phenol compounds amount, etc. and on the other hand it affects the technological and the sensory properties. So, finding a compromise between nutritional and sensory aspects become necessary.

Food by-products can be used as value-added ingredients through direct addition in food formulations as flour, after being dried and reduced to powder, or as extract after having applied a proper extraction technique. The advantage of using powders instead of extracts from the by-products is that less processing is required, which is a more sustainable approach as it consumes less energy and does not generate secondary by-products. On the other hand, the disadvantage of using powders is that higher doses are needed to achieve significant polyphenol fortification levels, which penalizes the organoleptic properties of the products (Iriondo-DeHond et al., 2018). A substantial number of scientific studies exists where many authors have tried to enrich the most frequently consumed foods through direct addition of plant by-products flour at various concentrations, in order to increase the phenolic compounds content and the antioxidant activity level (García et al., 2009; Özvural and Vural, 2011; Sant'Anna et al., 2014). Others, instead, focused their attention on optimization of extraction methods in order to recover interest bioactive substances with health-promoting properties from vegetable material. Since these active compounds are usually present in low concentrations and they are generally very complex, researchers are focused on the development of more effective and selective extraction methods for their recovery from the raw materials.

1.5 Extraction techniques of bioactive compounds

The extraction process is essential and is the first step when bioactive phytochemicals are used in the preparation of dietary supplements, nutraceuticals and food ingredients. In the scientific literature several extraction techniques have been proposed. Along with conventional methods, numerous new methods have been established but no single method is regarded as universal for extracting all bioactive compounds from plants. The efficiencies of conventional and non-conventional extraction methods mostly depend on the critical input parameters (solvent, temperature, pressure and time) and on the nature of

plant matrix and chemistry of bioactive compounds. Consequently, the quantitative and qualitative quality of the extract is affected by extraction method. In general, all these techniques have some common objectives, (a) to extract targeted bioactive compounds from complex plant sample, (b) to increase selectivity of analytical methods (c) to increase sensitivity of bioassay by increasing the concentration of targeted compounds, (d) to convert the bioactive compounds into a more suitable form for detection and separation, and (e) to provide a reproducible method that is independent of variations in the sample matrix (Azmir et al., 2013). Regarding classic methods, most of these techniques are based on the extracting power of different solvents with different polarities (methanol, ethanol, acetone, ethyl acetate and water, pure or mixed) and the application of heat and/or mixing. They are Soxhlet extraction, maceration and hydro-distillation (water distillation, water and steam distillation and direct steam distillation). In the last years the conventional extraction is increasingly replaced by new techniques, because of some its limitations, such as longer extraction time, high consumption of expensive and toxic solvent, evaporation of the huge amount of solvent, low extraction selectivity and thermal decomposition of thermo labile compounds. Thus, new and promising extraction techniques are introduced. These new approaches are referred as non-conventional extraction techniques and some of the most promising are ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE). They are considered more environmental friendly and energy efficient technologies and are preferred because of their great advantages compared to the traditional extraction. These new methodologies are able to shorten the extraction time, reduce the organic solvent waste, increase the extraction yield, enhance the quality of extracts and also use water as solvent (Azmir et al., 2013; Dai and Mumper, 2010; González-Centeno et al., 2014).

Ultrasound-assisted extraction (UAE) is a potentially useful technology, as it does not require complex instruments and is relatively low-cost. The UAE is based on sound mechanical waves, called ultrasounds that go beyond human hearing, whose frequencies are superior to 20 kHz. This type of extraction requires a liquid medium carefully selected that allows ultrasonic wave to propagate up to the product, creating the pressure fluctuations: compression and expansion. This process produces a phenomenon called cavitation, which means production, growth and collapse of gas/vapor-filled bubbles. The implosion of these microbubbles results in a number of mechanical effects, such as turbulent streaming, particle collisions, cell wall disruption and surface microfractures, which globally lead to a greater penetration of solvent into the sample matrix and,

consequently, to a significant increase in the mass transfer rates of bioactive compounds to the extraction solvent (Dai and Mumper, 2010; González-Centeno et al., 2014). Thus, the extraction mechanism by ultrasound involves two main types of physical phenomena, (a) the diffusion across the cell wall and (b) rinsing the contents of cell after breaking the walls. Moisture content of sample, milling degree, particle size, solvent and solvent-to-sample ratio are very important factors for obtaining efficient and effective extraction. Furthermore, temperatures, pressure, frequency and time of sonication are the governing factors for the action of ultrasound (Azmir et al., 2013). UAE is a method suitable for all types of compounds, it is able to preserve the integrity of the molecules that can be thermolabile, thermostable, hydrosoluble, and liposoluble. Recently, UAE has been widely used in the extraction of various bioactive compounds from different parts of plants such as leaves, stalks, fruits and plant seeds (Eh and Teoh, 2012; Khan et al., 2010; Londoño-Londoño et al., 2010). Li et al. (2005) found better recovery of chlorogenic acid from fresh leaves, fresh bark and dried bark of Eucommia ulmodies Oliv. by UAE at optimized condition (70% methanol, 20:1 solvent, sample ratio and 30 min time) than classical extraction techniques. Anthocyanins and phenolic compounds were extracted from grape peel using UAE and the extraction process was optimized with reference to solvent, extraction temperature and time (Ghafoor et al., 2009; Ghafoor et al., 2011).

Supercritical fluid extraction (SFE) is based on the use of supercritical fluids as extraction solvent. A substance can attain the supercritical state only if it is subjected to temperature and pressure beyond its critical point. Critical point is defined as the characteristic temperature (Tc) and pressure (Pc) above which the difference between gas and liquid phases does not exist, i.e. the specific properties of gas and/or liquid become vanished. In the critical region, there is only one phase, which possesses properties of both a gas and a liquid. Thus, supercritical fluids possess gas-like diffusion, viscosity and surface tension properties, and liquid-like density and solvation power. Due to their low viscosity and relatively high diffusivity, supercritical fluids have enhanced transport properties than liquids, can diffuse easily through solid materials. These properties make them suitable for extracting compounds in a short time with higher yields (Azmir et al., 2013; da Silva et al., 2016; Wijngaard et al., 2012). Carbon dioxide (CO_2) is the main supercritical solvent used in SFE due to its particular characteristics, such as moderate critical conditions (31ºC and 74 bar) and ready availability. Its low critical temperature and pressure offers the possibility to operate at room temperature and at moderate pressures, generally between 100 and 450 bar. In addition, CO_2 is cheap, environmentally friendly and generally

recognized as safe by FDA and EFSA. However, this technique is limited to compounds of low or medium polarity, because the main drawback of CO_2 is its low polarity, problem that can be overcome employing polar modifiers (ethanol, methanol, water and acetone), called co-solvents, to change the polarity of the supercritical fluid and to increase its solvating power towards the analytes of interest, that is phenolic compounds (Garcia-Salas et al., 2010; Herrero et al., 2010). Generally, ethanol (EtOH) is used as co-solvent considering its good miscibility with CO2, non-toxicity and permissible use in the food industry. In SFE the yield results (phenolic and total) increase directly with solvent polarity and so the use of EtOH as a co-solvent is particularly useful to enhance the phenolic fraction yield. At constant temperature, the rise in pressure increases the yield due to density enhancement. At constant pressure, the phenolic and the total yield decrease with rising temperatures due to the solvent density reduction which in turn results in lower solubility of analytes (Garcia-Salas et al., 2010). The successful extraction of bioactive compounds from plant materials rely upon several parameter of SFE which need to be precisely controlled for maximizing benefits from this technique. The major variables influencing the extraction efficiency are temperature, pressure, particle size and moisture content of feed material, time of extraction, flow rate of CO_2 and solvent-to-feed-ratio (Azmir et al., 2013). Lastly, SFE is of enormous interest today, with more than 200 references in the literature dealing with this topic. A high variety of samples, type of materials, target compounds and procedures have been published in the last years. SFE can be useful to extract carbohydrates, crude vegetable oils, essential oils, fatty acids and bioactive compounds from fruits and vegetables. SFE has been widely used to value food industry by-products generated during food manufacturing. SC-CO_2 has been mainly used for the recovery of apolar bioactive compounds, in particular carotenoids from several food by-products, such as tomato peel, apricot pomace and carrot cake. Other researchers studied the extraction of polyphenols from grape pomace, permanganate seeds, olive leaves, orange pomace, etc. (Herrero et al., 2010; Wijngaard et al., 2012).

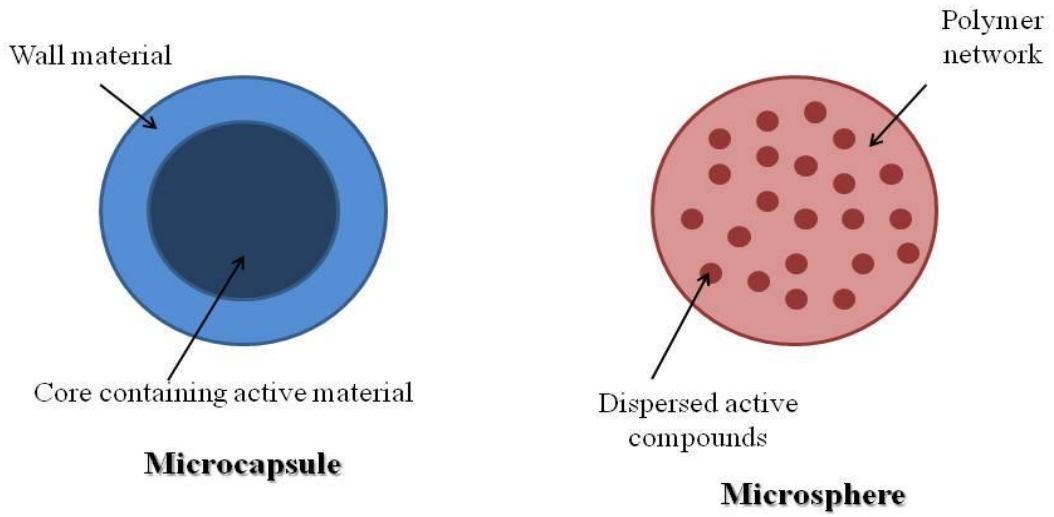
Pressurized liquid extraction (PLE) is also known by several names: pressurized fluid extraction (PFE), accelerated solvent extraction (ASE), enhanced solvent extraction (ESE) and high-pressure solvent extraction (HSPE). When 100% water is used as solvent, PLE is generally called superheated water extraction or subcritical water extraction (SWE) (Azmir et al., 2013; Wijngaard et al., 2012). This technique is based on the applications of high pressure (3.3-20.3 MPa) and temperatures (40-200°C). High pressure allows keeping solvents in the liquid state at temperatures greater than their normal boiling point and then

facilitates the extraction process, while higher extraction temperatures can promote higher analyte solubility by increasing both solubility and mass transfer rate and, also decrease the viscosity and surface tension of solvents, thus improving extraction rate. Therefore, extraction solvents including water which show low efficiency in extracting phytochemicals at low temperatures may be much more efficient at elevated PLE temperatures. In SWE, water is heated up to 200 °C and the change in the dielectric constant of the water with the temperature leads water to behave like an organic solvent. For example, the dielectric constant of water at 200 °C is equal to 36 which is close to methanol. In addition, PLE technique requires less time and small amounts of solvents than conventional techniques, because of the combination of high pressure and temperatures which provide faster extraction processes (Azmir et al., 2013; Dai and Mumper, 2010). Applications of PLE technique for obtaining natural products are frequently available in literature and it is generally used to extract polar compounds. In recent years, PLE has been successfully applied to the extraction of polyphenols from different plant materials such as grape seeds and skin, apples, spinach, eggplants and barley flours (Dai and Mumper, 2010). King (2007) for example showed the potential of the use of PLE in order to extract polyphenols from fruit and vegetable by-products. The main parameters in PLE optimizing are temperature, pressure, flow, rate and extraction time and various researchers optimized the extraction to recover the maximum yield of bioactive compounds from by-products. Monrad et al. (2010) tried to extract procyanidins from red grape pomace and reported that a temperature higher than 80 °C at a pressure of 6.8 MPa was required to enable an efficient procyanidin extraction from red grape pomace when using PLE. García-Marino et al. (2006) studied the recovery of catechins and proanthocyanidins from grape seeds using superheated water and assessed the effect of temperature in the extraction. Instead, Ju and Howard (2003) showed that in PLE at 80–100 °C, acidified water was as effective as acidified 60% methanol in extracting anthocyanins from grape skins.

1.6 Microencapsulation of bioactive compounds

If on the one hand the application of active compounds deriving from vegetable world has recently attracted great interest in the food sector to develop functional and nutraceutical foods, on the other hand the effectiveness of these substances depends on the stability, bioactivity and bioavailability of the active ingredients.

Furthermore, the unpleasant taste of most phenolic compounds also limits their application. Therefore, in the recent years the microencapsulation process and its application in food field have been widely studied. The utilization of encapsulated polyphenols, instead of free compounds, can effectively overcome problems of their instability, alleviate unpleasant tastes or flavors, as well as improve their in vivo and in vitro bioavailability (Fang and Bhandari, 2010). Microencapsulation is a technique in which a material or mixture of materials is covered by other materials or material systems. The coated material is called active or core material, while the coating material is called shell, wall material, carrier or encapsulant. This technique consists of the isolation of active substances within a micrometric-size particle. Usually, the active material is shielded from the surrounding environment by one or more layers of wall material. The structure of microparticles is generally classified into microcapsules with a single core surrounded by a layer of wall material; microspheres with the core dispersed in a continuous matrix network (Madene et al., 2006).



In the food industry, this technique can be applied for different purposes: protecting sensitive substances (flavors, antioxidants, polyunsaturated oils, vitamins, drugs, etc.) from the external medium, particularly, moisture, pH and light; masking of unpleasant taste and odor of the substances; retaining aroma in a food product during storage; guarding against

light-induced reactions and oxidation; dilution of the core material when only small amounts are required; increasing flavours shelf-life and allowing a controlled release (Madene et al., 2006; Nesterenko et al., 2013). The process for encapsulation of sensitive compounds consists of two steps: the first starts with mixing the core and wall materials to obtain a dispersion, a solution or an emulsion. The second step involves the actual production of the microcapsules by chemical or mechanical processes. Among the chemical processes are coacervation, gelation, liposome entrapment, inclusion complexation, interfacial polymerization and emulsion polymerization. Among the mechanical process we can find spray drying, spray cooling/chilling, fluidized bed, centrifugal suspension separation, lyophilization, co-crystallization and extrusion. The choice of microencapsulation technology will depend on different factors such as the physicochemical properties of core and coating; the desired physicochemical properties of the microcapsules (size, charge, yield, encapsulation efficiency); the stability, release and biological activity of the active material; and process costs. Process efficiency and product stability can be affected by the kind of wall material, ratio of the core material to wall material, encapsulation method and storage conditions. Wall material particularly affects the stability of microparticles and the degree of protection of the active core (Fang and Bhandari, 2010; Madene et al., 2006; Nesterenko et al., 2013). Materials commonly used as microcapsule coating for food applications are biomaterials such as carbohydrates, fats, waxes, and animal- and plant-derived proteins. Regarding carbohydrates, polysaccharides are chosen as coating components due to their good solubility in water and barrier properties. Some of these components are maltodextrin, starch, gum arabic, chitosan, alginates and inulin. Proteins from animal sources (whey proteins, gelatin, casein) and from vegetables (soy proteins, pea proteins, cereal proteins) are also widely used for encapsulation of active substances. These natural polymers offer several advantages: biocompatibility, biodegradability, good amphiphilic and functional properties such as water solubility, and emulsifying and foaming capacity. Lipophilic substances such as glycerides, oils, phospholipids, carotenoids, and waxes are also used as carrier materials. They permit the creation of barriers against moisture and allow for the carrying of hydrophobic substances in aqueous media (Nesterenko et al., 2013). Encapsulation technology is now well developed and accepted within the pharmaceutical, chemical, cosmetic and foods industries. In food products, fats and oils, aroma compounds and oleoresins, vitamins, minerals, colorants and enzymes have been encapsulated.

1.7 By-products for food enrichment

According to dietary guidelines fruit and vegetables are critical for a correct and healthy life style due to their valuable chemical composition. In fact, it is well known they are able to reduce risk of several chronic diseases because containing significant amounts of functional compounds as carotenoids, dietary fibres, polyphenols, tocopherols, vitamins and other substances. Recently, their consumption has increased due to the consumers' major awareness of diet related health problems, therefore they require natural and nutritious ingredients and foods with health-promoting properties. The food industry uses widely fruit and vegetables for the beverage and fruit-based foods manufacture generating a large amount of residue, which is frequently discarded, representing a serious problem for the environment. Classically, the outer layers and extremities of fruits and vegetables are removed during processing, mainly by peeling and pressing; they comprise essentially stalks, peels, seeds and crashed pulp which still contain large amounts of bioactive molecules and biopolymers, resulting in a considerable nutritional loss (Ferreira et al., 2015a). However, owed to their rich composition characterized by compounds with antimicrobial, antioxidant, anti-proliferative or anti-inflammatory effects, fruit and vegetable by-products could be used in other food processing process in order to minimize food waste. The applications of these by-products in foods can be divided in two categories: those with technical purposes, which include the improvement of shelf life, safety, stability, sensory quality, etc.; and those with biological purposes, which aim to enhance health-promoting effects for their conversion into functional foods (Iriondo-DeHond et al., 2018). Plant-based residues represent novel, natural and economic sources of flavoring, colorants, protein, dietary fiber, antimicrobials and antioxidants, which can be used in the food industry as natural additives with high nutritional value (Gowe, 2015). Over the past two decades, the literature has discussed the problem of waste generated by the food sector and the possibility of reuse them not only for animal feed or organic fertilization but also as raw material for novel food products. Their incorporation into foods eaten daily allows to increase the consumption of beneficial substances and then to reduce the risk of chronic diseases, and at the same time to reduce the environmental pollution problem. Food researchers have tried to exploit different kinds of vegetable origin by-products to enrich common foods, so improving their nutritional properties. Among the industrial interest substances, one of the main important compounds is represented by dietary fibre able to reduce cholesterol, diabetes, coronary heart disease and

ease constipation. Antioxidants are other great interest compounds for food sector because are able to inhibit or delay the oxidation reactions and furthermore these allow to substitute synthetic antioxidants. Depending on plant species, variety and tissue, high levels of health protecting antioxidants, such as vitamin C and E, phenolic compounds including phenyl-propanoids, flavonoids and carotenoids (lycopene) can be found. Phenols obtained by food wastes can serve not only as antioxidant agents but also as potential natural antimicrobial substances; several works report the estimation of their antimicrobial activity by *in vitro* tests. Many reports in the literature have also investigated the nutritional properties of bioactive compounds extracted from different parts of fruit and vegetable by-products. These new trends concerning the use of fruit and vegetables wastes as by-products for production of food additives or supplements with high nutritional value have gained increasing interest because these are high value products and their recovery may be economically attractive (Gowe, 2015). The main applications of these by-products to cereal-based food, meat products, fish and dairy products are listed below.

