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Ciclo XXXV

ARTICHOKE INDUSTRY BY-PRODUCTS UP-CYCLING FOR FOOD FORTIFICATION

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A Matilde,

e alla futura Donna che sarai…

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ABSTRACT

The upcycling of food by-products as functional ingredients for food fortification, particularly those derived from fruit and vegetables, represents a promising solution to the issue of food wastage.

This thesis focuses on the transformation of the globe artichoke by-products (stems, bracts and leaves) derived from the Sardinian ecotype "Spinoso sardo", into upcycled ingredients (as extracts and powders), to enhance the quality, sustainability, and economic value of staple savory baked goods, particularly breadsticks. The work is structured around three case studies.

The first case study aimed to produce edible and sustainable extracts with potential applications as food antioxidants. Two extraction techniques (maceration and ultrasound-assisted) were optimized to achieve maximal recovery of polyphenols from artichoke byproducts. A response surface methodology was employed to examine the impact of varying the amount of ethanol and the reduction of extraction time on extraction efficiency. The maximum yields were maintained at the shortest extraction time (10 minutes for sonication and 60 minutes for maceration), except for sonicated bracts (41 minutes). Furthermore, intermediate ethanol percentages (42%–64%) were identified as optimal for both techniques, except for sonicated leaves (20%). The extracts of leaves, bracts, and stems obtained through the optimized extraction methods, which facilitated the maximum recovery of antioxidant compounds while simultaneously reducing time and cost, were utilized in the second case study to enhance the stability to lipid oxidation of breadsticks. Two concentrations (1000 ppm and 2000 ppm) of each by-product extract were incorporated into a conventional formulation, resulting in an increased antioxidant capacity and oxidative stability

without affecting the dough's workability. Although a slight deterioration in texture was observed, the shelf-life of breadsticks was significantly extended, particularly at the highest levels of addition of stem extract, without any visible alteration in their appearance. The third case study assessed the impact of incorporating powders derived from artichoke stems and outer bracts (at concentrations of 3 and 5%) on the conventional breadsticks' nutritional, bioactive, textural, aromatic and organoleptic properties. All fortified samples exhibited augmented nutritional and volatile profiles, particularly at elevated addition levels. The fortification resulted in an improvement in the friability of the breadsticks, without a perceptible alteration in their final color. The fortified samples were perceived as more bitter, astringent, and herbaceous by consumers, yet also as healthier and more sustainable. It can be concluded that the use of upcycled ingredients obtained from artichoke by-products represents a successful strategy for pursuing a circular economy, enhancing the competitiveness of the artichoke supply chain and benefiting snack manufacturers, who would be able to offer a novel and more durable functional product in a growing market.

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1. INTRODUCTION

1.1 Overview of food system

The food supply chain, also known as food system or food industry, is a complex of several stages – production, processing, transport and distribution, retail and consumption – where different actors intervene to transform raw materials (fruits, vegetables, cereals, milk, etc.) into food suitable for human consumption (Baldwin, 2009; Murphy, 2014). Throughout this supply chain, large amounts of loss and waste are generated. The terms "food loss" and "food waste" are often confused and used indistinctly, but based on the FAO's definitions, "food loss" can be described as the edible food mass discarded in the first part of the supply chain, before reaching the final user (during production, post-harvest and processing), while that produced at the end of the supply chain, which relates to retail and consumption, is known as "food waste" (FAO, 2011). Given the considerable volume of wastage recorded and due to the steadily increasing human population and consequently the food production, the food industry has faced several challenges in recent decades, including ensuring safe, nutritious and healthy food for people and reducing the economic and environmental impact of food waste and loss. As a matter of fact, the increased demand for and production of food is leading to the depletion of natural resources, causing biodiversity loss, soil erosion and environmental pollution (Garcia et al., 2020).

Furthermore, the production of food that will not be consumed results in a squandering of inputs such as energy, water and soil, and an unnecessary production of CO_2 (FAO, 2011). Considering that the human population is expected to reach around 10 billion people in 2050, feeding them will require a balance between sustainability, food

security, food safety, waste valorization, and a responsible and conscious use of food already produced (Vågsholm et al., 2020). In response, in 2015, United Nations member states signed on to the 2030 Agenda, which focuses on 17 sustainable development goals (SDGs) to be achieved by 2030, including the eradication of poverty and hunger, sustainable land and water use, clean water security, responsible production and consumption, mitigation of climate change and pollution, and the promotion of sustainable lifestyles (Figure 1). In particular, under SDG 12 "Responsible Consumption and Production", there are two specific targets for food loss and waste management to be achieved by 2030: target 12.3 "Halve per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including post-harvest losses" and target 12.5 "Substantially reduce waste generation through prevention, reduction, recycling and reuse" (United Nations, 2015).

