



Polyphenols Extraction From Vegetable Wastes Using a Green and Sustainable Method

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Food systems have the potential to support human health, respecting the environmental sustainability principles. Food sustainability, enclosed in the concept of environmental sustainability, involves different aspects, including the recycling of food waste derived from the agri-food production chain, the use of biotechnologies ensuring the sustainability of the recovery processes of bioactive compounds from food waste and, last but not least, the awareness of having to consume and waste less food. Food loss and waste is generated during the whole supply chain, from production to household utilization. The utilization of agricultural wastes as an abundant, renewable and low-cost source for the production of high value-added products is currently explored. The bioactive compounds present in these sources have been proved to possess a wide range of biological activities; therefore, research is needed into the application of environmentally friendly traditional and advanced techniques with low production costs in the extraction, isolation and purification of phytochemical compounds from agricultural wastes in high yields and at maximal quality. Authors of this manuscript propose and discuss an innovative and sustainable extraction system of polyphenols from vegetable waste, based on an enzymatic pre-treatment coupled with a solid-liquid extraction by using a particular extractor (Naviglio Estrattore®). This extraction system, organic solvent free, allowed to extract relevant amount of polyphenols (flavonoids in particular) from several vegetable waste products.

Keywords: food system sustainability, food waste, food waste recycling, bioactive compounds, enzymatic extraction, polyphenols

INTRODUCTION

Food Loss and Waste in the Food Supply Chain

The reduction of food loss and waste is considered by the United Nations and many international institutions as one of the main ways to proceed toward the protection of the environment and for the well-being of humanity, as also reaffirmed by the UN 2030 Agenda for Sustainable Development [ODD-ONU Italia (unric.org)]. Furthermore, food waste is a key determinant, inter alia, of the loss of biodiversity, the dispersion of greenhouse gases in the atmosphere, the pollution of water, soil and other resources (www.isprambiente.gov.it). The main global factors, responsible of the large amount of food waste, are:

- the increase in the world population and urbanization,
- the great availability of fossil energy sources,
- the economic and cultural diffusion of mass agro-industrial systems,
- the transformation of (food) lifestyles
- assigning a relatively low economic and socio-cultural value to food (Clapp, 2002; FAO, 2011; Gille, 2012).

Several evidences also show that the increase of food waste, especially when produced during sales and consumption, is directly proportional to the increase of the levels of economic development (29, 30). It is extremely hard to exactly quantify the lost and wasted food in the world today and, even more difficult, is to prevent the food losses. Moreover, currently, there is not much research in the area, which is quite surprising considering that food production must significantly increase to meet future global demand. Not much attention appears to be paid to current global food supply chain losses (Gustavsson et al., 2011). The issue of food loss and waste represents an important topic in the efforts to reduce hunger, raise income and improve the food security in the world's poorest countries. Food losses have a relevant impact on food security for poor people, on food quality and safety, on economic development and on the environment. According to the Food and Agriculture Organization (FAO), 1.300.000.000 tons of food gets yearly lost or wasted in the all the world, representing approximately one-third of the edible parts of food produced for human consumption.

Food loss and waste is generated throughout the entire supply chain: the 54% of total loss and waste occurs during the upstream processes (including production and postharvest) and 46% of total loss and waste occurs during the downstream processes (including processing, distribution, and consumption) (Mirabella et al., 2014). In particular, in developing countries more than 40% of these food losses occur at post-harvest and processing levels, while in industrialized countries, around the 45% of the food losses occurs at retail and consumer levels. In Europe every year, 280–300 kg per capita of food at different stages are wasted and lost, mainly concerning the vegetables and fruit sector (Gustavsson et al., 2011; FAO et al., 2014). According to the report by the FAO of the United Nations (FAO et al., 2014), 45% of fruit and vegetable wastes and by-products from the fruit and vegetable processing industry are generated around the world throughout the entire food supply chain. Most of fruit and vegetable wastes is disposed in landfills or rivers, which represents a threat to the environment due to their high biodegradability, leachate, and methane emissions (Misi and Forster, 2002). However, these resources have a great potential to be used for the recovery of value-added products (Wadhwa and Bakshi, 2013).