1.7.1 Cereal based-products

Cereal products, such as pasta, bread and bakery products, have an important role in human nutrition. Generally, these are considered to be a good source of energy for the human body and represent staple foods of the population, widely consumed worldwide. Therefore they are seen as the best vehicle for fortification with bioactive compounds in order to develop pro-health products. Since bread made with white flour is a food with a low antioxidant capacity, many scientists focused on enrichment of the wheat bread with natural raw materials rich in phenolic antioxidants such as seeds, spices, green parts of plants, fruit or vegetable by-products from the food industry, these raw materials are often cheap and a very good source of antioxidants, especially phenolic acids (Dziki et al., 2014). The aim of the study presented by Gawlik-Dziki et al. (2013) was to investigate the effect of adding ground onion skin (OS) on the antioxidant properties and sensory value of bread. OS has been shown to be an excellent source of quercetin and its derivatives. The antioxidant potential of bread with OS was significantly higher than the activity noted in the control. The 2-3% OS addition into wheat flour caused a significant improvement of bread antioxidant abilities, but further increases in the OS supplement did not increase the activity of bread. Sensory evaluation showed that the replacement of wheat flour in bread with up to 3% onion powder also gave satisfactory consumer acceptability. El-Megeid et

al. (2009) studied the potential use of ground dried green tea leaves (TL) for bread enrichment and to evaluate the sensory characteristics and the nutritive value of bread. The results showed that the best level of fortification which had the highest scores was recorded for 2% TL enriched bread followed by 4% TL-fortified bread, while bread with 6% TL had the lowest scores, and so was excluded from the biological study. They also proved that TL polyphenol is effective against renal failure in rats. Based on the evidence available, it appeared that green tea-fortified bread was beneficial to renal function and eliminated oxidative stress by virtue of its antioxidant properties. Grape seeds are rich in phenols (mainly flavonols and proanthocyanidins or condensed tannins) endowing them with a high antioxidant capacity, thus Peng et al. (2010) studied the antioxidant activity change in breads with added grape seed extract (GSE). The results showed that bread with the addition of GSE had stronger antioxidant activity than that of control bread, and increasing the level of grape seed extract addition further enhanced their antioxidant capacity. Meanwhile, except for an acceptable colour change, adding GSE to bread had only a slight effect on the quality attributes. The wine-making by-products were also used in the work of Sant'Anna et al. (2014) in which the incorporation of 25, 50 and 75 g/kg of grape marc powder in fettuccini pasta preparation was evaluated over its cooking, nutraceutical and sensory properties. The results show that the incorporation of the dried by-product did not interfere in the water absorption and in the solid loss of the pasta after cooking. The addition of grape marc powder increased the total phenols, condensed tannins, monomeric anthocyanin and antioxidant capacity concentration in the cooked pasta due to the incorporation of polyphenols stemmed from grape. Sensory analysis showed that the incorporation of grape marc powder reduced the acceptance of aroma, aftertaste, flavor and appearance, regardless of the concentration of the dried residue added. The incorporation of 25 g/kg of grape marc powder presented the best overall acceptance. In the study performed by Boubaker et al. (2016), artichoke stem powder (ASP) was used to substitute 2.5%, 5%, 7.5% and 10% of wheat flour for making breads. Bread qualities and total phenols content were analyzed and compared with those of wheat bread. Results show that ASP addition considerably modified the bread quality: altered appearance and texture, darker crumb and more intense odor were observed. From the sensory evaluation, tastes of bread with higher content of ASP (7.5 and 10%) were the most acceptable for assessors. Total phenol contents of breads significantly increased with the addition of ASP. Therefore, ASP may be considered as valuable ingredients for industrial manufacture of functional foods. Also biscuits represent a potential candidate for the addition of functional

ingredients. Pasqualone et al. (2014) characterize the physico-chemical, sensory and volatile profile of functional biscuits enriched with grape marc extract. The enriched biscuits showed higher phenolic content and antioxidant activity than control sample. Moreover, they had higher sensory scores for color, fruity odor, and sour taste, coupled with lower friability. In the study proposed by Turksoy et al. (2011) the enrichment of cookie with pumpkin and carrot pomace powders was investigated. Mildner-Szkudlarz et al. (2011) reported that the white grape pomace (WGP) might be utilised for the novel formulation of biscuits as an alternative source of dietary fibre and phenols. The incorporation of 10% WGP caused an approximately 88% increase in total dietary fibre content as compared with the control, it increased the content of phenolic compounds and greatly enhanced the antioxidant properties of biscuits. Apple pomace (AP) is an interesting source of pectins and fibres and can be considered as a raw material for direct preparation of dietary fibre. The addition of hydrated apple powder (HAP) in the cookie formulation significantly increased the rheological properties but reduced physical properties of cookies. The cookies with 5% HAP showed acceptable physical, textural and sensory properties. Moreover, it was reported that the addition of apple pomace avoids the use of any other flavouring ingredients because has a pleasant fruity flavour (Lauková et al., 2016). Apple juice industry by-product, in particular apple peel powder (APP), was also used as substitute for the durum semolina at 10% and 15% during pasta making. Results showed that addition of APP increased cooking loss and amount of absorbed water of pasta samples and decreased sensory attributes (hardness and adhesiveness). However, the addition of apple by-products at 15% significantly increased total polyphenol content and antioxidant capacity, especially when polyphenols were extracted with ultrasound assisted extraction and methanol (Lončarić et al., 2014).

1.7.2 Meat-based products

Nowadays meat-based foods are considered unhealthy because characterized by high saturated fats amount and a low antioxidants level, thus many authors have tried to improve their nutritional aspect exploiting different fruit and vegetable by-products from industrial processing. Many studies published in literature focused on application of tomato industry by-products (seeds and peels) as natural ingredients in meat products. Calvo et al. (2008) used the tomato peels as source of lycopene in dry fermented sausages manufacture. The peel, after being dried and reduced in flour, was added to the meat mixture at several

concentrations (0%, 0.6%, 0.9% and 1.2%, w/w) to obtain a new product enriched in lycopene. They studied the effects of direct addition of dry tomato peel on the textural and sensory properties of sausages and assessed the changes in lycopene concentration during ripening. A slight loss of lycopene was detected after 21 days ripening, however, levels remained between 0.26 and 0.58 mg of lycopene/100 g of sausage. The sensory and textural properties and overall acceptability of all sausages were good, indicating that tomato peel could be added to dry fermented sausages to produce a meat product with healthy properties. Also, the same group of researchers (García et al., 2009) evaluated the direct addition of dry tomato peel (DTP) to raw and cooked hamburgers at different amounts (0–6.0% w/w) and the effects on the physico–chemical and sensorial characteristics of the new product were studied. Addition of DTP increased the colour parameters and modified all textural properties probably because of the presence of fibre, while the hardness values of cooked samples was significantly higher in the sample containing 6% DTP than control. The lycopene concentration of hamburgers manufactured with 4.5% (w/w) DTP contains approximately 4.9 mg of this carotene per 100 g of product; this amount is close to the daily intake of lycopene recommended as healthier. In the work of Savadkoohi et al. (2014), different levels of bleached tomato pomace (1, 3, 5 and 7 kg/100 kg farce) were applied in beef frankfurter, beef ham and meat-free sausage to investigate influence on the textural properties, sensory attributes and colour changes. Results highlighted that the texture and colour parameters of test sausages were not significantly different as compared to commercial sausages or sometimes even better. The tomato pomace-added sausages had higher water holding capacity compared to commercial samples. Instead, in the study proposed by Özvural and Vural (2011), grape seed flour (GSF) obtained from wine by-products was incorporated into frankfurters at various concentrations up to 5%. The addition of this flour in the frankfurters led to a decline in the oxidation level and enhanced the protein, total dietary fibre content and water holding capacity. Meanwhile, Rodríguez-Carpena et al. (2011) determined the effectiveness of peel and seed extracts from two avocado varieties as inhibitors of lipid and protein oxidation and colour deterioration of raw porcine patties during chilled storage. Results highlighted that avocado by-products extracts inhibited discoloration, oxidative reactions and protein carbonyls formation of treated patties than the controls. Das et al. (2016) tested antioxidant efficacy of litchi (*Litchi chinensis* Sonn.) pericarp extract in cooked sheep meat nuggets during 12 days of refrigerated storage. The extract at 1.5% significantly increased the phenolic content and it was effective in inhibiting the lipid

peroxidation of cooked nuggets similar to synthetic antioxidant at 100 ppm. As reported by Selani et al. (2016), pineapple by-products (peel and pomace) and canola oil were used in low-fat beef burger to develop healthier meat products. In particular, the burger formulation with 10% fat, 1.5% of pineapple by-products and 5% of canola oil improved oxidative stability, cholesterol content and fatty acid profile. Kanatt et al. (2005) used potato peel waste as a source of natural antioxidants and test its effectiveness in reducing lipid peroxidation of radiation-processed meat. Radiation processing is one of the most effective technologies for sterilization in raw meat and meat products. However, meat on irradiation may undergo oxidative changes that influence the sensory quality of meat. Potato peel extracts retard lipid peroxidation of radiation-processed meat without affecting its flavor. Riazi et al. (2016a; 2016b) described the effect of dry red grape pomace (DRGP - 1 and 2% w/w) as a nitrite substitute on the microbiological and physicochemical properties of beef sausages. The authors confirmed that the addition of grape pomace (1%, w/w) in combination of reduced nitrite levels to the beef sausage samples decreased TBARS content and lipid oxidation. Moreover, it inhibited the microbial growth during 30 days of storage and increased the shelf-life of the sausages as compared to the samples treated with nitrite and without nitrite. Olive waste extract (100, 200 or 400 mg gallic acid equivalents/kg muscle), was applied by Muíño et al. (2017) to increase the shelf-life of lamb meat patties enriched with omega-3 fatty acids. Extract application reduced lipid and protein oxidation while maintaining an acceptable colour for a longer period.

1.7.3 Fish-based products

Spinelli et al. (2016a) developed a new enriched product, as fish-burger, through direct addition of microencapsulated extract of brewers's spent grain, beer production by-product. Extraction by supercritical fluids and then microencapsulation of the extract was carried out, to mask its unpleasant and bitter taste. The authors evaluated the nutritional aspect of the fish-burger and the results showed that fortified fish-burgers were richer in polyphenols and flavonoids and therefore they also had a better antioxidant activity than the control sample. In another study, Spinelli et al. (2017) microencapsulated orange epicarp extract by spray-drying to enrich fish burger. The microencapsulation enhanced the bioaccessibility of bioactive compounds and the fish burger enriched with 50g/kg remained acceptable from the sensory point of view and showed high concentration of β-carotene, polyphenol and flavonoid content. Oil industry produces large volume of solid waste, in

particular dry olive paste which was used in the study proposed by Cedola et al. (2017) to enrich fish burger and thus enhance its quality characteristics. The addition of olive by-products led to an increase of the phenolic and flavonoid contents and the antioxidant activity; however, it also provoked a deterioration of sensory quality. Therefore, in order to balance quality and sensory characteristics of fish burgers, a pre-treatment of dry olive paste by hydration/extraction with milk was necessary to perform. In fact, this significantly improved the burger sensory quality by reducing the concentration of bitter components. In the study proposed by Özalp Özen et al. (2011) the effects of pomegranate seed extract (PSE) and grape seed extract (GSE) addition to chub mackerel minced muscle on lipid oxidation during frozen storage were determined. The results suggested that the GSE is a very effective inhibitor of primary and secondary oxidation products in minced fish muscle and has a potential as natural antioxidant to control lipid oxidation during frozen storage of fatty fish. The antioxidant properties of grape seed extract (GSE) were also confirmed by Shi et al. (2014). The authors reported that the GSE addition to silver carp fillets retarded the increase of peroxide value and thiobarbituric acid value and extended by 3 days the sensory shelf-life of fillets compared to the control.

1.7.4 Dairy products

Many studies have been carried out on the application of fruit and vegetable by-products rich in natural antioxidants in dairy foods manufacturing to improve nutritional and therapeutic properties. Since fermented milk products are among highly-consumed food in the world, yogurt, one of the well-known fermented dairy products, has been mainly used as vehicle of beneficial substances deriving from plant material to increase their intake into human diet. Despite its nutritional characteristics (excellent source of protein, calcium, phosphorus, riboflavin, thiamin, vitamin B12, folate, niacin, magnesium and zinc) and importance in human diet, yogurt is not being considered as a major source of phenolic compounds. Hence, many researchers used fruit and vegetable by-products as natural additives to increase the phenolic antioxidants level in the yogurt (Alenisan et al., 2017; Gahruie et al., 2015). Different bioactive compounds recovered from food by-products have been used as an alternative to conventional antioxidants to prevent lipid oxidation of dairy foods and increase their shelf life. These efforts have been made especially in high-fat content dairy foods, such as cheese and butter, but also in yogurts (Iriondo-DeHond et al., 2018). Antioxidants from tomato processing by-products were used as agents against

lipid peroxidation in traditional Tunisian butter, showing a protective action during cold storage, extending its shelf-life up to two months(Abid et al., 2017). Winemaking by-products have been widely used as the main source of polyphenols. As reported by Marchiani et al. (2016) grape pomace powder (GPP) can be used as functional ingredient to increase total phenolic content (TPC), radical scavenging activity (RSA) of semi-hard and hard cheeses. Powders obtained from three grape pomaces (Barbera, Chardonnay before distillation, Chardonnay after distillation) were added at two concentration levels (0.8 and 1.6 % w/w) into semi-hard and hard cheeses. Cheeses fortified with Chardonnay after distillation powder showed at the end of ripening the highest TPC and RSA values. Wine pomace flour has been directly added to the yogurt formulation. Tseng and Zhao (2013) demonstrated that dried grape pomace added at 1% (w/w) could be used as an alternative source of antioxidants and dietary fibre to increase not only fibre and total phenolic contents but also to extend product shelf-life delaying the oxidation of lipids during refrigerated storage of yogurt. Unfortunately, the total phenolic content and radical scavenging activity of fortified yogurts decreased slightly during storage. Chouchouli et al. (2013) fortified yoghurt with grape seed extracts. The addition of the extract did not affect the pH or *Lactobacilli* count, also fortified yogurt contained more polyphenols and exhibited higher antiradical and antioxidant activity than the control, even after 3-4 weeks of cold storage. Instead, El-Said et al. (2014) used pomegranate peel extracts (PPE) to develop a fortified yogurt and determined the antioxidant activities, total phenolic content and total flavonoids content of yogurt fortified with 5%, 10%, 15%, 20%, 25%, 30% and 35% of the PPE, before and after inoculation with the traditional yogurt starter. The fortified yogurt had a higher antioxidant activity before inoculation than after inoculation with the starter. Also, increasing the percentage of the added PPE increased significantly the antioxidant activities of yogurt up to 25% and further increase in the percentage of added PPE led no significant effect. Addition of PPE had no significant effects on the sensory attributes (appearance, texture and flavor) compared to the control sample. Grape pomace extract (GPE) has been also used as source of antioxidants to make ice cream with good nutritional and functional properties (Salem et al., 2014). The authors found that GPE exhibited high free radical scavenging activity and significantly increased total anthocyanin and total phenolic content of the enriched ice cream. Meanwhile, Çam et al. (2013) proposed incorporation of pomegranate peel (PP) at the levels of 0.1% and 0.4% (w/w) and pomegranate seed oil (PSO) at the levels of 2.0% and 4.0% (w/w) into ice cream to increase the functional properties. Data indicated that the incorporation of PP (0.4%)

increased phenolic content including antioxidant and antidiabetic activities; the PSO (2%) into the ice cream improved the content of conjugated fatty acids. Several studies have shown that food by-products can be used against spoilage and pathogenic bacteria without interfering with the viability of starter cultures and other microorganisms involved in fermentation processes, ensuring that the quality of the developed products is maintained. The antimicrobial and antimycotic *in vitro* properties of extracts recovered from food by-products have been analysed. The antimicrobial action against foodborne pathogens has been associated with the polyphenols of plant based by-products, which may penetrate the cell wall causing membrane disruption, damage of membrane proteins and enzymes, and structural changes that lead to bacterial death (Iriondo-DeHond et al., 2018). Pomegranate peel and grape seed extracts proved to be effective natural preservatives against *Listeria monocytogenes, Staphylococcus aureus* and *Salmonella enterica* in cheese. Pathogen counts in cheese significantly decreased with the by-product extract addition (Shan et al., 2011).

In some scientific studies industrial food by-products were used as texturizing agents. Dietary fiber from orange by-products was used to maintain the texture of lemon ice-cream when reducing its fat content by 50% (Crizel et al., 2014) and as fat replacers in low-fat yogurt (Yi et al., 2014). As reported by Issar et al. (2017) apple pomace fibre (2.5, 5, 7.5 and 10%) was used in preparation of acidophilus yoghurt and sensory quality, pH, acidity, total solids and fibre contents were evaluated. With the increase in fibre concentration decreased acidity and fat contents and results showed that yoghurt containing 5% apple fiber was judged as the best and hence optimized for preparation of fiber-enriched acidophilus yoghurt. Sah et al. (2016) studied the influence of adding pineapple peel powder (PPP) as a fibre source on physicochemical, textural, rheological, and microstructural attributes of probiotic yogurt during refrigerated storage for 28 days. A gel structure with large pores and reduced cross-linking between casein micelles in yogurts was observed with 1% of pineapple peel powders, which was associated with lower yogurt firmness and weak rheological properties due to the incompatibility between milk proteins and polysaccharides from the pineapple peel powders.

The research data shown above highlight the high potential of valorising food by-products for the development of innovative and healthy foods. By-products used as sustainable ingredients or sources of bioactive compounds have been shown to be effective in a wide range of technological and nutritional purposes. Food enrichment seems to be a cheap way and at the same time sustainable for improving product quality. The addition of industrial

residues to common foods represents an environmental friendly way to manage industrial waste. In addition, it would allow fulfilling the requirements of consumers concerned about chemical residues in their foods that look for healthy foods with natural ingredients. However, there are some important factors that limit its effectiveness, because the incorporation of vegetable material affects the sensory aspect. A compromise between nutritional value and sensory quality need to be generally achieved.

2. Objective

This PhD thesis has been focused on the development of new food products enriched with beneficial substances deriving from industrial processing, in particular from fruit and vegetables by-products. An optimization process of the formulation was performed using different technological devices in order to obtain the sensory quality at an acceptable level adding the maximum possible concentration of by-products. In particular, bread was fortified with beneficial substances from artichokes by-products. A watermelon-based jelly candy, without generating waste, was realized. The optimization of candy formulation in terms of watermelon rind, pulp and juice was first carried out, then fortification with orange by-products were made. Durum wheat spaghetti enriched with red grape marc were also optimized, being this by-product rich in fiber and phenolic antioxidants. Furthermore, bioaccessibility of polyphenols, glucose, as well as the antioxidant activity of nutrients in the bioaccessible fraction were also assessed. In addition, the comparison between different extraction techniques applied to broccoli by-products were carried out. The extraction methods were compared in terms of extraction efficiency, total phenolic compounds and total flavonoid compounds contents, and antioxidant activities. In addition, a spray-drying process of broccoli by-product extract has been optimized to protect the bioactive compounds from the environment prior to any other potential application. Fish-burgers were chosen as model system for the addition of fortified microcapsules.

3. Materials and Methods

3.1 Case study: Wheat Bread Enriched with Artichoke By-Products

3.1.1 Raw materials

Artichoke by-products consisting of outer leaves were provided by Farris s.r.l., a local company of Foggia (Italy). The samples were dried at 35 °C in a dryer (SG600, Namad, Rome, Italy) for 48 hours. The dried leaves were reduced in a fine powder by a hammer mill and then stored at 4 °C until further utilization.

Commercial soft wheat flour, provided by Agostini Mill (Montefiore dell'Aso, Ascoli Piceno, Italy), was used in this study for bread manufacture. Fresh compressed yeast, salt, sodium bicarbonate, cream of tartar and extra virgin olive oil were bought from a local market.