Figure 1. 17 Sustainable development goals of the 2030 Agenda adopted by all United Nations Member States in 2015. Source: United Nations site https://sdgs.un.org/goals.

In 2011, the FAO estimated that one-third of the food produced for human consumption, equivalent to about 1.3 billion tons, is lost or wasted annually worldwide,

at a cost of around 990 billion dollars (FAO, 2011; Trigo et al., 2019). Subsequently, in order to highlight critical points in the entire food supply chain and to better understand where to intervene, two indices were introduced to quantify food losses (*food loss index,* to estimate how much food is lost in production or in the supply chain before the retail level) and food waste (*food waste index,* which gives a measure of food wasted by consumers or retailers). Through these indices, it was possible to understand that the majority of food losses occur at the production stage, with a global food loss index of around 14%, amounting to at least 400 billion dollars (FAO, 2019). In terms of specific food categories, the highest loss rates were achieved by roots, tubers and oilseed crops (25%), closely followed by fruits and vegetables (22%), meat and animal products (12%), and lastly by cereals and pulses (9%) (Socas-Rodríguez et al., 2021).The first report on food waste was presented by the United Nations Environment Program (UNEP) in 2021, which estimated that food waste from households, retail and the food service amounted to 931 million tons (approximately 570 million tons only in the domestic sphere) (UNEP, 2021). In fact, food is often discarded even though it is still suitable for human consumption, and this food wastage is most significant in medium- and high-income countries. In low-income countries, food losses arise mainly in the early and middle stages of the supply chain, with fewer losses occurring at the consumer level (FAO, 2019). Concerning the fruit and vegetable sector, wastage in developing countries is concentrated at the production, post-harvest, and distribution stages, due to seasonality and lack of proper storage strategies for perishable products. In developed countries, on the other hand, they are largely caused by overproduction and generated in the post-harvest evaluation stage due to the high quality standards required (FAO, 2011; Plazzotta et al., 2017).

The Fusion report of 2016 revealed that in Europe approximately 88 million tons food are lost and wasted every year, equivalent to 173 kg per person (143 billion euros of associated costs), from primary production to consumption, of which approximately 31 million tons came from primary production, processing, wholesale, and retail (Chiaraluce, 2021; Jiménez-Moreno et al., 2020). In Italy, around 3.4 million tons of waste were produced by the food and beverage industry in 2019, of which 311,000 tons were from agricultural activities (including agriculture, forestry and fishing) (Chiaraluce, 2021). Furthermore, in Italy, up to 87% of fruits, vegetables and cereals are disposed before reaching householders (Segrè & Falasconi, 2011).

Considering that, according to Ojha et al., 2020, food waste and loss can be classified into three comprehensive categories - avoidable, possibly avoidable and unavoidable (Figure 2) - a multidisciplinary approach by all stakeholders (academia, industry, logistic service, government agencies and consumers) is needed wherever possible.