Food Waste Recovery Improves Sustainability of Food System

A food system assembles different aspects (environment, people, inputs, processes, infrastructures, institutions, etc.) and activities related to the production, processing, distribution, preparation, and consumption of food, and their socioeconomic and environmental outcomes (HLPE, 2014). As mentioned

before, food waste has an important impact on food and nutrition security, food quality and safety, natural resources, and environmental protection. For these reasons, management of food loss and waste, with their co- and by-products have already attracted the attention of food scientists and industry over the last decades (Galanakis, 2015). In fact, recently several scientific reports, relevant to food waste and treating methods, were published, especially including strategies for reduction of waste production, the valorisation of co- and by-products, and improvement of waste management. This increasing attention is mainly due to the following reasons:

- a) the growing environmental concerns,
- b) the necessity to minimize the impact of waste on human health,
- c) the high costs of waste disposal that are limiting the profits of the food industry,
- d) the growing interest toward benefits deriving from potentially marketable components present in food wastes and co-products (Laufenberg et al., 2003).

For all these reasons, unlike the traditional linear economic model, based on a “take-make-consume-throw away” pattern, a circular economy model is conceived and based on sharing, leasing, reuse, repair and recycling, in an (almost) closed loop, where the contained products and materials are highly valued (Bourguignon, 2016).

Agriculture not only is ensuring global food security for over 7 billion people around the world, but also plays an important role in supporting and promoting the development of other industries, such as nutraceuticals, pharmaceuticals and cosmetics. In particular, agri-food industry produces a large amount of wastes and residues along the whole supply chain, still containing a significant quantity of valuable bioactive compounds, such as polyphenols (phenolic acids, flavonoids, proanthocyanidins, anthocyanins, glycosides...), carotenoids, saponins, tannins, alkaloids, sterols, steroids, triterpenes, peptides and carbohydrates properties (Moure et al., 2001; Llorach et al., 2002; Zhang et al., 2017), which, as largely demonstrated, possess several biological activities, including antioxidant, antibacterial, antifungal, antiviral, antimicrobial, antidiabetic, anticancer, antidiarrhoeal, antihypertensive, antimutagenic, anti-inflammatory, anticholesterol and protective properties of cardiocirculatory system (Balasundram et al., 2006; Santana-Méridas et al., 2012; Nguyen, 2017). Increasing the yield of the target compounds adopting the most appropriate food waste recovery strategies, the economic value gained from them could be maximized. Moreover, the recovery of high added-value material could result in the development of innovative products and/or materials (Galanakis, 2015) and highlights the importance of science-based innovation to improve the sustainability of food system (Defra, 2006). There is another important aspect to consider: the recovery of food wastes and its valuable bioactive compounds could also help in promoting the viability and diversity of rural and urban economies, contributing to create new job opportunities (Galanakis, 2015). Finally, reducing food waste, by recovering compounds with biological activity present in the waste, represents an important solution to increase the

sustainability of the food production system, also contributing to the reduction of waste management costs which can represent a serious problem, especially for smaller producers.