3.1.2 Chemicals

Folin-Ciocalteu reagent, gallic acid monohydrate, methanol, hydrochloric acid, ethanol, ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt), potassium persulfate ($K_2S_2O_8$), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), aluminium chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), sodium hydroxide solution (NaOH), quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were supplied from Sigma-Aldrich (Milan, Italy). Anhydrous sodium carbonate (Na_2CO_3) was supplied from Carlo Erba (Milan, Italy). For the preparation of the phosphate buffered saline (PBS), the following salts were used: sodium phosphate dibasic heptahydrate ($HNa_2O_4P{\cdot}7H_2O$) and sodium phosphate monobasic monohydrate ($H_2NaO_4P{\cdot}H_2O$). These were purchased from Sigma-Aldrich (Milan, Italy). All reagents were of analytical grade.

3.1.3 Bread making process

Bread making process was performed as described by Mastromatteo et al. (2014). Dough mixing, processing and baking were performed on laboratory-scale equipment. Bread containing only soft wheat flour, yeast, sugar, salt and water (Table 1) was used as reference sample for sensory analysis (CTRL_br). Instead, artichoke leaf flour (ALF) percentage, structuring agent, leavening agent, oil and the optimum amount of water were

taken into account in order to optimize the ALF-rich bread formulation. After different trials, the bread was optimized and its composition is shown in Table 1. For chemical determinations, a different reference bread was used, containing all ingredients of optimized enriched bread but without ALF (0ALF/CMC_br). All the bread samples investigated are listed in Table 1. The enriched bread samples are labeled as following "XALF_br", where X refers to ALF concentration.

Table 1 Formula of the bread samples.

	CTRL_br	**0ALF/CMC_br**	**15ALF/CMC_br**
soft wheat flour (%)	60.2	54.5	39.5
ALF (%)	-	-	15
yeast (%)	1.81	1.4	1.4
sugar (%)	0.6	0.5	0.5
salt (%)	1.2	0.9	0.9
CMC (%)	-	2	2
sodium bicarbonate (%)	-	0.5	0.5
cream of tartar (%)	-	1.1	1.1
oil (%)	-	2.3	2.3
water (%)	36.2	37	37

CTRL_br: control sample for sensorial analysis; 0ALF/CMC_br: control sample for chemical analysis; 15ALF/CMC_br: bread made from artichoke leaf flour (15%, w/w) and carboxymethylcellulose (CMC) at 2% (w/w).

The mixing phase was divided in two steps: at the beginning ALF, water, a part of wheat flour and yeast were mixed for 5 min at low speed; then the other ingredients were added and mixed for 20 min at high speed. The CTRL and ALF-rich doughs required a different leavening time: 60 and 90 min, respectively, and they were left to rest in a leavening chamber (Thermogel, Varese, Italy) at controlled temperature (30 °C) and humidity (85%). Finally, the doughs were baked for 15 at 230 °C and for 35min at 200 °C in a preheated electric oven (Europa Forni, Vicenza, Italy). Samples were left to cool at ambient

temperature for about 3 h, subsequently were cut into slices and subjected to following analysis. The bread making process was performed in triplicate.

3.1.4 Sensory analysis

The sensory analysis was performed according to Mastromatteo et al. (2015). After cooling, the bread samples were submitted to a panel of 10 trained tasters in order to evaluate the sensory attributes. The panellists were selected on the basis of their sensory skills (ability to accurately determine and communicate the sensory attributes as appearance, odour, flavour and texture of a product). The panellists were also trained in sensory vocabulary and identification of particular attributes by evaluating durum wheat commercial bread. Loaf samples were sliced with an electric slicing knife (thickness of 15 mm) (Atlantic; Calenzano, Firenze, Italy) without removing the crust and each sample was placed on white plates. The bread samples were evaluated for acceptance of attributes such as color, odor, taste, crust and crumb firmness, large, bubbles and overall quality using a 9-point scale, where 1 corresponded to extremely unpleasant, 9 to extremely pleasant and 5 to satisfactory.

3.1.5 Extraction of bioactive compounds

In order to determine total phenol compounds, flavonoids and antioxidant activity of the artichoke leaf flour and bread samples, the bioactive compounds extraction was necessary to perform with acidified methanol (80% MeOH acidified with 1% HCl) as described by Biney and Beta (2014). Before extraction, the bread was sliced, dried at 35 °C and grounded. An amount of 2 g of sample was mixed with 20mL of extraction solvent. The mixtures were included in 50mL centrifuge tubes and shaken at room temperature in darkness for 2 h at 300 rpm using an orbital shaker (HS 260 BASIC, IKA, Staufen, Germany). Next, the samples were centrifuged at 5°C for 15 minutes at 10000 rpm (5804R, Eppendorf, Milan, Italy) and supernatant was collected and filtered (PTFE, 0.45 μm, Teknokroma Sant Cugat del Vallés, Barcelona, Spain) prior to the analytical determinations. For each sample, the extraction was carried out in triplicate and the obtained extracts were used for the following chemical analyses.

3.1.6 Chemical analysis

3.1.6.1 Determination of Total Polyphenol Compounds

Total polyphenol compounds (TPC), expressed as mg of gallic acid equivalents (GAEs)/g dry weight (dw), were determined by UV–VIS spectrophotometry according to the Folin-Ciocalteu method. This method is based on the oxidability of phenols at basic pH, while the Folin–Ciocalteu reagent works as an oxidant agent. The total phenolic compounds were determined as described by Spinelli et al. (2015). Briefly, 0.5 mL of properly diluted extract and 2.5 mL of Folin–Ciocalteu reagent diluted in water in a 1:10 ratio were mixed and left to rest for 5 min. An amount of 2 mL of Na_2CO_3 (4 g/100 mL) was then added and the mixture was allowed to rest again for 2 h in darkness. The absorbance was then read using a spectrophotometer (UV1800, Shimadzu Italia s.r.l, Milan) at 740 nm and before reading, the samples were filtered by means of 0.45 μm PTFE filters. Total phenolic compounds were quantified by a calibration curve previously built (3–200mg/L; R^2 = 0.9989). For each sample, the analysis was carried out in triplicate.

3.1.6.2 Determination of Total Flavonoid Compounds

The total flavonoid content (TFC) in both ALF and bread extracts was evaluated by aluminum chloride colorimetric method, according to Spinelli et al. (2016b) with slight modifications. Extracts (0.5mL), prepared as previously described, were mixed with 2mL of distilled water and 150 μL of a 5% sodium nitrite ($NaNO_2$) solution. After 6 minutes, 150 μL of a 10% aluminum chloride ($AlCl_3$) solution was added and the mixture was allowed to stand for 6 minutes. Finally, 1mL of 1M sodium hydroxide (NaOH) and 1.2 mL of distilled water were added to the mixture and filtered through a 0.45 µm Nylon syringe filter (Teknokroma Sant Cugat del Vallés, Barcelona, Spain). For each sample the absorbance was read against blank at 415 nm using a spectrophotometer (UV1800, Shimadzu Italia s.r.l, Milan). The calibration curve was prepared using quercetin as standard in the range 6.25–400 mg/L (R^2 = 0.9994) and the total amount of flavonoids was expressed in mg of quercetin equivalents (QEs) per gram of dried sample (dw). The analysis was carried out in triplicate for each sample.

3.1.6.3 Determination of Antioxidant Activity

The antioxidant activity of ALF and bread samples was assessed using two methods: ABTS and DPPH assays. In both cases, a calibration curve was built and the antioxidant

activity was expressed as mg Trolox equivalents (TEs) for gram of dried weight (dw). The determinations were carried out in triplicate for each sample.

ABTS assay. This test is based on the ability of antioxidants to interact with the radical cation $ABTS^{\bullet+}$ (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) inhibiting its absorption at 734 nm, according to the method of Re et al. (1999). 7mM ABTS stock solution and 140mM potassium persulfate were utilized. The ABTS radical cation ($ABTS^{\bullet+}$) was obtained by reacting ABTS stock solution with 2.45mMpotassiumpersulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h. The $ABTS^{\bullet+}$ solution was diluted with 5mM phosphate buffered saline (PBS) at pH 7.4 to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Then, 2mL of $ABTS^{\bullet+}$ was added to 20 μL of each extract. The mixture was left to react for 6 minutes at 30°C at darkness and then was measured through a UV-Vis spectrophotometer (UV1800, Shimadzu Italia s.r.l, Milan) at 734 nm. A calibration curve was previously built using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as standard, at concentrations between 0.90 and 150 mg/L (R^2 = 0,9963).

DPPH assay. This method is based on using of a stable free radical, as 2,2'-Difenil-1-picrilidrazile (DPPH). By reacting DPPH with an antioxidant compound capable of yielding a hydrogen atom to the radical compound, there is a discoloration of the solution due to the disappearance of the radical, which can be monitored over time by spectrophotometry at the wave length of maximum absorption. It was carried out according to the procedure described by Mensor et al. (2001) with slightly modification. Briefly, 1.25mL of each sample was mixed with 0.5mL of 0.3mM DPPH in ethanol. The mixture was left to react for 30 minutes at room temperature and at darkness. Then, the absorbance was measured at 518 nm by means of a spectrophotometer (UV1800, Shimadzu Italia s.r.l, Milan). Ethanol (0.5 mL) plus sample (1.25 mL) was used as blank. DPPH solution (0.5 mL; 0.3 mM) plus ethanol (1.25 mL) was used as control. The percentage antioxidant activity (AA) was calculated using the following formula:

$$\% \text{ AA} = 100-[((A_{sample} - A_{blank})*100) / A_{control}]$$

The antioxidant activity was expressed as mg Trolox equivalents (TEs) for gram of dried sample using a calibration curve built between 0.6 and 20 mg/L (R^2=0.9916).

3.1.7 Glycaemic index of bread

In vitro digestion was carried out as described by Chillo et al. (2011) with slight modifications. Briefly, each bread sample (5 g), in particular 0ALF/CMC_br and 15ALF/CMC_br, was tipped into a digestion vessel with 50 mL of distilled water and 5 mL maleate buffer (0.2 M, pH 6.0, containing 0.15 g $CaCl_2$ and 0.1 g sodium azide per litre) in a water bath at 37 °C and allowed to equilibrate for 15 min. Digestion was started by adding 0.1 mL amyloglucosidase (A 7095; Sigma-Aldrich, Milan, Italy) and 1 mL of 2 g per 100 g pancreatin (P7545; Sigma-Aldrich) in quick succession, and the vessel was stirred at 130 r.p.m. At 0, 20, 60 and 120 min, 0.5 mL of digested samples was removed for analysis of released glucose. After the 120-min sampling, the digests were homogenised using an Ultraturrax (Ika, Germany) to convert them into slurries. The incubation continued for 1 h, and 0.5 mL of each digested sample was removed for the analysis of released glucose.

The samples removed during digestion were added to 2.0 mL of ethanol and mixed. After 1 h, the ethanolic subsamples were centrifuged (2000 g, 2 min; Biofuge fresco HERAEUS, Germany), and an aliquot (0.05 mL) of the supernatant was removed. This aliquot was added to 0.25 mL amyloglucosidase (E-AMGDF; Megazyme International Ireland Ltd, 1 mL per 100 mL in sodium acetate buffer 0.1 M, pH 5.2) for 10 min at 20 °C. 0.75 mL DNS solution (10 g 3,5-dinitrosalicylic acid, 16 g NaOH and 300 g Na-K tartrate (Sigma-Aldrich; made to final volume 1 L) were then added to the tubes. The tubes were heated for 15 min in boiling water and then cooled in cold water for 1 h, after which 4 mL of water (15 °C) was added. After mixing, the reducing sugar concentration was measured colorimetrically (530 nm) using a UV–vis spectrophotometer (UV1800, Shimadzu Italia s.r.l, Milan). Glucose standards of 10.0 mg/ ml were used. The results were then plotted as glucose equivalent (GE) (mg) per g of sample vs. time (Mastromatteo et al., 2014).

3.1.8 Statistical analysis

All experimental data were subjected to one-way analysis of variance (ANOVA). To the aim, a Fisher's test with the option of homogeneous groups ($P<0.05$) was carried out to determinate significance differences between samples. STATISTICA 7.1 for Windows (StatSoft Italia s.r.l.) was used.

3.2 Case study: Watermelon-based Candy fortified with Orange By-Products

3.2.1 Raw materials

Fruits used in this study and all ingredients to make jelly candies were obtained from a local market (Ipercoop, Foggia, Italy). Watermelons (*Cucurbita lanatus* cv. *Crimson*) and oranges (*Citrus sinensis* cv. *Navel*) were thoroughly washed in tap water to remove residuals, dipped for 1 min in chlorinated water (20 mL L^{-1}) and rinsed. After that, the watermelon peel was manually removed using a home knife and then cut into small pieces, while the watermelon pulp was placed in a fruit extractor (Delonghi, Italy) to produce the juice, essential component of candies. The obtained juice was stored at -20 °C until to be used, instead the watermelon rind and exhaust pulp were dried at 38 °C for 48 h using a vacuum stove and then ground in a laboratory blender to fine powder. The powders thus obtained were sieved to uniform the granulometry, immediately vacuum packed and stored at 4 °C until use. Oranges were peeled to obtain flavedo (the orange peripheral surface) and albedo (the white part inside of peel). Flavedo and albedo were reduced into smaller pieces, dried and milled finely with the same process used for watermelon. Lastly the orange by-products powders were vacuum packed and stored at 4°C. Other purchased ingredients were: lemons, which were washed with tap water and squeezed with a home juicer; sugar and gelatine foils.

3.2.2 Chemicals

Folin-Ciocalteu reagent, anhydrous sodium carbonate (Na_2CO_3), gallic acid monohydrate, methanol, hydrochloric acid, aluminium chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), sodium hydroxide solution (NaOH), quercetin, sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$), glacial acetic acid (CH_3COOH), 2,4,6-Tripyridyl-s-Triazine (TPTZ), ferric chloride ($FeCl_3$), ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H2O$), the ingredients for HBSS (potassium chloride, sodium chloride, disodium hydrogen phosphate di-hydrate, di-potassium hydrogen phosphate, sodium hydrogen carbonate, and calcium chloride) and the enzymes for *in vitro* digestion (porcine pepsin, porcine bile acid, pancreatin, alpha-amylase from *Bacillus* sp.), were supplied from Sigma-Aldrich (Milan, Italy). Amylo-glucosidase was purchased from Megazyme (Wicklow, Ireland). All reagents were of analytical grade.

3.2.3 Jelly candy formulation

Jelly candy was produced exploiting all parts of the watermelon fruit. It was prepared by mixing watermelon juice (125g), freshly squeezed lemon juice (25g) and sugar (50g). The mixture was boiled to dissolve completely the sugar and cooled to approximately 40 °C. At meantime, 6 g of a commercial jellying agent (Cameo S.p.A., Italy) was been soaked in cold water for 10 minutes and then added at cooled mixture. Finally, 2.9 g of watermelon rind flour (WRF) and 2.25 g of watermelon pulp flour (WPF) were added to the mixture. The latter was poured into a mould and left to solidify for 24 hours under refrigeration conditions; afterward, the jelly was cut in pieces into dimension of 30 mm x 20 mm x 10 mm to realize candy samples. The details of formulation (ingredients concentration %) of watermelon jelly candy (WMC) are reported in the following Table 2.

Table 2 Formula of the watermelon-based candy (WMC).

Ingredients	%
watermelon juice	59.2
lemon juice	11.8
sugar	23.7
foils gelatine	2.8
watermelon rind flour	1.4
watermelon pulp flour	1.1

To calculate the amount of watermelon by-products flours the following equations were used:

$$x = \frac{W_F}{W_J} \qquad W_F = W_J \cdot x$$

where x represents the average value deriving from the watermelon processing performed in triplicate, WF is the flour weight obtained from watermelon rind and exhaust pulp and WJ is the juice weight.

In a subsequent experimental step, in order to improve the nutritional quality of the watermelon jelly candy (WMC) orange by-products were also added to the formulation. Their optimal concentration was defined by adopting a two steps optimization method.

First different concentrations of albedo flour (1.2, 2.4, 3.6 and 4.8 %) were used to individuate the optimal concentration to be added. The candies are labelled as following: AL1.2, AL2.4, AL3.6 and AL4.8. After that, on the basis of recorded sensory results, two albedo flour concentrations (1.2 and 2.4 %) were combined with three different amounts of flavedo flour (0.6, 1.2 and 2.4 %) thus obtaining six formulations of candies: AL1.2-FL0.6, AL1.2-FL1.2, AL1.2-FL2.4, AL2.4-FL0.6, AL2.4-FL1.2, AL2.4-FL2.4. The fortified candies were prepared with the same procedure used for the watermelon jelly candy.

3.2.4 Sensory evaluation

Sensory evaluation was designed to measure the degree of liking of samples according to a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). A panel formed by 8 members of the laboratory performed sensory evaluation of jelly candies in terms of appearance, colour, odour, taste, firmness and overall quality. The panellists had at least several years of experience in sensory evaluation prior to this study; however, they were retrained for this study in a session of 2 h to be experienced in the product and terminology. They were also instructed to rinse their mouth with plain water in between tasting.

3.2.5 *In vitro* digestion of candy

In vitro digestion was performed according to the protocol described by Gille et al. (2016) with some modifications. Briefly, 0.5 g of candy sample was subjected to an in vitro digestion process that consisted of two phases: gastric and intestinal. To simulate the gastric phase, porcine pepsin (40 mg/ml of 0.1 N HCl) was added and the pH adjusted to 2.2-2.4, followed by shaking at 37 °C in a water bath for 1 h. Subsequently, porcine bile acid (12 mg/ml), pancreatin (11 mg/ml), and alpha-amylase (3.3 mg/ml) were added. The pH was adjusted to 7.2–7.6 and shaking at 37 °C in a water bath for 2 h. After digestion, the samples were centrifuged (4000 rpm x 10 min) and filtered. The filtrate was used for the analysis of the bioaccessible fraction.

3.2.6 Chemical analyses

The extraction of polyphenols from watermelon rind and pulp, flavedo and albedo, and from candy samples was based on the method described by Cappa et al. (2015) with some modifications. Briefly, acidified methanol (80% MeOH acidified with 1 % (w/w) HCl) was used as extraction solvent. An amount of 8 ml was added to 0.4 g of sample and shaken at

room temperature in darkness for 2 h at 300 rpm using orbital shaker (HS 260 BASIC, IKA, Staufen, Germany). Next, the samples were centrifuged at 5 °C for 10 min at 10000 rpm (5804R, Eppendorf, Milan, Italy) and the supernatant was stored while the pellet was re-extracted with 4 ml of solvent. The process was performed for three times. The supernatants were collected together and used for the analytical determinations. The extraction was carried out in triplicate. The evaluation of total phenols, flavonoids and antioxidant activity was performed on each part of watermelon, on orange by-products and on the experimental candy samples before and after digestion.

3.2.6.1 Determination of total phenolic compounds

The total phenols were determined according to the Folin-Ciocalteu method described by Spinelli et al. (2015). More details are shown in the paragraph 3.1.6.1. Total phenolic content (TPC) was expressed as mg of gallic acid equivalents (GAEs) per gram of sample. For each sample, the analysis was carried out in triplicate.