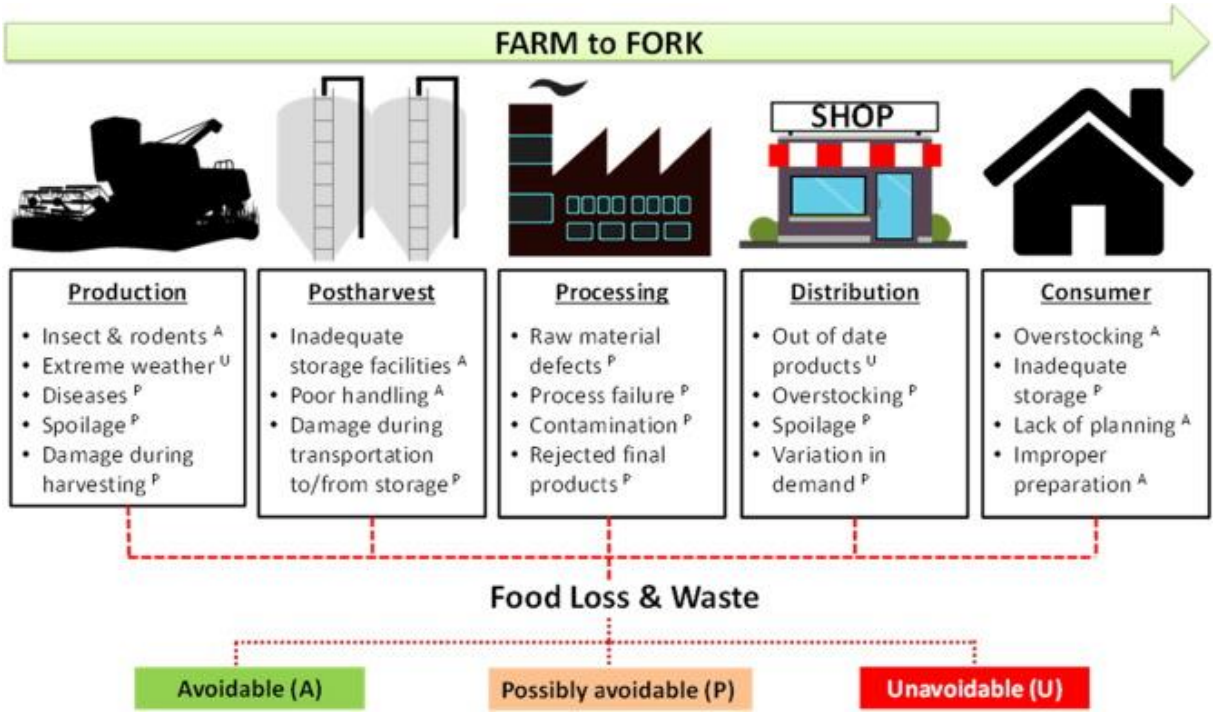

Figure 2. Avoidable, possibly avoidable and unavoidable food loss and waste throughout the food chain. Source: Ojha et al., 2020 https://doi.org/10.1016/j.wasman.2020.09.010

For instance, in the first stage of the agri-food supply chain, industry produces avoidable food losses, as a result of pre-harvest failures or problems during harvesting, processing, packaging transport and retail, which includes damaged or contaminated products, that cannot be harvested/sold (Ojha et al., 2020; Vågsholm et al., 2020). However, consumers are responsible for a large portion of preventable wastes. Partially avoidable food waste is mainly produced due to consumer practices and habits such as over-purchasing, excessive portion sizes, inadequate preparation techniques, poor storage, etc.. Unavoidable food waste includes inedible (e.g., bones, banana peels, etc.) and edible parts (e.g., potato peels) that are commonly generated during food preparation and consumption (Ojha et al., 2020).

Several strategies are being pursued to manage food loss and waste and rendering the system more sustainable, based on the transition from a linear to a circular economic model. In fact, traditionally, due to the large availability of raw materials and energy at low prices, the global industrial economy has been founded on a linear model built on the typical pattern "take -make-use-dispose" in which losses and waste were discarded without the possibility of being recovered. The circular model, on the other hand, is a regenerative system based on closed energy and raw material loops, in which resource use, waste, emissions and energy losses are minimized, until they are completely avoided (Chiaraluce, 2021). Therefore, in light of the declining resource accessibility, modern waste management focuses on the concept of “zero waste”, proposed by the Zero Waste International Alliance in 2004: “*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). To attain this

purpose, various waste management policies and frameworks, are put into practice, such as “4R” (Reduce, Reuse, Recycle, Recover) and the “waste hierarchy”, which vary from country to country according to their particular circumstances or policy strategies (European Commission, 2008; Sakai et al., 2011) (Figure 3). In both cases, prevention and reduction are prioritized, followed by the management of waste and losses management, which are no longer referred to as "waste" but rather as "resources".

Regrettably, the SDGs 2023 report found that despite companies and public procurement policies are working to improve the food system sustainability, the world is still falling behind in reducing per capita food waste and loss by 2030. Additionally, global crises such as the covid-19 pandemic and the conflict in Ukraine have hampered progress (United Nations, 2023). As a result, there is an urgent need to intensify efforts to guarantee that the SDGs remain on track and progress towards a sustainable future.