Sustainability of Extraction Processes

Food by-products and wastes contain highly complex components with a relevant biological and economical value, although these residual materials generally present a lower concentrations of valuable compounds respect to the initial sources. Fruit and vegetable wastes, in particular, contain a significant amount of biologically active compounds (value-added products) like polyphenols, glucosinolates, dietary fibers, essential oils, pigments, organic acids, etc. (Baiano, 2014; Kumar et al., 2017). In addition, in some vegetable materials, the content of bioactive compound in by-products is higher than in their major parts. These compounds can be extracted, purified, and characterized using emerging technologies, allowing to develop new commercial applications in food and non-food (pharmaceutical, biomedical, cosmetic, etc.) areas (Galanakis et al., 2012). Even in the process of recovering food waste, the reduction of energy and the optimization of raw materials can significantly reduce costs and at the same time increase the environmental sustainability of the food system. An efficient food waste recovery process has two meanings; firstly, it is necessary to use only that type of material that otherwise would have been thrown away and, secondly, to use / process that material as efficiently as possible, using technologies and extraction methods functional to the type of compounds to be extracted. Food wastes are generated in different forms and compositions, according to regional, seasonal, and processing characteristics in each case (Gustavsson et al., 2011). This implies that the cost for the processing could increase, as well as the recovery yield can decrease. Moreover, food wastes are susceptible to microbial contamination and require both proper preservation and fast treatment. Following all these considerations, the development of an economically feasible, sustainable, and safe recovery of bio-active compounds from food residue must take into account several parameters such as the abundance and distribution of food wastes (related to the presence of industry), the set-up of methodologies providing the highest recovery yield of different compounds, the utilization of green solvents, the preservation of the biological properties of selected compounds from source to final product, waste minimization prior to the recovery process, the application of environmentally friendly traditional and advanced techniques with low production costs in the extraction, isolation and purification of phytochemical compounds from agricultural wastes in high yields and at maximal quality (Varzakas et al., 2016; Nguyen, 2017). Hence, technological advances in extraction, separation and identification have been developed to produce natural products with potent biological activity (Van Lanen and Shen, 2006; Wang and Weller, 2006). The biggest research challenge is now the identification and set up of the best “extraction” conditions, i.e., the condition that improve release of the bioactive compounds from the vegetable matrix in which they are encased. The recovery process of bioactive compounds from fruit and vegetable waste includes several important stages in succession: preparation of dried

samples, extraction process, production of the powder extract, isolation and purification by chromatography (Pham, 2017). Each of these different phases involves the use of different technologies, aimed at the recovery of specific molecules with different physico-chemical characteristics; however, it is important that, in compliance with sustainability criteria, each of these processes has a reduced environmental impact both in terms of energy and the use of solvent. Regarding the extraction methods, conventional methods, commonly found in literature, are very numerous and long to list but the main used are liquid-liquid or solid-liquid extraction, primarily based on the use of organic solvents. Nowadays, in respect with “Circular Economy” principles, alternative and innovative extraction systems are used, depending on the characteristics of target compounds; the main emerging techniques, considered innovative, rapid, reproducible, cost/benefit, and clean, are the following:

- Ultrasound Assisted Extraction (UAE)
- Pulsed Electric Field (PEF)
- Microwave assisted Extraction (MAE)
- Pressurized Liquid Extraction (PLE)
- Supercritical Fluid Extraction (SFE)
- Enzyme Assisted Extraction (EAE)

Except for solvent extraction, whose sustainability is depending on the type of used solvent, the other listed technologies are considered “green,” although the cost of some equipment used in the extraction plants is sometimes high. Enzyme Assisted Extraction is perhaps the most environmentally and economically sustainable of those listed above. The main mechanism behind EAE involves cell wall degrading enzymes such as glucanase and pectinase which weaken or deconstruct the cell wall, making intracellular compounds more accessible for extraction.

STRATEGY FOR POLYPHENOLS EXTRACTION FROM VEGETABLE WASTE MATERIAL – AN EXPERIMENTAL EVIDENCE

An eco-friendly extraction system, alternative to conventional solvent-based and complex physical methods, was set up by the authors of this papers and it will be described below.

Some vegetable waste, obtained from manufacturing industry, were used as source of bioactive compounds such as polyphenols. Since many polyphenols are found to form complexes with plant cell-walls structures (proteins and carbohydrates), often recalcitrant to degradation, the treatment of plant materials with enzymatic mixtures containing cellulases, hemicellulases and pectinases, promotes the hydrolytic degradation of cell wall polymers, thereby favoring the release of secondary metabolites (Khandare et al., 2011; Puri et al., 2012). Cell wall polysaccharides, in fact, are known to interact with various polyphenols, modifying their bioaccessibility and bioavailability. The EAE treatment of the food waste is then complemented by a solid-liquid (water) extraction with the

Naviglio Estrattore® (NE), a system based on the generation of a negative pressure gradient between the outside and inside of solid matrix, followed by a sudden reinstatement of the initial balanced conditions. This pressure gradient leads to the forced extraction of compounds from the solid matrix in the aqueous phase. The preceding enzymatic treatment is expected to enhance the amount of extracted polyphenolic compounds.