3.2.6.2 Determination of total flavonoids

Flavonoids were quantified by aluminium trichloride method (see section 3.1.6.2) as described bySpinelli et al. (2016b), using quercetin as standard. Total flavonoid content (TFC) was expressed as mg of quercetin equivalents (QEs) per gram of sample. The analysis was carried out in triplicate for each sample.

3.2.6.3 Determination of antioxidant activity

The ferric reducing antioxidant power assay (FRAP assay) was carried out according to the procedure described by Benzie and Strain (1996) with slightly modification. The FRAP reagent was prepared by mixing 100ml of 300 mM acetate buffer at pH=3.6 (consisting of 3.1 g of sodium acetate trihydrate and 16 mL of acetic acid glacial per liter of buffer solution), 10 ml of 10 mM TPTZ solution (0.031g TPTZ in 10 ml of 40 mM HCl dissolved at 50°C) and 10 ml of 20 mM $FeCl_3$ aqueous solution. The FRAP solution was prepared on the day of analysis and held at 37°C. An amount of 3ml of FRAP reagent was added to 200 µl of each properly diluted extract. The mixture was left to react for 30 minutes at 37°C and then the absorbance was read at 593nm. The calibration curve was obtained using as standard $FeSO_4 \cdot 7H_2O$ at concentrations from 6.25 to 800 µM (R^2 = 0.9998). The antioxidant activity was expressed as µmoli of ferrous equivalent Fe (II) per gram of sample. All tests were carried out in triplicate.

3.2.7 Quality Index

A whole quality index (WQI) that accounted for both nutritional and sensory quality was proposed as following:

$$WQI = \left(\frac{\left| NQ^F(x) - NQ^C \right|}{NQ^C} \right) \cdot \left(\frac{OSQ^F(x) - OSQ^{\min}}{OSQ^C - OSQ^{\min}} \right) \quad (1)$$

where: x is a variable related to the food formulation, it is the concentration of orange by-products; NQF(x) is the fortified food nutritional quality at a given value of x; NQC is the nutritional quality of the control sample (candy without orange by-products); OSQF(x) is the fortified food overall quality at a given value of x, OSQC is the overall quality of the control sample; OSQmin is the sensory threshold for food acceptability. Equation (1) is given by the product of two distinct terms: the former one takes into account the nutritional quality of the fortified food, it always increases with the concentration of the healthy ingredient; the latter one is related to the sensory quality of the fortified food, it always decreases with the healthy ingredient content.

3.2.8 Statistical analysis

All experimental data were subjected to one-way analysis of variance (ANOVA). To the aim, a Fischer's test with the option of homogeneous groups ($P<0.05$) was carried out to determinate significance differences between samples. Statistica 7.1 for Windows was used.

3.3 Case study: Durum Wheat Spaghetti Enriched with Red Grape Marc

3.3.1 Raw materials

Red grape marc (RGM), made up of skins, seeds and stalks, was provided by a local company of Foggia (Southern Italy, vintage 2016). The sample was dried at 30–35 °C in a dryer (SG600, Namad, Rome, Italy) for 48 hours, a fine powder (≤ 500 μm) (RGM-500) was produced by a hammer mill (16/BV-Beccaria s.r.l., Cuneo, Italy) and then stored at 4 °C until further utilization. Then, the flour was sieved by Sieve Shakers (Mod AS 300 Retsch) to obtain medium sized particles (≤ 125 μm) (RGM-125). The commercial semolina was purchased from Agostini mill (Montefiore dell'Aso, Ascoli Piceno, Italy).

3.3.2 Chemicals

Folin-Ciocalteu reagent, anhydrous sodium carbonate, hydrochloric acid, and formic acid were obtained from Merck (Darmstadt, Germany); methanol and acetonitrile were purchased from VWR international (Darmstadt, Germany); ferric chloride hexahydrate was obtained from neoLab Migge GmbH (Heidelberg, Germany). Ferrous sulfate heptahydrate, the ingredients for HBSS (potassium chloride, sodium chloride, disodium hydrogen phosphate dihydrate, dipotassium hydrogen phosphate, sodium hydrogen carbonate and calcium chloride) were obtained from Carl Roth (Karlsruhe, Germany). Gallic acid monohydrate, sodium acetate trihydrate, glacial acetic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), polyphenol standards, enzymes for in vitro digestion (porcine pepsin, porcine bile acid, pancreatin, alpha-amylase from Bacillus sp.), and glucose assay kit were supplied by Sigma Aldrich (Taufkirchen, Germany). Amyloglucosidase was purchased from Megazyme (Gernsheim, Germany).

3.3.3 Spaghetti preparation

Durum wheat semolina was mixed with water (30 % w/w) in the rotary shaft mixer (Namad, Rome, Italy) at 25°C for 20 min to uniformly distribute water. Spaghetti based only on durum wheat semolina were manufactured and used as the reference sample (CTRL). To produce enriched spaghetti, the red grape marc flour was added to durum wheat semolina at a concentration of 15 % (w/w) and with a different particle size (500μm; 125μm). Three red grape marc flour preparations were used: (i) RGM-500, (ii) RGM- 125 and (iii) RGM-125 and 0.6 % (w/w) transglutaminase (TG) to produce three spaghetti

formulations: RGM-500, RGM-125, RGM/TG, respectively. TG powder was previously dissolved in water in order to ensure its solubility. In all steps, the dough was extruded with a 60VR extruder (Namad). Subsequently, the extruded pasta was dried in a dryer (SG600; Namad). The drying process conditions applied were in accordance with Padalino et al. (2013): I step, time 20 min at 55 °C; II step, time 580 min at 75 °C; III step, time 40 min at 60 °C; IV step, time 20 min at 45 °C; V step, time 840 min at 40 °C. The pasta manufacture has been made in triplicate.

3.3.4 Sensory analysis

Dried spaghetti samples were cooked in distilled water to optimal cooking time and then they were served to the panelists. The sensory test panel consisted of ten panellists, aged between 28 and 45 years, who were trained in developing a sensory vocabulary and in identifying particular attributes to evaluate durum wheat commercial spaghetti. For the sensory evaluation they were asked to indicate color, homogeneity, and resistance to breaking of uncooked spaghetti as well as elasticity, firmness, bulkiness, adhesiveness, color, homogeneity, odor and taste of cooked samples. For the evaluation a nine-point scale, in which 1 corresponded to "extremely unpleasant", 9 to "extremely pleasant", and 5 to "threshold of acceptability" was used to quantify each attribute. On the basis of the above mentioned attributes, panelists were also asked to score the sensory overall quality of both cooked and uncooked samples, using the same nine-point scale (Padalino et al., 2013).

3.3.5 *In vitro* digestion of spaghetti

In vitro digestion was performed according to protocol described by Gille et al. (2016) with some modifications. Briefly, 0.3 g of each cooked spaghetti sample was subjected to an *in vitro* digestion process that consisted of two phases: gastric and intestinal. To simulate the gastric phase, porcine pepsin (40 mg/mL of 0.1 N HCl) was added and the pH adjusted to 2.2-2.4, followed by shaking at 37 °C in a water bath for 1 h. Subsequently, porcine bile acid (12 mg/mL), pancreatin (11 mg/mL), and alpha-amylase (3.3 mg/mL) were added. The pH was adjusted to 7.2–7.6, followed by a treatment with nitrogen gas and shaking at 37 °C in a water bath for 2 h. After digestion, the samples were centrifuged (4000 rpm x 10 min) and filtered using 0.20 μm syringe filters. The filtrate was used for the analysis of the bioaccessible fraction.

3.3.6 Extraction of bioactive compounds

The uncooked and cooked spaghetti were submitted to extraction with acidified methanol (80 % MeOH in H_2O acidified with 1 % HCl) as described by Biney and Beta (2014). The details have been previously displayed in the section 3.1.5. Before extraction, the cooked spaghetti was dried by a freeze dryer and reduced in powder. The extraction was carried out in triplicate.

3.3.7 Chemical Analyses

3.3.7.1 Determination of bioactive compounds and antioxidant activity

The evaluations of total polyphenols, total anthocyanins and antioxidant activity were performed both on the methanol extracts and on digested samples. Total polyphenol content (TPC) was expressed as mg of gallic acid equivalents (GAEs)/g dry weight (dw), and were determined by UV–VIS spectrophotometry according to the Folin- Ciocalteu method previously described (see section 3.1.6.1) (Spinelli et al., 2015). Total anthocyanin content (TAC) was evaluated according to the spectrophotometric method described by Lee et al. (2005). It was expressed as mg malvidin 3-O-glucoside (mvd-glu) per gram of dw. The antioxidant activity (μmol Fe(II)/g dry weight) was assessed by FRAP (ferric reducing antioxidant power) assay, which was carried out according to the original study of Benzie and Strain (1996).

3.3.7.2 HPLC

After *in vitro* digestion, the CTRL and RGM/ TG samples were centrifuged, filtered, and if necessary concentrated using the solid phase extraction (SPE). The samples were run on an ELITE Lachrom HPLC system equipped with a diode array detector (DAD; Hitachi L-2455; Germany). The chromatographic separation was executed with a Phenomenex Luna 3 μ, C18 (2), 150 x 4.6 mm column. The temperature of the column oven was set at 40°C. The injection volume was 60 μL and the flow rate was 0.75 mL/min. The mobile phases used consisted of 100 % acetonitrile (solution A) and water containing 0.5 % (v/v) formic acid (solution B). A gradient elution was employed as follows: at 0 min A = 7 %; at 5 min A = 12 %; at 12 min A= 17 %; at 20 min A = 27 %; at 29 min A = 100 % for 5 min. At the end of the gradient, the column was equilibrated to the initial condition (A = 7 %). Detection was carried out at three different wavelengths: 280, 360 and 520 nm. Quantitative determinations were based on the peak area of different polyphenol standards present in red grape marc: gallic acid and p-coumaric acid, quercetin, pelargonidin,

malvidin 3-O-glucoside (mvd-glu) and delphinidin. Furthermore, the mvd-glu identification was confirmed by spiking, namely by adding the standard to the sample. Unidentified polyphenols (X1, X2, X3, X4 –anthocyanins) were detected at 520 nm and recognized as anthocyanins from their UV-Vis spectrum; similar to that of malvidin 3-O-glucoside and characteristic for anthocyanins. For their quantification, the calibration curve of mvd-glu was used.

3.3.8 Determination of bioaccessible glucose

The glucose amount available for absorption in the small intestine was evaluated using the glucose assay kit (Sigma Aldrich, Taufkirchen, Germany) after gastric phase (t = 0 min) and at the end of intestinal digestion (t = 2 h). Each time 0.5 mL of CTRL and RGM samples were mixed with 1 mL ethanol, centrifuged and filtered. Then 100 µL supernatant was added to 890 µL of HBSS and 10 µL of amyloglucosidase (3260 U/mL). The solution was incubated for 1 h at 37°C before glucose determination.

3.3.9 Statistical analysis

All experimental data were subjected to one-way analysis of variance (ANOVA) using Tukey-Kramer's test to determinate significance differences between samples ($P < 0.05$). SIGMA PLOT 12.3 for Windows was used.

3.4 Case study: Fish Burger with Extract from Broccoli By-Products

3.4.1 Raw materials and chemicals

Broccoli by-products (BRC), made of stems and leaves, were supplied by a local industry located in Puglia (Farris, Foggia, Italy). They were dried at 35°C in a dryer (SG600, Namad, Rome, Italy) for 48 hours. The dried broccoli were ground by a hammer mill (16/BV-Beccaria s.r.l., Cuneo, Italy) and then stored at 4°C until further utilization. Folin-Ciocalteu reagent, anhydrous sodium carbonate (Na_2CO_3), gallic acid monohydrate, sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), quercetin, DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt), potassium persulfate ($K_2S_2O_8$), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), absolute ethanol were supplied by Sigma-Aldrich (Milan, Italy). For the preparation of the phosphate buffered saline (PBS), the following salts were used: sodium phosphate dibasic heptahydrate ($HNa_2O_4P*7H_2O$) and sodium phosphate monobasic monohydrate ($H_2NaO_4P*H_2O$). These were purchased from Sigma-Aldrich (Milan, Italy). All reagents were of analytical grade. Sapio (Monza, Italy) supplied CO_2 with purity degree of 4.5 for SFE. While N_2 was provided by Air Liquide (Milan, Italy) with purity degree of 99,9%.

3.4.2 Supercritical fluid extraction (SFE)

The extraction process was performed according to the optimal conditions described by Arnáiz et al. (2016) using the supercritical fluid extractor Speed SFE-2 (Applied Separation, Allentown, USA). It was equipped with two pumps, one pushed the supercritical CO_2 throughout the system and the other allowed adding an organic co-solvent (ethanol). The system was set to the desired temperature (35°C) and pressure (150 bar) with suitable switches, while the CO_2 flow rate was regulated by means a metering valve and set at 2 L/min. The collection condition was at room temperature and atmospheric pressure. According to the performed overall extraction curve (OEC), the whole process lasted 140 minutes, divided into 7 cycles. Each cycle consisted of 10 min of static phase in order to try to maximize the contact of the supercritical solvent with sample material and 10 min of dynamic phase, in which the 20% ethanol was pumped. Briefly, 33g of finely ground broccoli were loaded into the 50 mL vessel that was placed in the extractor and was allowed to equilibrate to the desired temperature. After reaching the

desired temperature, pressurization was initiated to make the extraction process. The obtained extract was collected in a separator vessel while CO_2 was vented by a flow meter. The extract was placed overnight in vacuum oven at 30°C in order to remove ethanol. The solid residue was dissolved in 25 mL of absolute ethanol and centrifuged at 6000 rpm for 10 minutes (5804R, Eppendorf, Milan, Italy) before chemical analysis.

3.4.3 Ultrasound assisted extraction (UAE)

Broccoli extract was also obtained by means of UAE (USR-1500-50WL, Weal s.r.l., Milan, Italy), using only water as solvent, according to Marinelli et al. (2015). The system consists of a reactor of 25 liters capacity, in which the BRC flour was suspended in water at a ratio of 1:10 (w/v). The sample was ultrasonically treated for 60 minutes at acoustic frequency of 25 kHz and with ultrasonic power density of 50 W/L. The obtained extract was homogenized and a part of this placed overnight in vacuum oven at 30°C. The dry residue was dissolved in 25 mL of water and centrifuged at 6000 rpm for 10 minutes before analytical determinations.

3.4.4 Pressurized liquid extraction (PLE)

Pressurized liquid extraction was carried out with PLE-1 (LabService Analytica srl, Anzola Emilia, Bologna, Italy) according to the optimized method by Ares et al. (2015) with slight modifications. The extraction cell was filled with 15g of dried broccoli flour, mixed with inert matrix and glass beads to favor uniform distribution of the extraction solvent in order to maximize the extraction yields. The method was divided in more steps: (i) filling the cell with the extraction solvent (EtOH 70%) for 2.3 minutes in order to wet the sample; (ii) increasing of the pressure up to 1500 psi, (iii) heating for 5 minutes up to a temperature of 60; (iv) static extraction (one extraction cycle, 5 min); (v) depressurization for 30 seconds; (vi) washing the cell for 50 seconds; and finally (vii) purge of the solvent from cell with N_2 for 2 minutes. The extraction was performed in triplicate and between them a rinse was made to remove any residual. The obtained extract was dried under vacuum oven at 30°C overnight. Next, the solid residue was dissolved in 25mL of the same extraction solvent and centrifuged for 10 minutes at 6000 rpm before chemical analysis.

3.4.5 Microencapsulation of broccoli extract

Microencapsulation of broccoli extract was carried out with a drying process using a mini Spray Dryer B-290 (BUCHI Labortechnik AG, Flawil, Switzerland), optimizing the

following parameters: wall material (Capsul, Maltodextrins (MD) 14-16 DE), the concentration of wall material (10-20-30%), the ratio of core:wall material (1:2, 1:5, 1:10, 1:20) and the temperature (80, 100, 130,150,170°C). The optimization was based on chemical characterization (TPC, TFC, ABTS and DPPH assays) of the obtained power. The resulting formulations were spray dried at the following conditions: aspiration rate of 100% and pump flow rate 25%. At the end of each drying session, the powders were collected, placed in closed vials and kept at room temperature in a dry and dark place until to the analysis. For each considered parameter, the microencapsulation process was performed in triplicate.

3.4.6 Fish burger preparation

Fresh sea bass fillets were obtained from a local seafood company Minaba s.r.l. (Manfredonia, Foggia, Italy). In the laboratory the fish was cleaned and trimmed to remove bones and skin. The burgers with extract (BRC_FB) consisted of minced fish mixed with 5% w/w of optimized broccoli powder and whey protein, parsley, potato flour and salt. Then, they were shaped in a circular mould to produce fish burgers and cooked in an electric convention oven (H2810, Hugin, Milan, Italy) at 180°C for 15 min. Fish burgers without any powder were also prepared and used as reference samples (CTRL_FB). For each burger three replicates were made. Both raw and cooked fish burger were analyzed from chemical point of view.

3.4.7 Polyphenol compounds extraction from fish-burger

The extraction of polyphenols from raw and cooked fish burger samples was based on the method described by Biney and Beta (2014). For more details see section 3.1.5. Before extraction CTRL_FB and BRC_FB samples were dried at 30°C and grounded. The extraction process was carried out in triplicate.

3.4.8 Chemical analyses

The evaluation of total phenols, flavonoids and antioxidant activity were performed on the broccoli extracts, microencapsulated broccoli powder (50 mg dissolved in 20 mL of water) and on fish burgers.

3.4.8.1 Determination of total phenolic compounds

The total phenols were determined according to the Folin-Ciocalteu method described by Spinelli et al. (2015), as previously indicated (section 3.1.6.1) Total phenolic content

(TPC) was expressed as mg of gallic acid equivalents (GAEs) per gram of dried sample, according to a calibration curve (3.125-100 mg/L; R^2 = 0.9989). For each sample, the analysis was carried out in triplicate.

3.4.8.2 Determination of total flavonoid compounds

Flavonoids were quantified by aluminium trichloride method according to Spinelli et al. (2016b), previously described (section 3.1.6.2). Total flavonoid content (TFC) was quantified by a calibration curve built in the range 6.25-400 mg/L (R^2 = 0.9990) and expressed as mg of quercetin equivalents (QEs) per gram of dried sample. The analysis was carried out in triplicate for each sample.

3.4.8.3 Determination of antioxidant activity

The antioxidant activity of samples was assessed using two methods: ABTS and DPPH assays. ABTS assay was carried out according to the original work of Re et al. (1999). A calibration curve was built using Trolox as standard at concentrations between 0.94 and 100 mg/L (R^2 = 0.9995). Instead, DPPH assay was based on the study of Mensor et al. (2001). For antioxidant activity quantification, a calibration curve was built between 1.56 and 100 mg/L (R^2=0.9989) using Trolox as standard. In both cases, the antioxidant activity was expressed as mg Trolox equivalents (TEs) for gram of dried sample using. All samples were run in triplicate.

Both assays have been previously described in detail (section 3.1.6.3).

3.4.9 Statistical Analysis

All experimental data were subjected to one-way analysis of variance (ANOVA). To the aim, a Fisher's test with the option of homogeneous groups ($P<0.05$) was carried out to determinate significance differences between samples. STATISTICA 7.1 for Windows (StatSoft Italia s.r.l.) was used.

4. Results and Discussion

4.1 Case study: Wheat Bread Enriched with Artichoke By-Products

In this work, the formulation of bread enriched with artichoke leaf flour (ALF) was optimized. In particular, a compromise between the nutritional and sensory properties of bread was found. The aim was to add to the bread formulation as much ALF as possible in order to improve its nutritional aspect, keeping, at the same time, its sensory properties at an acceptable level.