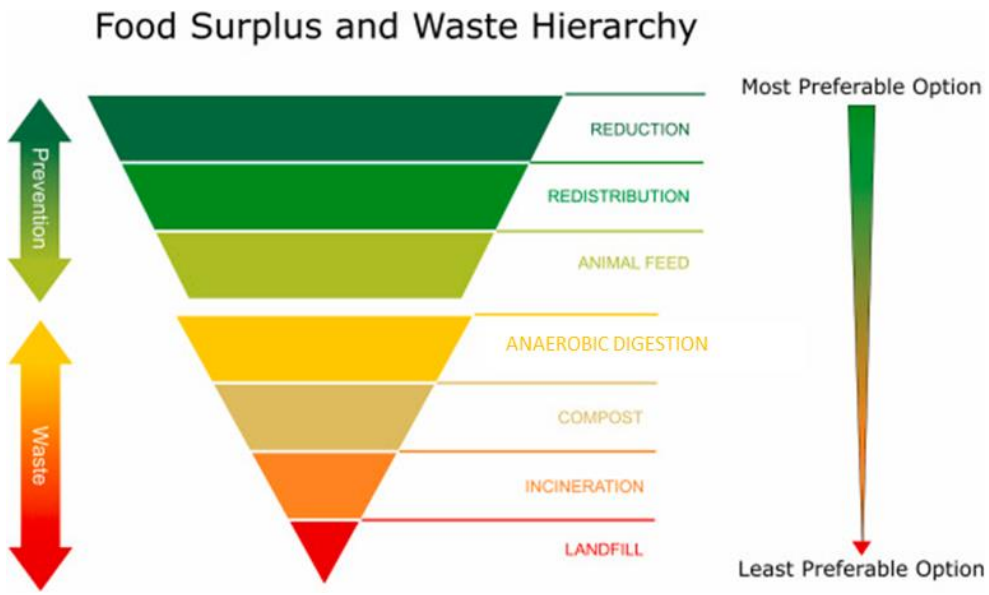

Figure 3. Waste hierarchy. Source: Parsa et al. 2023
https://doi.org/10.1016/j.jenvman.2023.118554

1.2 Food by-products and their valorization

Until recently, food waste and loss were considered as a negative issue that required treatment, minimization, and prevention due to the environmental and economic consequences of disposal. However, with the introduction of the principles of circular economy, sustainability, and the paradigms of "4R" and "waste hierarchy", food wastes are acquiring a positive value as a source of valuable nutraceuticals with high potential. For this reason, to complement the terms "food waste" and "food loss", "food by-product" has been introduced to indicate a final substrate, resulting from a production process, that can be used for the recovery of functional compounds and the development of new products with added value (Galanakis, 2012). These terms are often confused and used as synonyms. In order for a by-product not to be considered as waste, it must meet certain conditions (Fig.4). As reported in the Directive 2008/98/EC, the by-product is a secondary outcome of processing that is not the intended primary product, which can be legally reused in the same or another production process and/or used directly with or without additional treatment, as it is safe for human consumption and the environment (European Commission, 2008).