Vegetable wastes [*Petroselinum crispum* (Mill.) (parsley), *Brassica oleracea* L. italica var. (broccoli), *Brassica oleracea* L. botrytis var. (cauliflower), *Spinacia oleracea* L. (spinach), *Allium ampeloprasum* L. (leek), *Eruca vesicaria* L. (rocket salad), *Cichorium intybus* L. (chicory)] were collected from farms and food processing industries (AOP Unolombardia consortium), located in Lombardia region (Italy). Enzymatic mixtures were obtained from Novozymes®.

Preparation of Samples, Enzymatic Pre-treatment and Extraction System

The enzymatic mixtures (around 10 U/ml) used in this work include hemicellulases, mixture of different cellulases and pectinase mixture, optimized after preliminary experiments in smaller scale. Fragments of the vegetable residues (0.5 cm), obtained by using a mill, were placed in the plastic trays with distilled water and an aliquot of each commercial enzymatic mixture in stirring conditions.

After this pre-treatment (50°C for 3 h), the solid residue (around 5 kg) from each material is placed in Naviglio Estrattore® (N.E.), filled with only water, to complete the extraction process of polyphenols. The total amount of polyphenols and flavonoids was then measured using classical colorimetric methods (Folin-Ciocolteau and aluminum chloride methods, respectively), and the identification of the main polyphenols was performed by HPLC.