4.1.1 Formulation optimization

In order to assess the maximum ALF concentration to be added to bread, increasing concentrations of vegetable flour were tested (5 - 10 - 15 %, w/w) until the bread overall quality was deemed unacceptable. Table 3 shows the sensory parameters considered for each sample.

Table 3 Sensory characteristics of bread samples enriched with different concentrations of artichoke leaf flour.

Sample	Color	Odor	Taste	Crust firmness	Crumb firmness	Large bubbles	Overall quality
CTRL_br	$8.1^{a} \pm 0.4$	$8.2^{a} \pm 0.2$	$7.9^{a} \pm 0.24$	$7.9^{a} \pm 0.2$	$7.8^{a} \pm 0.3$	$7.6^{a} \pm 0.2$	$7.9^{a} \pm 0.3$
5ALF_br	$7.9^{a} \pm 0.3$	$8.1^{a} \pm 0.2$	$7.9^{a} \pm 0.34$	$7.8^{a} \pm 0.3$	$7.7^{a} \pm 0.2$	$7.6^{a} \pm 0.2$	$7.7^{a} \pm 0.4$
10ALF_br	$7.0^{b} \pm 0.3$	$7.8^{b} \pm 0.4$	$7.2^{b} \pm 0.24$	$7.4^{b} \pm 0.2$	$6.8^{b} \pm 0.3$	$7.0^{b} \pm 0.2$	$6.8^{b} \pm 0.3$
15ALF_br	$6.5^{c} \pm 0.2$	$6.9^{c} \pm 0.3$	$6.6^{c} \pm 0.28$	$6.9^{c} \pm 0.2$	$4.5^{c} \pm 0.3$	$4.6^{c} \pm 0.4$	$4.7^{c} \pm 0.3$
15ALF/CMC_br	$6.7^{c} \pm 0.2$	$7.0^{c} \pm 0.2$	$6.8^{c} \pm 0.26$	$7.2^{b} \pm 0.3$	$6.8^{b} \pm 0.4$	$6.8^{b} \pm 0.3$	$6.8^{b} \pm 0.4$

CTRL_br: control bread; XALF_br: X refers to artichoke leaf flour concentration; CMC: carboxymethyl cellulose. Results are expressed as means ± SD for $n = 3$. Data in columns with different superscripts are significantly different ($P < 0.05$).

As expected, results indicated that increasing the vegetable flour concentration, the bread overall quality decreased. In fact, the bread sample enriched with 5% (w/w) ALF recorded highest scores among the fortified samples tested in this work: good odor, color and taste,

good crust firmness and soft crumb with large bubbles. It was very appreciated by panelists and it was statistically similar to CTRL sample. Increasing the ALF concentration to 10% (w/w) the considered sensory attributes, in particular the crumb firmness and large bubbles, decreased compared to both CTRL and 5ALF_br samples. However, its overall quality resulted still acceptable (6.8 ± 0.3). This result is in line with the work of Hoye and Ross (2011), who observed a worsening in sensory and texture characteristics when the bread was enriched with grape seed flour > 7.5% (w/w). Instead, the ALF concentration at 15% (w/w) negatively affected every sensory attribute. In particular, the crumb was found to be firmer and heavier with small bubbles; consequently, the bread overall quality was evaluated as unacceptable (4.7 ± 0.3). This behavior is due to the fact that bread quality depends on its composition and mostly its protein content, which influences the strength of the dough (Kihlberg et al., 2004; Mastromatteo et al., 2014). A strong dough, which is able to stretch without breaking during the gas bubbles formation is necessary to make a good bread (Mir et al., 2016). Gluten network is a wheat protein complex formed by prolamine and glutenin following a mechanical action and in the presence of water. It is well known from the literature that the gluten is critical for the bread making process, because it is responsible for the viscoelastic properties of dough and promotes the retention of the CO2 produced during fermentation (Mir et al., 2016). Therefore, the replacement of semolina with 15% (w/w) vegetable flour greatly weakened the protein network of 15ALF_br sample compared to other samples and so the dough was not able to entrap the gas bubbles and consequently the loaf volume collapsed (Kihlberg et al., 2004). In order to improve the structure 15ALF_br sample, it was necessary to use structuring agents, such as hydrocolloids that represent the main class. They are water-soluble polysaccharides widely employed as additives in the food industry, used mostly in gluten-free bread production because they are able to improve dough structural proprieties (Gao et al., 2017; Guarda et al., 2004). Hydrocolloids can be used as gluten replacements because they are able to imitate the viscoelastic behavior of gluten due to their water-binding capacity and to formation of a gelatinous structure during baking. This strengthens the dough and improves gas retention during baking, leading to better bread volume (Kittisuban et al., 2014; Rojas et al., 1999). Different studies of gluten-free bread have shown the improving effect of hydrocolloids on bread quality, in particular on the crumb (Houben et al., 2012; Kittisuban et al., 2014; McCarthy et al., 2005). Taking into account the above, hydrocolloids can also be exploited to improve the sensory quality of the investigated wheat durum bread enriched with vegetable flour. In this work several structuring agents

were tested (data not shown). An improvement compared to 15ALF_br was observed using carboxymethyl cellulose (CMC) at 2% (w/w) (Table 3). The 15ALF/CMC_br sample showed a softer crumb with more bubbles (6.8 ± 0.4 and 6.8 ± 0.3, respectively). The overall quality was statistically improved when compared to that of sample without CMC. This result is in line with many studies that observed an increase in volume, crust and crumb porosity using CMC in bread formulation (Kohajdová and Karovičová, 2009). Mohammadi et al. (2014) studied the effects of xanthan gum (XG) and carboxymethyl cellulose (CMC) on quality parameters of gluten-free bread and noted an improvement of the porosity using CMC. The same effect was observed by Lazaridou et al. (2007). Furthermore, the texture improvement is also due to the presence of small amount of oil in the bread optimized formulation. According to Mancebo et al. (2017) the oil was able to improve the volume of gluten-free bread, its porosity and to reduce its hardness.

4.1.2 Nutritional properties

Among the experimental enriched samples, only 15ALF/CMC_br was taken into account for chemical characterization and compared to 0ALF/CMC_br. In addition, the nutritional properties of ALF was also assessed. The measured nutritional parameters, in terms of total polyphenols (mg GAEs/g dw), total flavonoids (mg QEs/g dw) and antioxidant activity measured by ABTS (mg TEs/g dw) and DPPH (mg TEs/g dw) assays, were shown in Table 4.

Table 4 Total phenols, total flavonoids and antioxidant activity of bread samples.

Sample	Total phenols (mg GAEs/g dw)	Total flavonoids (mg QEs/g dw)	ABTS (mg TEs/g dw)	DPPH (mg TEs/g dw)
ALF	16.54 ± 0.41^{a}	14.93 ± 0.39^{a}	20.58 ± 0.35^{a}	7.11 ± 0.04^{a}
0ALF/CMC_br	0.22 ± 0.01^{b}	0.07 ± 0.02^{b}	0.42 ± 0.01^{b}	0.62 ± 0.02^{b}
15ALF/CMC_br	0.92 ± 0.01^{c}	0.45 ± 0.01^{c}	1.20 ± 0.02^{c}	0.74 ± 0.02^{c}

ALF: artichoke leaf flour; XALF/CMC_br: X refers to artichoke leaf flour concentration; CMC: carboxy methyl cellulose. GAEs: gallic acid equivalents; QEs: quercetin equivalents; TEs: Trolox equivalents. Results are expressed as means ± SD for $n = 3$. Data in columns with different superscripts are significantly different ($P < 0.05$).

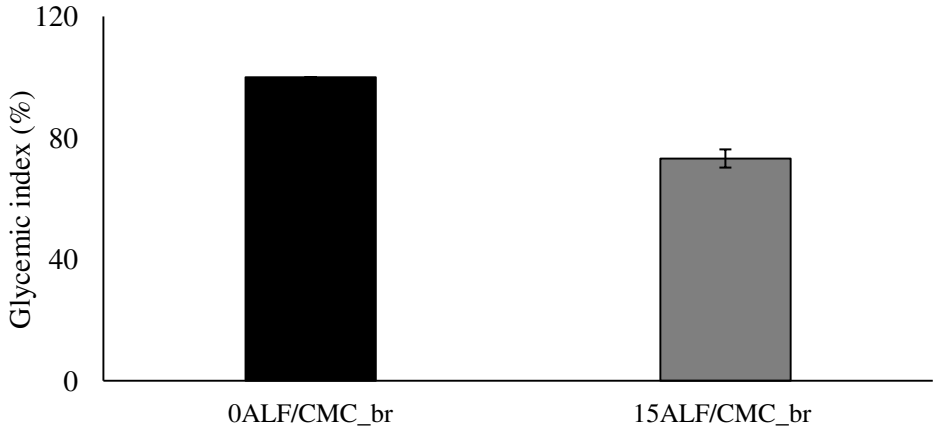
As can be seen from the data listed in the Table 4, for each parameter there is a statistically significant difference between 0ALF/CMC_br and 15ALF/CMC_br samples. Data suggest that the addition of 15% ALF to formulation greatly improved the bread nutritional quality.

In particular, the enriched bread had greater concentration of total polyphenols (0.92 ± 0.01 mg GAEs/g dw) and flavonoids (0.45 ± 0.01 mg QEs/g dw) if compared to the reference sample (0.22 ± 0.01 mg GAEs/g dw and 0.07 ± 0.02 mg QEs/g dw for total polyphenols and flavonoids, respectively). With regard to the antioxidant activity of bread, this is mainly linked to the presence of added phenolic substances and, as Alonso et al. (2002) showed, there is a positive correlation between the antioxidant activity and the sample total polyphenolic content. Consequently, the 15ALF/CMC_br sample also had a higher antioxidant activity (1.20 ± 0.02 and 0.74 ± 0.02 mg TEs/g dw for ABTS and DPPH assay, respectively) than the control. An increase in the antioxidant properties was also observed by other researchers that have tried to improve the nutritional aspect of wheat bread exploiting different raw materials, such as pseudo-cereals and other cereals, spices and plant by-products (Gawlik-Dziki et al., 2013; Ho et al., 2013; Peng et al., 2010; Sivam et al., 2010). However, even if a significant difference between experimental breads was observed, there was a loss of bioactive compounds during the bread-making process since the obtained values were lower than expected. In fact, considering the obtained data on ALF flour and the vegetable flour percentage in the bread formulation, a decrease in total polyphenol and flavonoid content of about 65 and 80% (w/w), respectively, was observed. Consequently, the antioxidant activity observed was lower than that expected. It is known that antioxidant compounds are sensible and reactive; therefore, some modifications could occur during the process. On the basis of the work of Holtekjølen et al. (2008) that studied the antioxidant properties of the bread enriched with barley flour before and after the baking process, the amount of bioactive compounds can change for different reasons: degradation at high temperature during baking; oxidation by oxidative enzymes present in other ingredients or by ambient oxygen and by reaction with proteins and carbohydrates. The reduction of TPC and antioxidant activity during the bread making process was also observed by Alvarez-Jubete et al. (2010). However, this effect is in disagreement with others works in which an increase in total polyphenol content after baking was recorded, following the release of bound phenol acids (Ciccoritti et al., 2017; Turkmen et al., 2005). In literature there are different controversies on this aspect. It is difficult to do a comparison with other data reported by other authors, because different extraction and measurement methods are often used and, moreover, it should be considered that the spectrophotometric methods, Folin-Ciocalteau and DPPH/ABTS assays, are not specific, therefore the same false positives could be estimated.

4.1.3 Glycemic index

Since carbohydrates are considered as one of the factors of obesity, diabetes and other chronic diseases, the reduction of the amount of quickly digestible carbohydrates and then of food glycemic index, which indicates how carbohydrate-rich foods affect the blood glucose level, could have a positive effect on human health (Augustin et al., 2015). Therefore, in this work the influence of artichoke vegetable flour on the glycemic index of 15ALF/CMC_br was also evaluated and compared to control bread (0ALF/CMC). Figure 1 shows the effect of ALF adding on bread glycemic index: a decrease of about 27% was observed; 15ALF/CMC_br recorded a statistically significant lower value than the reference sample.

Figure 1 Glycemic index of the bread samples.



0ALF/CMC_br: control bread; 15ALF/CMC_br: bread containing artichoke leaf flour (ALF; 15% w/w) and carboxymethylcellulose (CMC; 2% w/w).

Most probably the above results are a direct consequence of the fact that artichoke is a vegetable rich in inulin, which is a soluble fiber and cannot be absorbed in the intestine due to its chemical structure characterized by β-2,1 bonds between the fructose units (Yu et al., 2018). Different scientists have found that the inulin is able to improve the glucose metabolism, thus lowering the blood sugar level (Liu et al., 2016; Yang et al., 2012). The effect observed on our experimental breads is in agreement with Yokoyama et al. (1997), according to which a high fiber content is able to reduce the glycemic response of food. The same trend was confirmed by Padalino et al. (2013) who also found that the addition of pepper flour (containing a high level of dietary fibers) reduced the glycemic index of maize-based pasta.

4.2 Case study: Watermelon-based Candy fortified with orange by-products

During the first part of this study, a watermelon-based jelly candy was realized using all the parts of the watermelon fruit including the by-products (rind and exhaust pulp) obtained in the juice production. Sensory and chemical quality were evaluated. Subsequently, in order to improve the candy nutritional content, orange by-products were added. First albedo flour was added at four different concentrations to the watermelon-based jelly candy formulation. Based on the sensory results two concentrations were chosen out of four. They were combined with four different amount of flavedo flour. The fortified final candy samples were also assessed for sensory and chemical quality. In the subsequent paragraphs, the results recorded in each step were provided.

4.2.1 Quality of watermelon jelly candy

4.2.1.1 Sensory quality

Ratings for sensory attributes of watermelon jelly candies are presented in Table 5.

Table 5 Sensory attributes of watermelon jelly candy (WMC).

Sensory quality	
Appearance	8.5 ± 0.5
Colour	8.00 ± 0.01
Odour	8.00 ± 0.01
Taste	8.1 ± 0.2
Firmness	8.00 ± 0.01
Overall quality	8.1 ± 0.2

As can be inferred, the WMC was attractive for the quality attribute of appearance (score 8.5) and similarly, for colour and odour, the product was marked as more than acceptable by all the panel members. The taste attribute was pleasant and the panellists recognized the fruity aroma typical of watermelon (score 8.1). The gelatine gave a gummy firmness characterized by suitable hardness and transparency as confirmed also in the literature (Hartel and Hartel, 2014; Marfil et al., 2012). The firmness was slightly affected by the presence of watermelon flours that conferred a certain fibrous and graininess to the candy, even if most of the sensory scores were in the category of ‘like very much’ (score 8).

Results of sensory analysis indicated that the product was appreciated for all attributes with an overall quality more than 8.

4.2.1.2 Chemical quality

Table 6 shows the total phenolic and flavonoid contents and the antioxidant activity, assessed using the FRAP assay, of the watermelon candy (WMC) and its ingredients.

Table 6 Chemical characterization of watermelon jelly candy and its ingredients.

Sample	TPC mg GAEs/g	TFC mg QEs/g	FRAP µmoli FeSO4 ·7H2O/g
Rind	4.83 ± 0.06	1.87 ± 0.07	46.8 ± 3.2
Pulp	3.8 ± 0.1	1.67 ± 0.03	32.7 ± 2.4
Watermelon juice	0.202 ± 0.002	0.026 ± 0.001	1.093 ± 0.017
Lemon juice	0.71 ± 0.01	0.155 ± 0.001	10.5 ± 0.12
WMC	0.44 ± 0.05	0.16 ± 0.02	2.53 ± 0.10

WMC: watermelon-based candy.

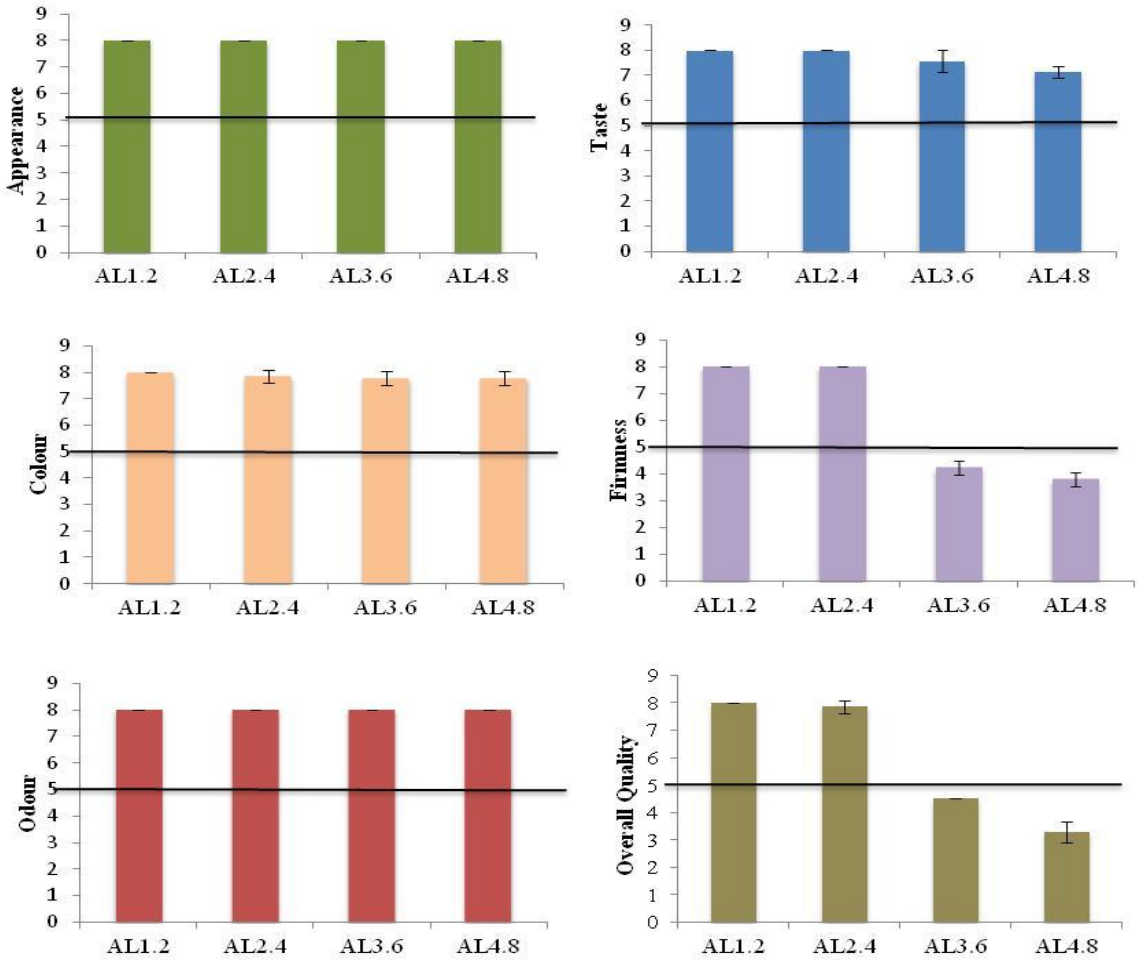
According to our findings, the rind is the component with the highest contents of total polyphenols (TPC) and flavonoids (TFC) (4.83 ± 0.06 mg GAEs/g and 1.87 ± 0.07 mg QEs/g, respectively) as well as the antioxidant activity (46.84 ± 3.17 µmoli $FeSO_4 \cdot 7H_2O$/g), followed by the pulp. Compared to rind and pulp, the other ingredients are very poor in bioactive compounds. The values of TPC and TFC recorded for the developed candy, reported in the last row of the Table 6, are higher than expected data (0.31 mg GAEs/g TPC and 0.08 mg QEs/g TFC). The same trend was also observed by Lee et al. (2010) who developed a jelly using banana peels and observed more polyphenols and flavonoids than calculated values. This behavior is probably due to cooking during candy making that promotes the release of active compounds by breaking the plant cell wall. According to Choi et al. (2006), who studied the influence of heat treatment on the antioxidant activity and polyphenol compounds of Shiitake mushroom, the phenol substances increased with increasing heating temperature. In terms of antioxidant activity, a value corresponding to that expected has been recorded. Taking into account the candy recipe and the chemical composition of its ingredients, each element influenced in different way the jelly chemical quality. The watermelon juice gives the highest phenolic contribution, equal to 39%, while the flavonoids are mostly attributed to the rind, which contributes for about 32.5%.