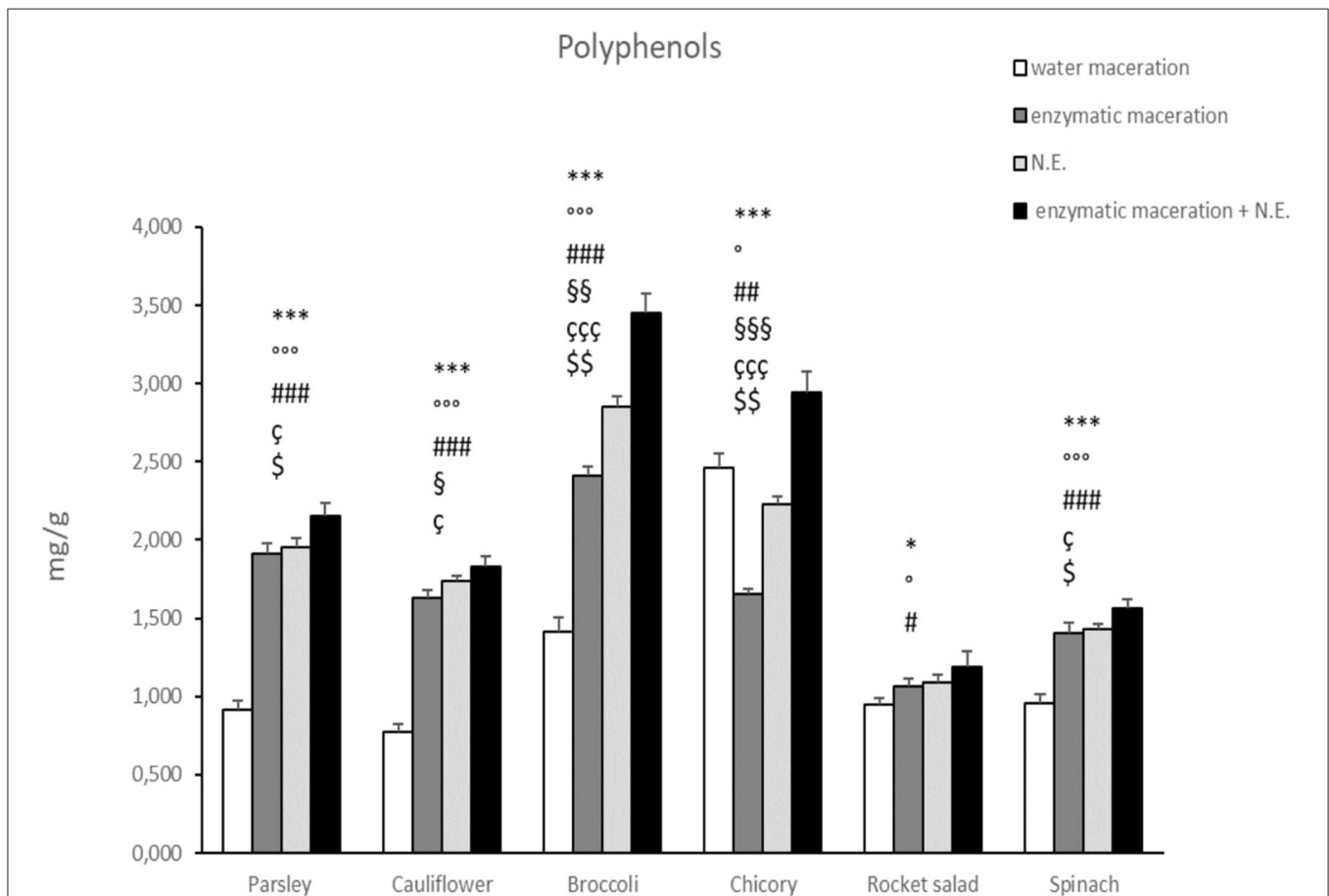
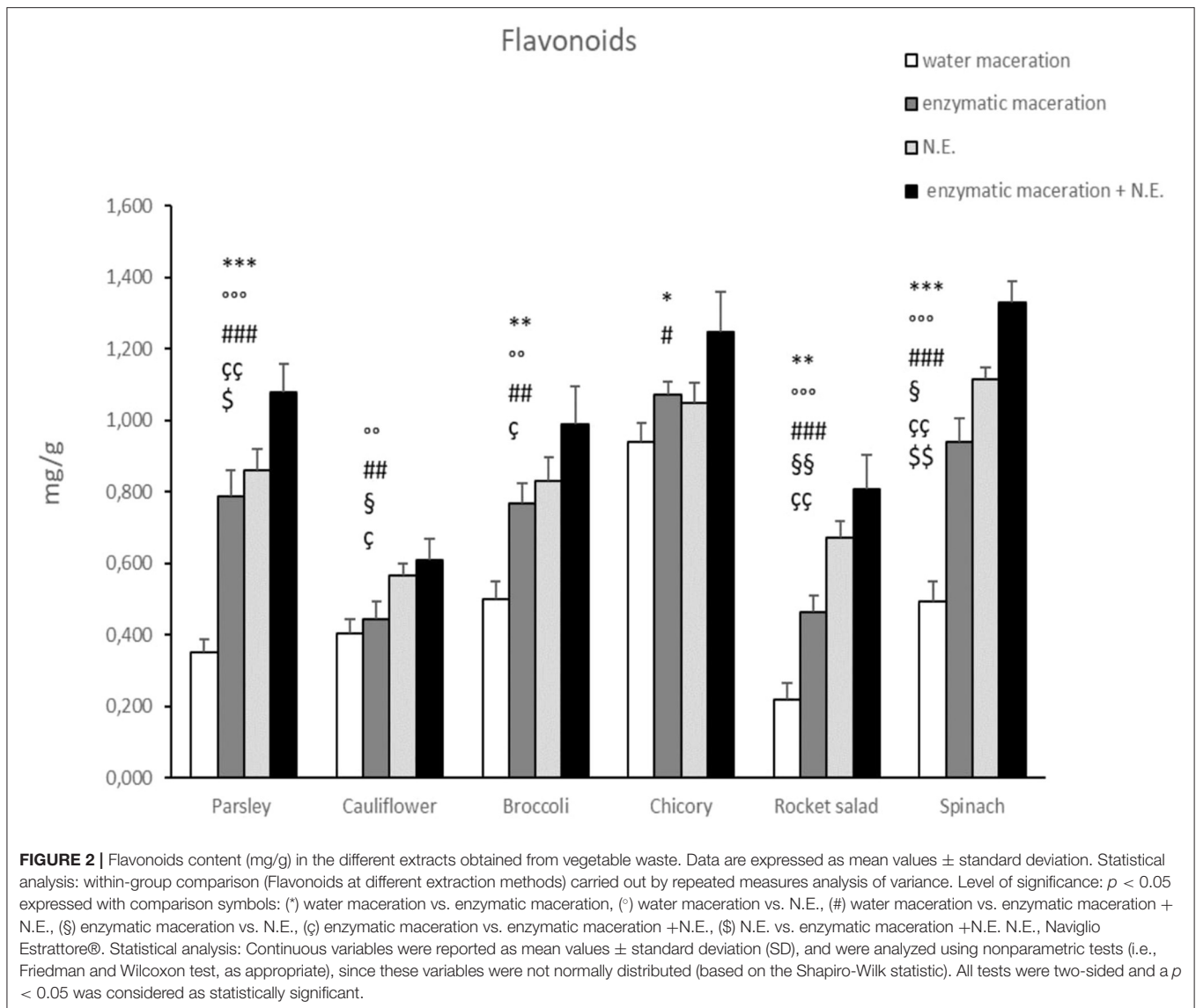


FIGURE 1 | Polyphenolic content (mg/g) in the different extracts obtained from vegetable waste. Data are expressed as mean values \pm standard deviation. Statistical analysis: within-group comparison (Polyphenols at different extraction methods) carried out by repeated measures analysis of variance. Level of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ expressed with comparison symbols: (*) water maceration vs. enzymatic maceration, (°) water maceration vs. N.E., (#) water maceration vs. enzymatic maceration + N.E., (§) enzymatic maceration vs. N.E., (ç) enzymatic maceration vs. enzymatic maceration + N.E., (§) N.E. vs. enzymatic maceration + N.E. N.E., Naviglio Estrattore®. Statistical analysis: Continuous variables were reported as mean values \pm standard deviation (SD), and were analyzed using nonparametric tests (i.e., Friedman and Wilcoxon test, as appropriate), since these variables were not normally distributed (based on the Shapiro-Wilk statistic). All tests were two-sided and a $p < 0.05$ was considered as statistically significant.



RESULTS

The total polyphenols content (TPC) and the total flavonoids content (TFC) contents are showed in **Figures 1** and **2**, respectively. The data are presented as mg/g fresh weight (FW), mean \pm standard deviation (SD, $n = 3$). It is possible to observe as the enzymatic pre-treatment followed by the extraction with Naviglio Estrattore® (N.E.), allowed to recover a higher concentration of polyphenols in all the plant waste material and especially from broccoli and chicory residues. In these two types of material, the amount of extracted polyphenols was clearly higher compared to that one observed using the only N.E. or the only enzymatic maceration. When the flavonoid concentration was tested, in all the examined material a noticeable increase using the N.E. after the enzymatic pre-treatment was measured (**Figure 2**). This observed difference in pre-treatment efficacy and in the

phenolics extraction from vegetable waste is principally due to the biochemical characteristics and the cell wall composition of each material.

By using HPLC analysis, many phenolics were identified after the complete extraction system (enzymatic pre-treatment + N.E.) (**Table 1**).

The data obtained in this work are difficult to compare to those found in literature for different reasons. There are huge differences in vegetables polyphenol quantification depending on seasonal harvesting (Arabbi et al., 2004; Hertog et al., 2007), different cultivar (Heimler et al., 2007; Koh et al., 2009), the climate where they grow (Podsedek, 2007), the cultivation site (D'Acunzo et al., 2017) endogenous circadian rhythms (Soengas et al., 2018), soil and pest-control treatment (Valverde et al., 2015). Moreover, the use of solvent is a variable that greatly affects the extraction efficiency. In all the scientific papers regarding the extraction of polyphenols from the same vegetable matrix, several

TABLE 1 | Phenolic compounds found in the vegetable extracts, obtained after the extraction process by N.E. following the enzymatic pre-treatment ($n = 3$).