4.2.2 Quality of watermelon jelly candy fortified with orange by-products

4.2.2.1 Sensory quality

As regards the sensory quality of the candies enriched with orange by-products, at first the candies with four different concentrations of albedo flour (1.2% - 2.4% - 3.6% - 4.8%) were evaluated to know the maximum amount of albedo that could be added without compromising the quality of sample. The results for each sensory attribute are shown in the following Figure 2.

Figure 2 Sensory attributes of watermelon candies enriched with albedo flour.



AL1.2: watermelon candy enriched with 1.2% albedo; AL2.4: watermelon candy enriched with 2.4% albedo; AL3.6: watermelon candy enriched with 3.6% albedo; AL4.8: watermelon candy enriched with 4.8% albedo.

As can be observed, there are not many differences between experimental samples enriched with the different amounts of albedo flour. All of them are above the threshold of acceptability, except for firmness. Indeed, the firmness was the cause responsible for

unacceptability of samples with 3.6% and 4.8% of albedo flour, due to the pasty conferred by the excessive presence of flour that increased the feeling sticky on the palate. The other attributes (appearance, colour, odour) were not influenced by the albedo flour and exhibited values ranging around 8. The taste in all the samples was slightly reduced due to the bitterness conferred by the albedo, even though it remained agreeable. As concern the overall quality of the candy samples, the two highest concentrations of albedo flour ($AL_{3.6}$ and $AL_{4.8}$) compromised the sensory quality, recording a negative score, below the acceptability threshold (score below 5). Instead, comparable and acceptable results were recorded with both 1.2 and 2.4% of albedo addition, with no significant difference. On the basis of the results recorded, AL1.2 and AL2.4 were chosen and combined with the flavedo flour. In Table 7 are reported the sensory attributes for the six formulations of fortified jelly candies.

Table 7 Sensory attributes of watermelon jelly candy with and without orange by-products.

Samples	Appearance	Colour	Odour	Taste	Firmness	Overall Quality
$AL_{1.2}$-$FL_{0.6}$	7.8 ± 0.4^{a}	7.71 ± 0.5^{a}	$7.3 \pm 0.5^{a,b}$	$7.2 \pm 0.5^{a,b}$	6.7 ± 0.4^{e}	7.4 ± 0.3^{e}
$AL_{1.2}$-$FL_{1.2}$	7.9 ± 0.2^{a}	7.9 ± 0.4^{a}	$7.2 \pm 0.5^{a,b}$	6.6 ± 0.9^{b}	$6.4 \pm 0.4^{c,d}$	$6.9 \pm 0.4^{d,e}$
$AL_{1.2}$-$FL_{2.4}$	7.6 ± 0.2^{a}	7.7 ± 0.4^{a}	$7.3 \pm 0.5^{a,b}$	6.4 ± 1.0^{b}	$5.9 \pm 0.4^{b,c}$	$5.8 \pm 0.5^{b,c}$
$AL_{2.4}$-$FL_{0.6}$	7.6 ± 0.4^{a}	7.6 ± 0.4^{a}	7.0 ± 0.6^{a}	6.6 ± 0.8^{b}	$6.0 \pm 0.6^{b,c}$	$6.4 \pm 0.4^{c,d}$
$AL_{2.4}$-$FL_{1.2}$	7.5 ± 0.4^{a}	7.6 ± 0.4^{a}	7.0 ± 0.6^{a}	6.5 ± 0.7^{b}	5.4 ± 0.5^{b}	5.7 ± 0.6^{b}
$AL_{2.4}$-$FL_{2.4}$	7.3 ± 0.4^{a}	7.4 ± 0.4^{a}	7.0 ± 0.6^{a}	4.9 ± 0.4^{a}	4.4 ± 0.2^{a}	4.50 ± 0.01^{a}

$AL_{1.2}$-$FL_{0.6}$: watermelon candy enriched with 1.2% albedo and 0.6% flavedo; $AL_{1.2}$-$FL_{1.2}$: watermelon candy enriched with 1.2% albedo and 1.2% flavedo; $AL_{1.2}$-$FL_{2.4}$: watermelon candy enriched with 1.2% albedo and 2.4% flavedo; $AL_{2.4}$-$FL_{0.6}$: watermelon candy enriched with 2.4% albedo and 0.6% flaved; $AL_{2.2}$-$FL_{1.2}$: watermelon candy enriched with 2.4% albedo and 1.2% flavedo; $AL_{2.4}$-$FL_{2.4}$: watermelon candy enriched with 2.4% albedo and 2.4% flavedo. Results are expressed as means ± SD for $n = 3$. $^{a-e}$ Data in columns with different superscripts are significantly different ($P < 0.05$), as determined by ANOVA followed by the Fischer's test.

Data highlight that all types of fortified candy samples, except AL2.4-FL2.4, show an overall quality values above the threshold. Sample AL2.4-FL2.4 were found unacceptable in terms of firmness and taste. As expected, an increase in flavedo concentration brought about a reduction in overall quality. Taste and firmness are the attributes mainly responsible of this reduction. In fact, increasing the flavedo flour concentration a different undesirable intensity of the taste was perceived. Many studies report that an increase in

viscosity or hardness globally reduces both perceived taste and aroma intensities (Baines and Morris, 1987; Boland et al., 2006; Kälviäinen et al., 2000). The adding of albedo and flavedo flours did not influence significantly the appearance, colour and odour attributes of jelly candy samples. It is worth noting that comparing all the samples the combination AL1.2-FL0.6 did not affect at great level the whole sensory quality of the candy.

4.2.2.2 Chemical quality

Albedo and flavedo were chemically characterized in terms of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity by means FRAP assay. According to obtained data, shown in Table 8 flavedo had higher total phenol and flavonoid amounts and consequently a higher antioxidant activity (22.6 ± 2.02 mg GAEs/g, 10.14 ± 0.18 mg QEs/g and 319.7 ± 4.1 µmoli FeSO4 ·7H2O/g, respectively) compared to albedo (17.4 ± 1.4 mg GAEs/g, 9.12 ± 0.44 mg QEs/g and 166 ± 1.6 µmoli FeSO4 ·7H2O/g, respectively).

Table 8 Chemical characterization of orange by-products.

	Albedo	**Flavedo**
TPC **mg GAEs/g**	17.4 ± 1.4	22.6 ± 2.02
TFC **mg QEs/g**	9.12 ± 0.44	10.14 ± 0.18
FRAP **µmoli $FeSO_4 \cdot 7H_2O$/g**	166.0 ± 1.6	319.7 ± 4.1

Anyhow, the chemical composition of both orange by-products in terms of antioxidants was better than watermelon fruit (see Table 6), thus justifying their addition to jelly candy based on watermelon.

The chemical characterization of the fortified watermelon candy samples is shown in Table 9. Only five of the six formulations of enriched products accepted from the sensory point of view were also studied for their chemical composition (AL1.2-FL0.6, AL1.2-FL1.2, AL1.2-FL2.4, AL2.4-FL0.6, AL2.4-FL1.2). As can be observed from data listed in Table 9, the AL1.2-FL2.4 sample was the best one, characterized by 1.26 ± 0.03 mg GAEs/g,

0.76 ± 0.03mg QEs/g and 10.5 ± 0.5 µmoli FeSO4 ·7H2O/g, followed by AL2.4-FL1.2, AL2.4-FL0.6, AL1.2-FL1.2, AL1.2-FL0.6. Anyhow, all the enriched samples were very comparable from the chemical point of view.

Table 9 Chemical characterization of watermelon-based candies fortified with orange by-products.

Sample	TPC mg GAEs/g	TFC mg QEs/g	FRAP µmoli FeSO4 ·7H2O/g
$AL_{1.2}$-$FL_{0.6}$	0.94 ± 0.03^{a}	0.46 ± 0.02^{a}	6.6 ± 0.3^{a}
$AL_{1.2}$-$FL_{1.2}$	0.94 ± 0.03^{a}	0.54 ± 0.03^{b}	7.8 ± 0.4^{b}
$AL_{1.2}$-$FL_{2.4}$	1.26 ± 0.03^{d}	0.76 ± 0.03^{e}	10.5 ± 0.5^{d}
$AL_{2.4}$-$FL_{0.6}$	1.13 ± 0.03^{b}	0.58 ± 0.06^{c}	8.0 ± 0.2^{b}
$AL_{2.4}$-$FL_{1.2}$	1.16 ± 0.03^{c}	0.68 ± 0.03^{d}	9.6 ± 0.1^{c}

$AL_{1.2}$-$FL_{0.6}$: watermelon candy enriched with 1.2% albedo and 0.6% flavedo; $AL_{1.2}$-$FL_{1.2}$: watermelon candy enriched with 1.2% albedo and 1.2% flavedo; $AL_{1.2}$-$FL_{2.4}$: watermelon candy enriched with 1.2% albedo and 2.4% flavedo; $AL_{2.4}$-$FL_{0.6}$: watermelon candy enriched with 2.4% albedo and 0.6% flaved; $AL_{2.4}$-$FL_{1.2}$: watermelon candy enriched with 2.4% albedo and 1.2%. Results are expressed as means ± SD for $n = 3$. $^{a-e}$ Data in columns with different superscripts are significantly different ($P < 0.05$), as determined by ANOVA followed by the Fischer'test.

It is interesting to highlight that also the candy with the lowest orange by-products concentration (AL1.2-FL0.6) recorded an important improvement of the antioxidant activity compared to the control sample (see Table 6 and 9).

In order to have positive effect on human health, it is important that the bioactive compounds in candy are available for absorption in the gastrointestinal tract. Therefore, to understand if the jelly chemical properties are compromised by digestion conditions, in this study the in vitro digestion of the jellies was also performed. Table 10 shows the total phenols, flavonoids and antioxidant activity of jellies after digestion. It is worth noting that comparing data in the Table 10 an increase in TPC, TFC and above all in antioxidant activity also remained after digestion for the candies fortified with orange by-products. Each enriched sample was statistically different than WMC. Even after *in vitro* digestion the trend was similar to that before digestion: the $AL_{1.2}$-$FL_{2.4}$ sample resulted the best one, with the highest concentrations of TPC, TFC and antioxidant activity.

Table 10 Chemical characterization of candy with and without orange by-products after digestion.

Sample	TPC mg GAEs/g	TFC mg QEs/g	FRAP µmoli FeSO4 ·7H2O/g
WMC	0.5 ± 0.3^{a}	0.03 ± 0.02^{a}	2.9 ± 0.6^{a}
$AL_{1.2}$-$FL_{0.6}$	0.88 ± 0.06^{bc}	0.25 ± 0.03^{b}	5.7 ± 0.2^{b}
$AL_{1.2}$-$FL_{1.2}$	0.9 ± 0.2^{bc}	0.35 ± 0.05^{c}	6.3 ± 0.5^{cd}
$AL_{1.2}$-$FL_{2.4}$	1.0 ± 0.3^{c}	0.54 ± 0.06^{d}	8.2 ± 0.7^{e}
$AL_{2.4}$-$FL_{0.6}$	0.9 ± 0.2^{b}	0.28 ± 0.05^{b}	5.9 ± 0.5^{bc}
$AL_{2.4}$-$FL_{1.2}$	1.0 ± 0.2^{bc}	0.37 ± 0.09^{c}	6.7 ± 0.8^{d}

WMC: watermelon-based candy; $AL_{1.2}$-$FL_{0.6}$: watermelon candy enriched with 1.2% albedo and 0.6% flavedo; $AL_{1.2}$-$FL_{1.2}$: watermelon candy enriched with 1.2% albedo and 1.2% flavedo; $AL_{1.2}$-$FL_{2.4}$: watermelon candy enriched with 1.2% albedo and 2.4% flavedo; $AL_{2.4}$-$FL_{0.6}$: watermelon candy enriched with 2.4% albedo and 0.6% flaved; $AL_{2.4}$-$FL_{1.2}$: watermelon candy enriched with 2.4% albedo and 1.2%. Results are expressed as means ± SD for $n = 3$. $^{a-e}$ Data in columns with different superscripts are significantly different ($P < 0.05$), as determined by ANOVA followed by the Fischer'test.

With the aim to develop an acceptable and nutritional new candy product, a whole quality index (WQI) was also calculated. This index took into account the score of overall quality and the TPC value recorded after digestion (Table 11). As can be seen, the WQI greatly changed among samples and allowed to select the best one. In fact, the highest WQI value was referred to the AL1.2-FL0.6 sample but it is also possible to infer that the flavedo amount could be increased up to 1.2% without compromising the product quality.

Table 11 Whole Quality Index (WQI) of enriched candies.

Sample	WQI
$AL_{1.2}$-$FL_{0.6}$	0.50
$AL_{1.2}$-$FL_{1.2}$	0.44
$AL_{1.2}$-$FL_{2.4}$	0.22
$AL_{2.4}$-$FL_{0.6}$	0.27
$AL_{2.4}$-$FL_{1.2}$	0.19

$AL_{1.2}$-$FL_{0.6}$: watermelon candy enriched with 1.2% albedo and 0.6% flavedo; $AL_{1.2}$-$FL_{1.2}$: watermelon candy enriched with 1.2% albedo and 1.2% flavedo; $AL_{1.2}$-$FL_{2.4}$: watermelon candy enriched with 1.2% albedo and 2.4% flavedo; $AL_{2.4}$-$FL_{0.6}$: watermelon candy enriched with 2.4% albedo and 0.6% flaved; $AL_{2.4}$-$FL_{1.2}$: watermelon candy enriched with 2.4% albedo and 1.2%.

4.3 Case study: Durum Wheat Spaghetti Enriched with Red Grape Marc

The aim of this study was to improve the nutritional properties of pasta by adding red grape marc (RGM), which is rich in polyphenols and low in digestible carbohydrates, to increase phenolic antioxidants and decrease the glycemic load/energy intake.

4.3.1 Sensory quality

The aim was to maximally increase the concentration of red grape marc in the spaghetti and to keep the sensory quality at least at an acceptable level at the same time. Preliminary studies indicated that the increasing concentration of red grape marc flour affected the overall quality of the spaghetti, so it was possible to add a maximum of 15% (w/w) of grape pomace. Here it was investigated whether a smaller particle size of red grape marc and addition of TG can improve the sensory quality and nutritional characteristics of spaghetti produced using 15 % (w/w) of red grape marc. Specifically, RGM flour with particle size (≤ 125 μm) (RGM-125) without or with transglutaminase at 0.6 % (w/w) level (RGM/TG) were used to try to improve the quality of pasta compared to the RGM flour with particle size ≤ 500 μm (RGM-500). Data reported in Table 12 show that the uncooked spaghetti sample with RGM-500 flour had the lowest overall quality value due to the poor resistance to breakage and the unpleasant dark purple color. In contrast, the addition of RGM-125 flour caused a noticeable improvement of the pasta color as compared to RGM-500. Furthermore, addition of TG to RGM- 125 slightly increased the overall quality score of the uncooked sample due to the increase in the break resistance value. Regarding the cooked pasta, the RGM-500 sample again showed the lowest overall quality value as compared to the other investigated samples. In particular, the incorporation of grape marc flour negatively influenced parameters, such as elasticity, adhesiveness and bulkiness. Table 12 shows that the RGM/TG sample recorded an acceptable value (6.0) of overall quality. Specifically, this sample exhibited significantly higher sensory scores for elasticity and firmness as well as a significant decline of both adhesiveness and bulkiness, when compared to the other fortified samples (RGM-500 and RGM- 125). No significant differences in color, odor and taste were observed between the RGM-125 and RGM/TG sample. Based on its sensory quality, the RGM/TG spaghetti sample was selected for the successive investigations.

Table 12 Sensory properties of uncooked (a) and cooked spaghetti (b). The spaghetti samples were evaluated by 10 trained panelists.

a)

Uncooked Spaghetti				
	CTRL	**RGM-500**	**RGM-125**	**RGM/TG**
Color	7.3 ± 0.2^{a}	5.8 ± 0.2^{c}	6.3 ± 0.3^{b}	6.3 ± 0.3^{b}
Homogeneity	7.3 ± 0.3^{a}	5.0 ± 0.3^{c}	6.3 ± 0.3^{b}	6.3 ± 0.3^{b}
Break Resistance	7.2 ± 0.2^{a}	5.38 ± 0.3^{c}	5.5 ± 0.3^{bc}	5.8 ± 0.3^{b}
Overall Quality	7.5 ± 0.2^{a}	5.3 ± 0.3^{c}	6.0 ± 0.3^{b}	6.2 ± 0.3^{b}

b)

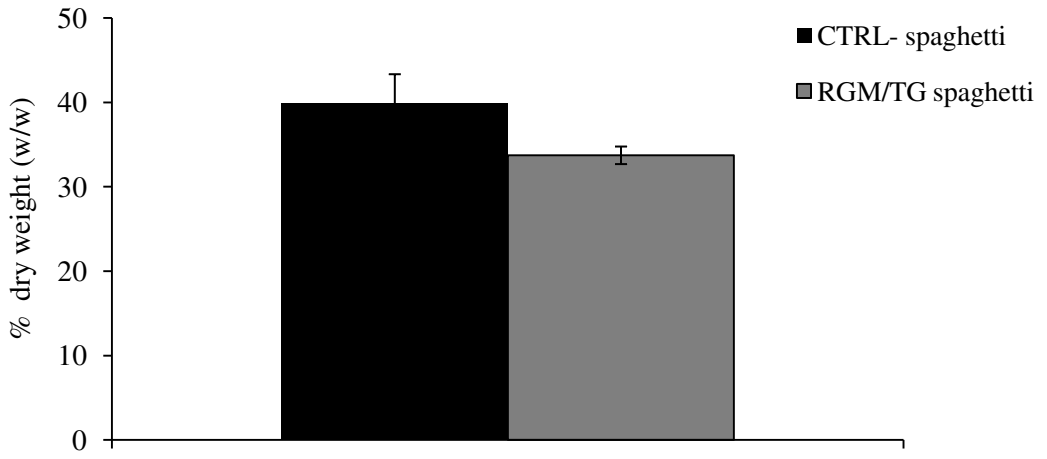
Cooked Spaghetti				
	CTRL	**RGM-500**	**RGM-125**	**RGM/TG**
Elasticity	7.5 ± 0.2^{a}	5.0 ± 0.3^{c}	5.3 ± 0.3^{c}	5.8 ± 0.3^{b}
Firmness	7.2 ± 0.2^{a}	5.0 ± 0.2^{c}	5.3 ± 0.3^{c}	6.0 ± 0.3^{b}
Bulkiness	7.2 ± 0.2^{a}	4.0 ± 0.3^{d}	5.0 ± 0.3^{c}	6.0 ± 0.3^{b}
Adhesiveness	7.4 ± 0.2^{a}	4.0 ± 0.3^{d}	4.9 ± 0.3^{c}	5.9 ± 0.3^{b}
Color	7.5 ± 0.3^{a}	4.5 ± 0.3^{c}	6.0 ± 0.3^{b}	6.2 ± 0.3^{b}
Odor	7.8 ± 0.3^{a}	6.2 ± 0.3^{b}	6.3 ± 0.3^{b}	6.3 ± 0.3^{ba}
Taste	7.7 ± 0.3^{a}	5.3 ± 0.3^{c}	6.5 ± 0.3^{b}	6.5 ± 0.3^{b}
Overall Quality	7.3 ± 0.3^{a}	4.5 ± 0.3^{d}	5.3 ± 0.3^{c}	6.0 ± 0.3^{b}

CTRL: control spaghetti; RGM-500: spaghetti made from red grape marc flour (15% w/w, particle size ≤ 500 µm); RGM-125: spaghetti made from 125µm red grape marc flour (15% w/w, particle size ≤ 125 µm); RGM/TG: spaghetti made from 125µm red grape marc flour (15% w/w) and 0.6% (w/w) transglutaminase. Different letters in each column indicate statistically significant differences ($P \leq 0.001$), as determined by ANOVA followed by the Tukey-Kramer test.