Polyphenols	Broccoli (mg/kg)	Cauliflower (mg/kg)	Chicory (mg/kg)	Rocket salad (mg/kg)	Spinach (mg/kg)	Parsley (mg/kg)
Chlorogenic acid	70 ± 0.5	320 ± 15	150 ± 11	100 ± 13	80 ± 7	10 ± 0.9
Catechins	90 ± 3	130 ± 8	380 ± 10	20 ± 2	9 ± 0.9	70 ± 5.5
Caffeic acid	40 ± 5	–	170 ± 6	–	6 ± 0.5	3 ± 0.6
Cumaric acid	–	5 ± 0.5	–	–	5 ± 0.3	16 ± 4
Vitexin	3 ± 0.4	–	–	–	–	30 ± 7.5
Orientin	190 ± 8	16 ± 2	–	–	160 ± 15	22 ± 5
Rutin	12 ± 0.9	9 ± 0.8	–	–	–	50 ± 5
Quercetin	–	–	8 ± 0.6	–	–	13 ± 0.8
Cinnamic acid	–	3	–	–	–	1 ± 0.3
Luteolin	–	–	6 ± 0.5	–	–	8 ± 0.5
Kaempferol	–	–	7 ± 0.3	7 ± 0.5	5 ± 0.4	–

solvents like alcohols (methanol, ethanol), acetone, diethyl ether and ethyl acetate, often mixed with different proportions of water, were used.

DISCUSSION

The recovery of food by-products is a way to re-use the waste of the agri-food production chain, recovering their precious compounds, complying with the requirements of a circular economy, increasing the sustainability of the food production system. The set-up of innovative and sustainable extraction systems of natural products is currently a hot research topic involving different areas. The aim is to reduce or to eliminate the use of hazardous extraction solvents and ensure a safe and high quality of extract/product, in order to protect both the environment and consumers. Consequently, a sustainable and eco-friendly recovery of bioactive compounds from fruit and vegetable by-products for application in food, medical, cosmetic, pharmaceutical or agrochemicals industries is crucial to enhancing their added value and to reducing the pollution risk for the environment. For many years, the conventional techniques based on the use of solvent have been widely accepted, mainly because of their ease of use, efficiency, and wide-ranging applicability (Stalikas, 2007). In recent years, the use of organic conventional solvent is highly discouraged and legal limitations are becoming more and more rigorous, especially for food and pharmaceutical industry. Processes involving the use of organic solvents have a high negative impact on health and environment; beside this important aspect, the residues of solvent may also remain in the final products. This requires long additional purification steps and has repercussions on the total process cost. Additionally, by using pure organic solvents, very polar phenolic acids (benzoic, cinnamic acids) cannot be extracted completely. Therefore, the possibility to use important amounts of industrial by-products of agri-food chain to extract bio-active compounds as commercial goods with high marketing potential, in compliant with the current European legislation that strongly encourages the food industry to find new end-uses for by-/co-products, was explored in the experimental work presented in this paper.

Since the biggest research challenge is the identification of the best “extraction” conditions, in order to improve the release of bioactive compounds from the vegetable waste matrix reducing the use of organic solvents and respecting the principles of sustainability of the whole extraction process, the enzymatic pre-treatment coupled with aqueous extraction using Naviglio Estrattore® represent a sustainable strategy allowing to recover a good amount of phenolics, in particular. Due to the use of water as extraction solvent, phenolics compounds represent the main phytochemicals extracted and analyzed from the selected vegetable by-products.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

ED: writing—original draft. ED and DB: work planning. EB and AM: performed research. MV, DB, and ED: data curation. DB: statistical analysis. MD and DB: funding acquisition. All authors contributed to the article and approved the submitted version.

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