In this way it was showed that the substitution of wheat durum semolina by 125 μm RGM flour improved the sensory characteristics of spaghetti such as color (light purple color) and taste as compared to RGM-500 (500 μm) spaghetti. This result is in agreement with previous observations showing that a spaghetti sample enriched with medium particle size tomato peel flour had a pleasant orange color as compared to that with coarse particle size tomato peel flour (Padalino et al., 2017). The addition of the 125 μm RGM flour also improved the taste of the cooked pasta possibly due to small fibrous sensation during mastication. This result is also in line with previous reports (Padalino et al., 2017). The significant decline of overall quality in terms of elasticity, adhesiveness, and bulkiness recorded for RGM-500 cooked spaghetti samples might be explained by the dilution of the gluten strength by RGM leading to interrupted and weakened overall structure of pasta (Rayas-Duarte et al., 1996; Rekha et al., 2013). Conversely, the addition of RGM flour with a lower particle size (125 μm) and transglutaminase (RGM/TG) improved the sensory characteristics. Possibly, lower particle size can help to form a more stable network able to bind starch granules with untraditional flour and prevent a loss of solids during cooking, reducing pasta adhesiveness and bulkiness (Padalino et al., 2017; Rekha et al., 2013). Furthermore, transglutaminase catalyzes covalent crosslinks strengthening the gluten network which prevents texture deterioration during cooking, increases hardness, elasticity and decreases stickiness of cooked pasta (Yeoh et al., 2011).

According to data, the fortification resulted in a reduction of dry matter in the RGM-enriched cooked spaghetti of about 6 % (Figure 3). This was probably due to a higher solid release during cooking from the enriched sample. Semolina was substituted with red grape marc flour, which does not contain gluten. Therefore, the structure of RGM spaghetti is weaker than that of the control sample. It has been reported that gluten is critical for a strong protein network that helps to reduce loss of solids during cooking (Biney and Beta, 2014; Rayas-Duarte et al., 1996). Furthermore, the fiber contained in RGM can also react with the protein network and break protein-starch bonds, thereby leading to a greater release of solids in the water (Tudorică et al., 2002).

Figure 3 Dry weight (%) of CTRL and RGM cooked spaghetti.



CTRL: control spaghetti; RGM/TG: spaghetti made from 125µm red grape marc flour (15% w/w) and 0.6% (w/w) transglutaminase.

4.3.2 Polyphenol concentration and antioxidant activity: effect of cooking

Regarding the nutritional characterization, it were investigated the total polyphenol and anthocyanin concentrations and the antioxidant activity of spaghetti enriched with 125 µm red grape marc powder at 15 % (w/w) and containing 0.6 % TG in comparison to the control spaghetti made from commercial wheat durum semolina.

Table 13 Total polyphenol content and antioxidant activity (FRAP assay) of CTRL and RGM/TG spaghetti before and after cooking.

	Uncooked spaghetti		**Cooked spaghetti**	
	CTRL	**RGM/TG**	**CTRL**	**RGM/TG**
Total polyphenols (mg GAEs/g dw)	0.54 ± 0.08^{a}	2.27 ± 0.20^{b}	0.28 ± 0.02^{a}	2.1 ± 0.2^{b}
Total anthocyanins (mg mvd-glu /g dw)	ND	0.095 ± 0.008^{a}	ND	0.084 ± 0.002^{a}
Antioxidant activity (µmol Fe(II)/g dw)	3.1 ± 0.3^{a}	54.5 ± 7.7^{b}	2.75 ± 0.05^{a}	56.2 ± 9.7^{b}

ND: not detectable. CTRL: control spaghetti; RGM/TG: spaghetti containing red grape marc flour (15% w/w; particle size ≤ 125 µm) and 0.6% (w/w) transglutaminase.
Results are expressed as means ± SD, $n = 3$. ^{a,b}Data in each row with different letters are significantly different ($P < 0.05$), as determined by ANOVA followed by the Tukey-Kramer test.

Table 13 shows the total polyphenol content of uncooked and cooked spaghetti samples. It is clear that the enrichment with RGM/TG flour enhanced the levels of total polyphenols significantly in both raw and cooked spaghetti compared to the CTRL sample. The uncooked pasta contained 0.54 ± 0.08 and 2.27 ± 0.20 mg GAEs/g dw in CTRL and RGM/TG spaghetti, respectively; on the contrary, the cooked pasta had 0.28 ± 0.02 mg GAEs/g dw in the reference sample and 2.1 ± 0.2 mg GAEs/g dw in the enriched sample. There was no statistically significant difference between raw and cooked pasta for both CTRL and RGM/TG pasta, thus indicating no significant effect of cooking on the content of bioactive compounds. This is probably due to fact that RGM polyphenols are mostly in glycosidic form localized in cellular vacuoles and other organelles, that may protect them from leaching or decay during cooking process (Sakihama et al., 2002). In addition, according to obtained data RGM represent a anthocyanin source. In fact, in the fortified spaghetti the content of anthocyanins was: 0.095 ± 0.008 and 0.084 ± 0.002 mg mvd-glu / g dw for uncooked and cooked pasta, respectively, while no anthocyanins (TAC) were detected in the CTRL sample before and after cooking (Table 13). This is in line with data from Sant'Anna et al. (2014). Similar to the total polyphenols, the cooking process had no significant effect on the total anthocyanin concentration in the RGM/TG spaghetti. Finally, as can be seen from Table 13, the uncooked and cooked RGM/TG spaghetti exhibited a high antioxidant activity, 54.5 ± 7.7 before cooking and 56.2 ± 9.7 μmol Fe(II)/g dw after cooking, compared to the control sample. Again, the cooking procedure had no effect, in fact no loss of antioxidant activity of either CTRL or RGM/TG was observed after cooking. These findings are in line with the observations for the total polyphenol and anthocyanin contents. Fares et al. (2010) even observed an greater antioxidant capacity in the pasta samples enriched with the debranning fractions after cooking; possibly this is due to the better extraction efficiency of bound polyphenols like phenolic acids. A similar effect was observed by Turkmen et al. (2005), who studied the effect of the cooking method on total polyphenols and antioxidant activity of different vegetables**.**

4.3.3 Bioaccessibility of polyphenols and glucose

In order to affect human health beneficially, it is important that the added phytochemicals in food products are available for absorption in the gastrointestinal tract. Therefore, the bioaccessible total polyphenol and anthocyanin content as well as the antioxidant activity were evaluated in this study. Data are shown in Table 14 and as can be seen RGM/TG

spaghetti contained a statistically significant higher amount of bioaccessible total polyphenols, available for absorption in the intestine, than CTRL spaghetti: 5.53 ± 0.61 vs. 4.16 ± 0.50 mg GAEs/g dw. Furthermore, in both experimental samples (RGM/TG and CTRL) the polyphenol concentration increased after *in vitro* digestion compared to cooked pasta before digestion, while maintaining a similar difference observed between cooked and non-digested RGM and CTRL samples. A possible explanation may be the difference in extraction efficiency from samples (*in vitro* digested *vs.* non-digested). As Gawlik-Dziki et al. (2015) showed, the use of gastrointestinal digestive enzymes led to release of bound phenol acids as well as amino acids from wheat proteins such as cysteine, tryptophan, and tyrosine. These amino acids can react with Folin-Ciocalteu reagent and mimic the reaction of polyphenols (Abdel-Aal and Hucl, 2002; Everette et al., 2010). The same trend was also observed by Miranda et al. (2013), who compared the polyphenol amount extracted from potatoes by means of the chemical method and *in vitro* digestion.

Table 14 Total polyphenol content, antioxidant activity (FRAP assay) and total anthocyanins in the bioaccessible fraction of cooked CTRL and RGM/TG spaghetti after *in vitro* digestion.

	Cooked spaghetti	
Bioaccessible fraction	**CTRL**	**RGM/TG**
Total polyphenols (mg GAEs/g dw)	4.2 ± 0.5[a]	5.5 ± 0.6[a]
Total anthocyanins (mg mvd-glu /g dw)	ND	0.037 ± 0.001
Antioxidant activity (μmol Fe(II)/g dw)	6.3 ± 2.6[a]	25.3 ± 4.9[b]

ND: not detectable. CTRL: control spaghetti; RGM/TG: spaghetti containing red grape marc flour (15% w/w; particle size ≤ 125 μm) and 0.6% (w/w) transglutaminase.
Results are expressed as means ± SD, $n = 3$.
[a,b]Data in the same row with different letters are significantly different ($P < 0.05$), as determined by ANOVA followed by the Tukey-Kramer test.

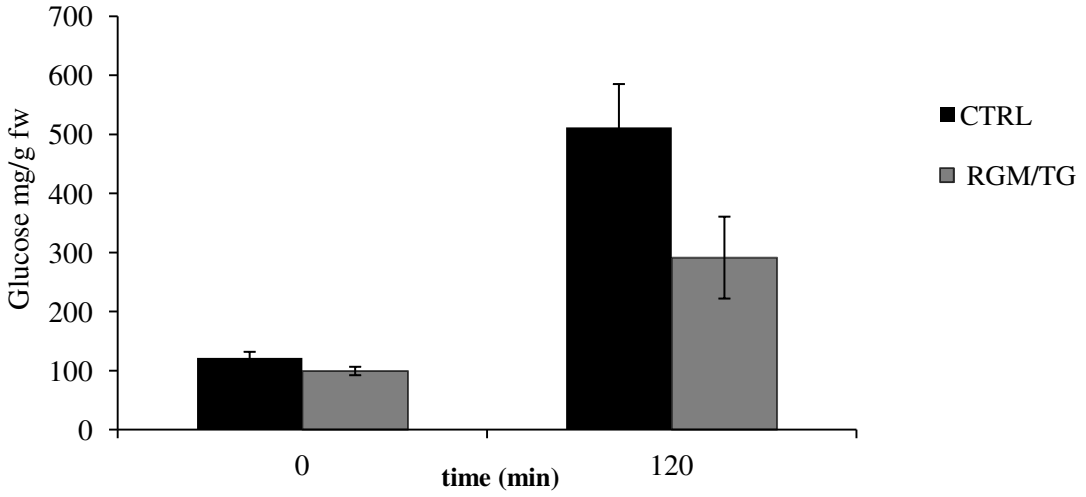
Furthermore, it was observed a significant amount of bioaccessible anthocyanins in the digested RGM/TG- samples but no anthocyanins in the CTRL spaghetti, assessed spectrophotometrically as well as by HPLC. Both these methods used showed similar values of total anthocyanins in RGM/TG sample: 0.037 ± 0.001 mg mvd-glu/g dw and

0.039 ± 0.005 mg mvd-glu /g dw, respectively. In contrast to the total polyphenols, the bioaccessible total anthocyanins after *in vitro* digestion was lower than that observed in the cooked spaghetti before digestion. Probably, a part of the anthocyanins was destroyed or remained in the food matrix. Several authors found that the total polyphenols increased while the anthocyanin amount decreased during digestion, due to the instability of anthocyanins in the neutral/alkaline intestinal environmental (pH = 7.2-7.6) (McDougall et al., 2005; Podsędek et al., 2014; Tagliazucchi et al., 2010).

Regarding the antioxidant activity of the bioaccessible fraction (Table 14), the RGM-enriched pasta showed a significantly higher antioxidant activity than the CTRL sample: 25.3 ± 4.9 vs. 6.3 ± 2.6 μmol Fe(II)/g dry weight (dw), respectively. However, there was an increase in the activity of the CTRL sample (6.3 *vs.* 2.75 μmol Fe(II)/g dw,) and a decrease in the RGM/TG spaghetti (25.3 *vs.* 56.2 μmol Fe(II)/g dw) compared to the respective cooked, non-digested samples (Table 13 - 14). In the CTRL spaghetti the observed increase is probably due to the release of amino acids from wheat durum semolina proteins and liberated phenolic acids such as ferulic acid, substances characterized by antioxidant activity, during digestion by enzymes (Gawlik-Dziki et al., 2015; Miranda et al., 2013). In contrast, the antioxidant activity of the fortified sample (RGM/TG) decreased from approx. 56 to approx. 25 μmol Fe(II)/g dw. This decrease can be due to loss of active but also sensitive to *in vitro* digestion RGM-polyphenols and the efficiency of antioxidant extraction from the matrix. The cooked spaghetti was extracted using methanol/water/HCl. In contrast, the bioaccessible fraction was obtained using gastrointestinal enzymes, water and physiological temperature, pH. This can lead not only to different concentrations of polyphenols but maybe most importantly to a different composition of individual polyphenols in these extracts. The antioxidant activity varies enormously among polyphenols. Despite the higher total polyphenol level in the bioaccessible fraction seems that the individual polyphenols in this fraction exhibit lower antioxidant activity than those in the methanol/ water/HCl extract before *in vitro* digestion. Possibly, effective polyphenol antioxidants like anthocyanins are partly destroyed under physiological pH at the small intestine conditions yielding polyphenols with lower antioxidant activity. Reducing energy density and glycemic load can have a positive effect to prevent obesity and associated diseases (Rolls, 2009). Spaghetti is rich in complex carbohydrates that are effectively digested to glucose in the small intestine. Therefore, in the last part of this work the glucose amount available for absorption in the small intestine phase was also investigated in order to show that the enrichment of

spaghetti with red grape marc can cause a significant reduction of bioaccessible glucose release in the small intestine model after 2h of digestion. Data are shown in the Figure 4. At t=0 (directly after the gastric phase and before the small intestine phase of digestion), the bioaccessible glucose concentration was 121.7 ± 10.2 and 99.4 ± 7.1 mg Glu/g fw for CTRL and RGM/TG spaghetti, respectively. No statistically significant difference between experimental spaghetti samples was detected. But at the end of the small intestinal phase of digestion (after 2h) the enriched spaghetti showed a significantly lower concentration of bioaccessible glucose (291.4 ± 69.3 mg Glu/g fw) compared to the control spaghetti (511.5 ± 73.7 mg Glu/g fw). This is due to the fact that in the fortified sample the wheat flour starch was substituted with RGM flour, which does not contain starch or other digestible carbohydrates. Similar data were reported by Biney and Beta (2014).

Figure 4 Bioaccessible (available for absorption) glucose during *in vitro* digestion of CTRL and RGM/TG spaghetti.



CTRL: control spaghetti without the addition of red grape marc flour; RGM (RGM/TG): spaghetti containing red grape marc flour (15 % w/w; particle size ≤ 125 µm) and 0.6 % (w/w) transglutaminase; Glu: glucose; fw: fresh weight. t=0 min (before digestion of the spaghetti samples in the small intestinal phase).

4.4 *Case study: Fish Burger fortified with Extract from Broccoli By-Products*

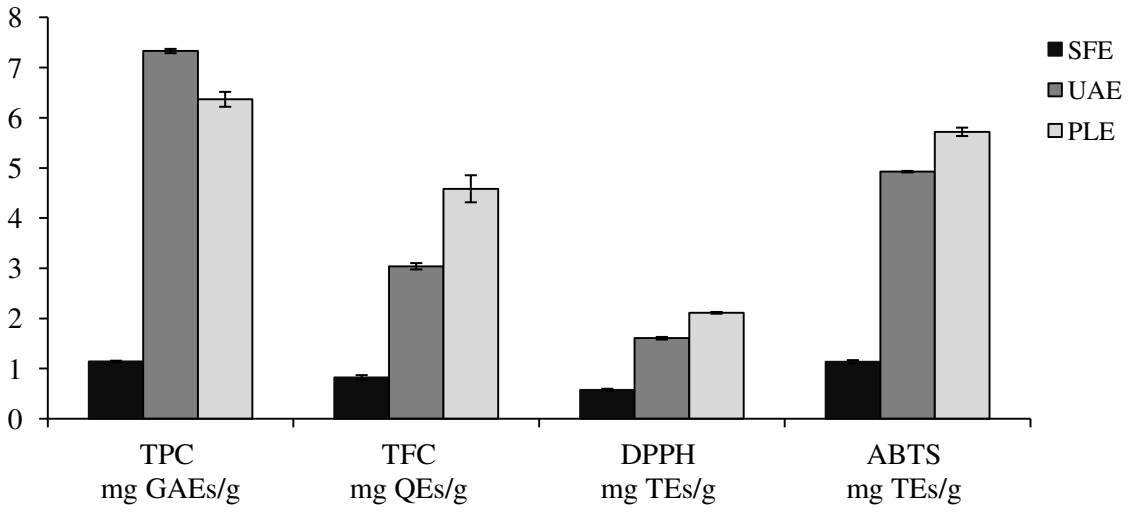
In this study the use of extract from broccoli by-products (steams and leaves) has been proposed, being reach in active compounds. To this aim, in the first step three extraction techniques have been compared in terms of extraction efficiency. Then, a microencapsulation process of the extract has been optimized considering the following parameters: wall material (Capsul, maltodextrin 14-16 DE), concentration of wall material (10-20-30%, w/v), ratio of core:wall material (1:2, 1:5, 1:10, 1:20) and inlet temperature (80, 100, 130,150,170°C). Finally, the obtained broccoli powder has been added at 5% (w/w) in fish burgers.

4.4.1 Identification of the most efficient extraction technique

In order to produce high-value ingredient, it is important to identify an extraction method that gives high concentration of bioactive compounds in the extract. Therefore, in this work three extraction techniques were compared in terms of extraction efficiency from broccoli by-products. Then, total phenolic compounds (TPC), total flavonoid compounds (TFC) and antioxidant activities (DPPH and ABTS) of the extracts were measured. As can be seen in Figure 5, the highest total phenolic content was obtained with UAE (7.33 ± 0.04 mg GAEs/g dw), followed by PLE (6.37 ± 0.15 mg GAEs/g dw) and SFE (1.15 ± 0.01 mg GAEs/g dw).

This result is in accordance with other data in the literature. Nayak et al. (2015) compared different non-conventional extraction techniques to recover bioactive compounds from Citrus sinensis peels and observed that UAE gave a higher amount of total phenols compared to PLE. Also, Liazid et al. (2010) noted a higher yield of polyphenols with UAE than PLE. This behavior could be attributed to the cavitation phenomena generated by the ultrasonic waves (González-Centeno et al., 2014). The PLE differed slightly from UAE, while SFE showed a much lower yield than the other two methods, probably because it is a more selective technique and its efficiency depends on solubility of compounds in the supercritical fluid, that is governed by the density of the fluid, specifically by applied pressure and temperature (Cheah et al., 2010). Furthermore, even if supercritical fluids have similar characteristics to liquids, they are less able to break the bonds between matrix and analyte.

Figure 5 Total phenols, total flavonoids and antioxidant activity of the extract obtained from different extraction techniques (SFE, UAE and PLE).



As far as the other parameters is concerned (TFC, DPPH and ABTS), the PLE technique represented the best extraction method. Specifically, high amount of flavonoids (4.59 ± 0.27 mg QEs / g dw) and antioxidant activity (2.11 ± 0.02 and 5.72 ± 0.08 mg TEs / g dw, for DPPH and ABTS, respectively) were recorded. The high content of total flavonoids could be linked to a greater sensibility of these substances to the extraction time. Long time could cause their degradation. In fact, the PLE was shorter than the other two extraction techniques. The extraction process took about 20 min, 1 h and 140 min for PLE, UAE and SFE, respectively. This result was also confirmed by Casazza et al. (2010) who compared several non-conventional extraction methods (UAE, MAE and HPTE) vs classic solid–liquid extraction in terms of extraction yield and antioxidant power of the extract and observed that prolonging the time over 30 min the yield of total flavonoids decreased. Furthermore, the PLE gave a better extract from the qualitative point of view, since it contained a greater antioxidant activity compared to SFE and UAE extracts. It is known that the antioxidant activity depends more significantly from the quality of the phenolic compounds and not from their quantity (Otero-Pareja et al., 2015). According to the obtained data, we can conclude that the PLE represented the more efficient extraction technique to recover bioactive compounds from broccoli by-products. It showed only a little difference in TPC compared to UAE, but according to Nayak et al. (2015) it could be due to interactions between phenolic and non phenolic compounds during the extraction.

4.4.2 Extract microencapsulation

As Jafari et al. (2008) have stated, the successful of the microencapsulation process of nutraceutical extracts relies on achieving the maximum retention of the core within the powdered material. Hence, for the optimization of the microencapsulation of broccoli extract, different parameters were considered: wall materials (Capsul and maltodextrins), their concentrations (10, 20, 30%), core/wall ratio (1/20, 1/10, 1/5 and 1/2) and inlet temperature (170, 150, 130, 100, 80 °C), making a total of 28 experiments.

Table 15 Chemical characterization of the broccoli by-products powder obtained at different core/wall ratio and concentration of wall material.

	TPC mg GAEs/g dw	**TFC** mg QEs/g dw	**DPPH** mg TEs/g dw	**ABTS** mg TEs/g dw
		1:20		
Capsul 10%	2.10±0.09c	0a	1.37±0.04c	4.99±0.07f
MD 10%	3.50±0.07e	2.69±0.40c	4.86±0.06f	4.23±0.03e
Capsul 20%	1.29±0.05b	0a	1.07±0.02b	3.60±0.10d
MD 20%	2.51±0.11d	1.42±0.31b	4.50±0.06e	2.32±0.03b
Capsul 30%	0.86±0.12a	0a	0.90±0.04a	2.99±0.08c
MD 30%	1.97±0.10c	0.54±0.23a	4.36±0.04d	1.67±0.03a
		1:10		
Capsul 10%	4.56±0.17e	1.15±0.23a	2.13±0.03a	6.82±0.15e
MD 10%	5.93±0.25f	2.89±0.20d	5.26±0.06f	7.01±0.07e
Capsul 20%	2.54±0.05b	1.21±0.12a	2.51±0.01b	5.34±0.09d
MD 20%	3.35±0.02d	2.42±0.23c	4.82±0.06e	4.11±0.11b
Capsul 30%	1.81±0.07a	1.01±0.12a	2.26±0.03c	4.42±0.11c
MD 30%	2.90±0.09c	1.55±0.51b	4.59±0.05d	3.08±0.11a
		1:5		
Capsul 10%	7.92±0.10d	4.23±0.23c	3.04±0.04c	9.76±0.19d
MD 10%	9.31±0.22e	5.71±0.00d	5.62±0.06f	10.50±0.15e
Capsul 20%	4.62±0.04b	3.16±0.31b	2.92±0.01b	7.91±0.02c
MD 20%	5.37±0.07c	3.49±0.00b	5.03±0.02e	6.67±0.17b
Capsul 30%	3.21±0.02a	2.09±0.20a	2.70±0.02a	6.45±0.09b
MD 30%	4.47±0.02b	1.68±0.35a	4.87±0.03d	5.10±0.14a
		1:2		
Capsul 10%	13.97±0.11e	12.48±0.31e	5.66±0.01e	13.39±0.08d
MD 10%	16.04±0.14f	12.14±0.20e	5.68±0.09e	15.23±0.20e
Capsul 20%	8.53±0.16c	6.51±0.35c	6.54±0.01b	11.73±0.14c
MD 20%	10.40±0.15d	7.72±0.20d	5.56±0.00d	11.60±0.12c
Capsul 30%	6.10±0.09a	4.50±0.40b	3.25±0.01a	9.79±0.06b
MD 30%	7.33±0.15b	3.56±0.12a	5.30±0.03c	8.84±0.19a

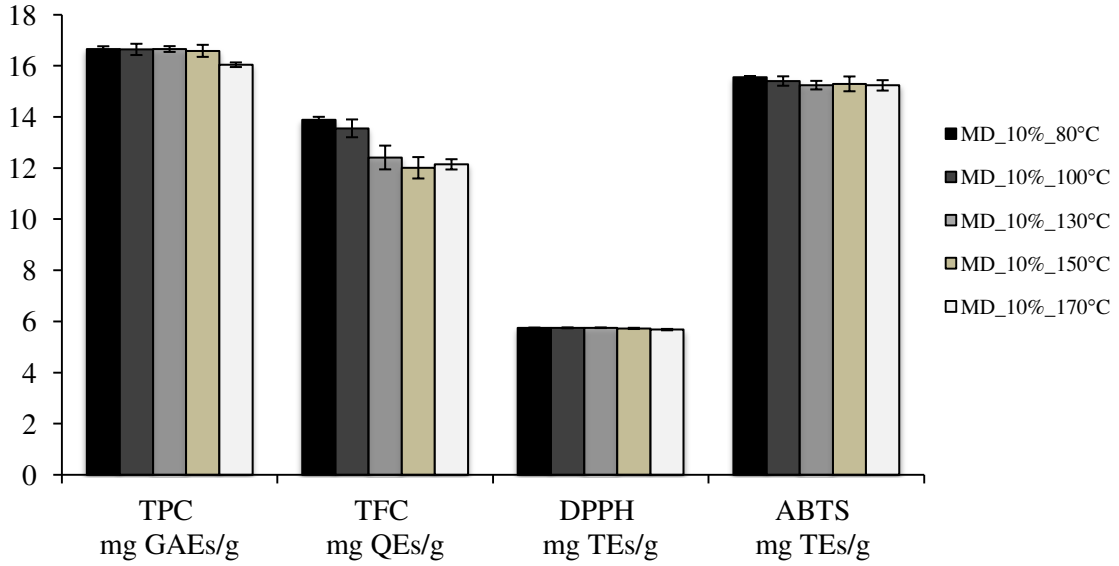
Results are expressed as means ± SD for $n = 3$. a-f data in columns are significantly different ($P < 0.05$), as determined by ANOVA followed by the Fischer'test.

As Table 15 and Figure 6 show, broccoli powders were chemically characterized in terms of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity by means DPPH and ABTS assays. The inlet temperature was fixed at 170 °C and the effects of the concentration of Capsul and maltodextrins on the bioactive compounds content were investigated. As can be seen in Table 15, the properties of selected wall materials statistically affected the encapsulation efficiency of samples. Specifically, it was observed low retention of bioactive compounds by increasing the concentration of both wall materials from 10 to 30% (w/v), probably due to the increase in total solids content and consequently high viscosity of the infeed emulsion. Some authors also noted that there is an optimal level of total solids concentration (Jafari et al., 2008; Reineccius and Bangs, 1985). So, if more wall material was added, the solution become over-saturated and therefore the un-dissolved wall material does not have any protective effect, thus reducing the core compounds retention. Furthermore, increasing the solids amount, the emulsion viscosity increases. This finding improves the retention up to an optimal level, beyond which the retention decreases due to long spray-drying process and slow droplet formation(Jafari et al., 2008; Rosenberg et al., 1990). Finally a more viscous emulsion generates larger drops. The larger the particle size, the lower the retention, because the formation time of droplets membrane is longer and thus the loss of bioactive substances increased. Another reason for which core compounds retention decreased with increasing wall material concentration is probably the presence of agglomerations and cakes in the powder, as also noted by Sansone et al. (2011). They identified the cause of this behavior in the delay of a semi-permeable layer development. In agreement with the results by Sansone et al. (2011), the less concentration of wall material (10%) was found the most appropriate to microencapsulate broccoli by-product extract. Maltodextrins and Capsul are encapsulating agents widely used in the food industry, however, even though Capsul has good retention, stability and emulsifying properties (Aburto et al., 1998), in Table 15 it can be observed that the preservation of bioactive compounds was significantly greater with the use of maltodextrins. According to Bakowska-Barczak and Kolodziejczyk (2011), maltodextrins are produced from the acid hydrolysis of corn starch, showing high water solubility and low viscosity. Most likely, thanks to these structural chemical differences, maltodextrins appeared to be wall material that offered greater bioactive compounds protection than Capsul. In previous works it has been also confirmed the microencapsulation efficiency of maltodextrins. Sansone et al. (2011) for example studied the capacity of maltodextrin/pectin matrix to spray-dry polyphenol-rich extracts,

and Silva et al. (2013) demonstrated the feasibility to microencapsulate jaboticaba extracts with 30% maltodextrins. As reported in the study of Wu et al. (2014) it was shown that maltodextrins could provide a good sulforaphane retention after spray-drying. Hence, maltodextrins have been found more suitable for microencapsulate broccoli by-product extract. Microencapsulation efficiency was not only affected by the type of wall material but also by the core/wall ratio, term commonly used to indicate the concentration of core material and that has great effect on the properties of powder (Hogan et al., 2001). In general, there is an optimal and specific core-to-wall ratio, although in most of the previous studies a core/wall ratio of 1:4 was usually adopted. In this regard, Jafari et al. (2008) have proposed to optimize the core concentration in order to obtain different benefits such as less wall material, yield and output increase and positive economic impact. As shown in Table 15, results indicated that increasing core/wall ratio from 1/20 to 1/2, TPC, TFC, DPPH and ABTS increased. Most probably, the reason may be associated with the instability of prepared mixture when core/wall decreased from 1/4 (Shu et al., 2006). Furthermore, this trend was confirmed by the findings of Goula and Adamopoulos (2011) who, developing a new technique for lycopene microencapsulation by spray-drying, noted that increasing core/wall from 1/19 to 1/3, the encapsulation efficiency increased. Therefore, the most advantageous core-to-wall ratio for microencapsulating broccoli by-products extracts was identified in 1/2. Finally, keeping core/wall ratio of 1/2 and using 10% maltodextrins as wall material, the effect of different inlet temperatures was also assessed. As can be seen in Figure 6, when the inlet temperature increased from 80 °C to 170 °C, DPPH (5.78-5.75 mg TEs/g powder) and ABTS (15.2-15.5 mg TEs/g powder) remained almost unchanged. On the contrary, TPC (16.04-16.66 mg GAEs/g powder) and especially TFC (12.01-13.89 mg QEs/g powder) were more affected by the temperature. Several authors have argued that with high air inlet temperature premature release and degradation of the encapsulated ingredients can occur (Cai and Corke, 2000; Goula and Adamopoulos, 2011; Silva et al., 2013; Tonon et al., 2011; Wu et al., 2014). Specifically, Zakarian and King (1982) reported that elevated values of temperature cause the formation of cracks in the membrane, inducing an excessive evaporation of pigments. Contrarily, lower temperatures produced powders with agglomerations that reduce the exposition to oxygen, defending the pigment against the oxidation (Quek et al., 2007). Based on the results obtained, 80 °C represented the most suitable inlet temperature for production of microcapsules as stable source of bioactive compounds. In conclusion, the optimal conditions for the microencapsulation of

broccoli by-product extract were established as follows: concentration of maltodextrins at 10%, ratio of core to wall material of 1/2 and inlet temperature of 80 °C. Applying these experimental conditions, the highest values of TPC (16.6 ± 0.1 mg GAEs/g powder), TFC (13.9 ± 0.1 mg QEs/g powder), DPPH (5.75 ± 0.01 mg TEs/g powder) and ABTS (15.55 ± 0.05 mg TEs/g powder) were found (Figure 6).

Figure 6 Chemical characterization of the broccoli by-products powder obtained at different inlet temperatures.

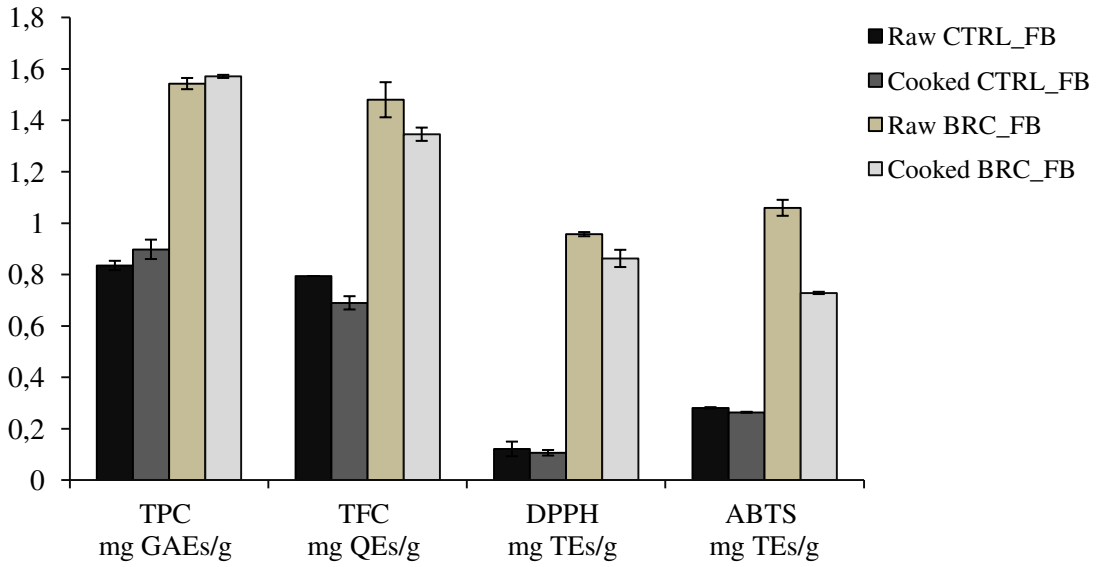


4.4.3 Application of microencapsulate extract to fish burger

Figure 7 shows the total phenolic content, total flavonoid content and antioxidant capability of raw and cooked fish-burgers with and without extract.

As expected, the incorporation of 5% microencapsulated extract significantly enhanced the concentration of bioactive compounds and the antioxidant activity when compared with control ($p < 0.05$). After cooking the TFC and the antioxidant activity of the fortified samples were slightly reduced (raw BRC_FB presented 1.48 ± 0.07 mg QEs g^{-1}, 0.96±0.01 mg TEs g^{-1} for DPPH and 1.06±0.03 mg TEs g^{-1} for ABTS while cooked BRC_FB 1.35 ± 0.03 mg QEs g^{-1}, 0.86 ± 0.03 TEs g^{-1} for DPPH and 0.73 ± 0.00 TEs g^{-1} for ABTS) but no significant variation ($p > 0.05$) was found in terms of TPC concentrations in both raw and cooked samples (1.54 mg GAEs g^{-1} in the raw burger and 1.57 mg GAEs g^{-1} in the cooked sample), thus confirming the attractive recycling of broccoli by-product as food functional ingredient.

Figure 7 Chemical characterization (TPC, TFC, DPPH and ABTS) of raw and cooked fish-burgers with and without broccoli powder at 5% (w/w).



CTRL_FB: control fish-burger; BRC_FB: fish-burger enriched with broccoli powder.

5. Conclusions

The case-studies described in this research thesis have shown the high potential of food by-products for development of innovative and healthy foods. Obtained data have proved that fruit and vegetable by-products represent valid raw materials to be used as food ingredients, allowing to produce foods with health properties, satisfying a market niche based on functional and sustainable product and reducing at the same time the environmental pollution and waste disposal problems. In addition, these raw materials are often cheap and interesting source of antioxidants, especially phenolic compounds. The exploitation of fruit and vegetable by-products as source of functional compounds and their application in food is a promising field, also offering new ways to diversify the food production. Products enrichment seems to be a very easy and inexpensive way for valorizing by-products, however, there are some important factors that still limit their application. One of the most important is linked to the sensory aspects that are very often compromised by this addition. Therefore, in every case study addressed the nutritional and sensory quality were both taken into account and a proper compromise was found. Both bread and pasta were enriched with 15% (w/w) vegetable by-products. Specifically, bread was fortified with artichoke leaf flour, while spaghetti with red grape marc. The improvement of sensory aspect was obtained using in the first case a structuring agent, carboxy methyl cellulose, while in the second study the sensory quality was improved by using a smaller particle size of red grape marc flour and proper amount of transglutaminase. In the case-study concerning the watermelon-based candy, a whole quality index which takes into account both nutritional and sensory quality, was calculated to determine the best combination of orange by-products to be added to develop a product with additional chemical properties without compromising its acceptability. As regard the last case study, several extraction techniques (SFE, PLE, UAE) were evaluated to record broccoli by-product extract and among them, PLE was found the best method because gave the greatest content of bioactive compounds and the best antioxidant activity of the relative extract. Furthermore, spray-drying process was investigated to protect the extract. The powder with high concentration of phenols, flavonoids and antioxidant activity was obtained using maltodextrins at 10% as wall material and keeping core/wall ratio of 1/2 at 80 °C as inlet temperature. Finally, the obtained powder was used as food ingredient and added to fish burger formulation.

In all the case studies addressed, the findings showed that the enrichment with by-products increased the polyphenol compounds and the antioxidant activity of the product, highlighting the possibility to reuse plant industrial residues in other production system through appropriate technological devices. In the abundant scientific literature, the current approach to the development of fortified foods based on using by-products as novel ingredients has focused on selecting specific concentrations of by-products to improve the technological and healthy properties of products. However, in many scientific studyes these substances have been often added in low concentrations to not alter the sensory aspect and not all the studies included sensory analyses of developed product. Therefore, this PhD-thesis have emphasized the nutritional evaluation, increasing the vegetable residues concentration at the highest levels without neglecting the sensory properties. Furthermore, it has been also given importance to the bioaccessibility of added phytochemicals in the food product after complete digestion. Obviously, further investigations are still necessary, such as clinical studies that confirm the positive effects of different antioxidant components added to foods on human health. In addition, concerns regarding food quality and safety that may arise from using industrial residues in food formulations should be also better examined. Finally, in the future it would be also interesting to study how the new sustainable product impacts on the environment.

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