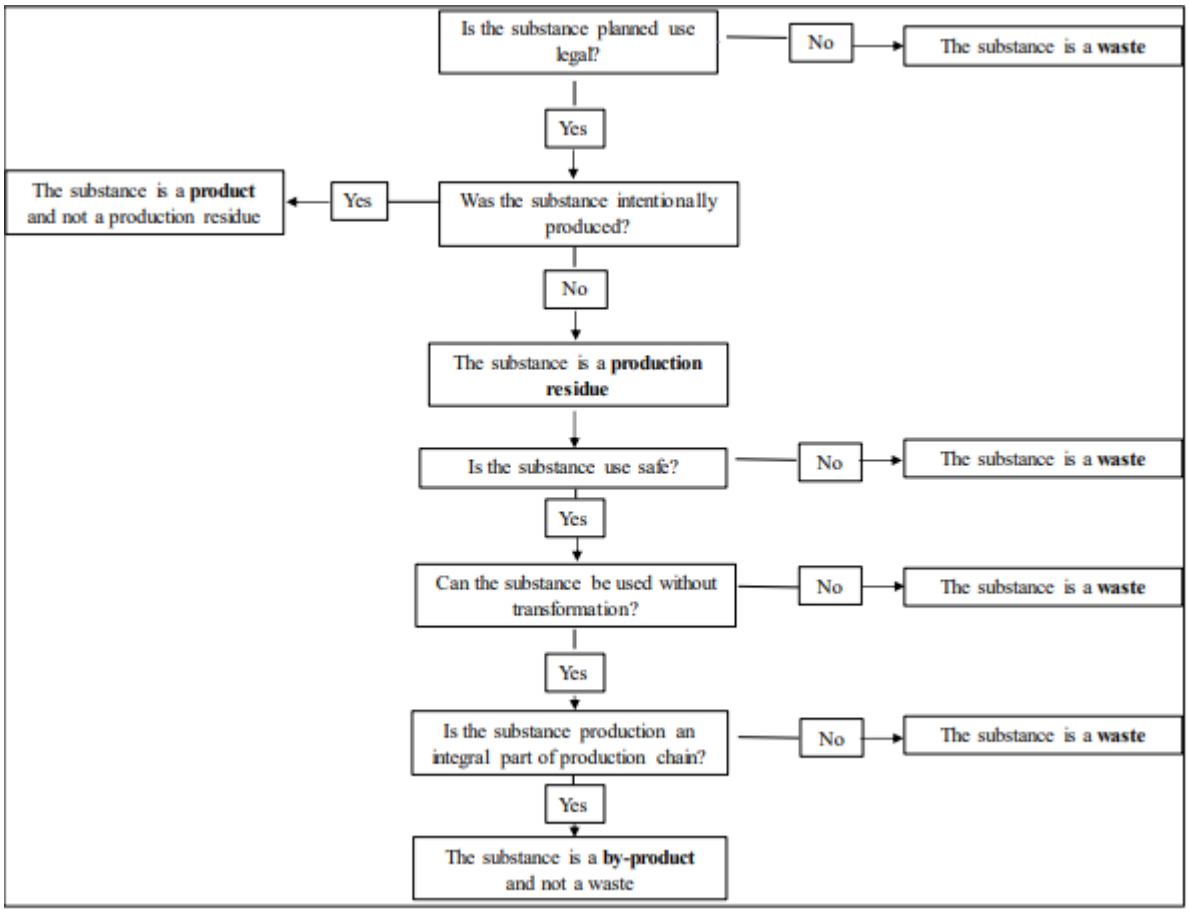

Figure 4. Decision tree to distinguish "waste" or "by-product" according to Directive 2008/98/EC Source: Marzocchi S, 2018

So, while a traditional waste is destined for landfill or incineration, resulting in air, water, and soil pollution, the by-product is a secondary product with new end-uses. In this perspective, the European Union is implementing policies to promote waste reduction and by-product valorization. In this context, agri-food wastes from different sectors (vegetables, fruits, meat, beverages, aquaculture, and marine products, etc.) are an attractive and cheaper source of high added value ingredients and bioactive compounds, which can have a wide range of applications as nutraceuticals, food additives, food active packaging, cosmetics, etc. (Soares Mateus et al., 2023; Socas-Rodríguez et al., 2021). Fruits and vegetables, due to their perishability and high water content, generate a significant amount of waste, which is moreover difficult to dispose of due to microbiological instability, off-odors and leachate formation (Plazzotta et al.,

2017; Soares Mateus et al., 2023). Therefore, it is preferable to reuse these wastes for other purposes without or with slight transformations (e.g. soil amendment, animal feed, etc.), as well as to recycle them after major modifications (Plazzotta et al., 2017). Conventionally, horticultural by-products are subjected to composting for organic fertilizer production, processing into powders that can be employed to adsorb pollutants from water and ground, briquetting, and anaerobic digestion for energy recovery (Ganesh et al., 2022; Plazzotta et al., 2017). These traditional approaches to waste management require large volumes of organic waste to produce value-added products, resulting in operational problems, costs, and environmental impacts (Ganesh et al., 2022). Furthermore, the processing of fruits and vegetables (e.g. juicing, canning, pickling, production of preserves, fermented products, syrups) produces by-products with great potential, such as stems, leaves, roots, peels, pomace and seeds, which are richer in bioactive compounds (phenolic compounds, dietary fibers, organic acids, proteins, essential minerals etc.) than the edible parts (Kainat et al., 2022; Soares Mateus et al., 2023). Indeed, in recent years, new perspectives have emerged for the use of fruit and vegetable wastes, allowing even modest amounts to be processed compared to conventional methods (Ganesh et al., 2022). These new approaches involve the exploitation of compounds with biological activity in by-products that can be used to improve quality in conventional foods (nutritional, technological and sensorial properties), to produce functional foods with nutritional benefits, to obtain nutraceuticals, to isolate bioactive components and to formulate bioactive food packaging (Lemes et al., 2022). In the following sections, the approaches to valorize these compounds will be reviewed, focusing on their reuse in food matrices.

1.2.1 Fruit & vegetable by-products as functional ingredients

Fruits and vegetables (F&V) by-products, which vary from commodity to commodity (leaves, roots, tubers, peels, pulp, pomace, seeds, etc.), have traditionally been considered a major issue for industry in terms of cost and environmental impact due to their disposal. Nowadays, these matrices are considered as a cheap source of bioactive compounds that can be used as functional ingredients in a wide variety of food products (Sagar et al., 2018).

Bioactive compounds are a diversified group of natural or synthetic compounds, which can exert a wide range of beneficial effects in the living tissues (anti-microbial, antioxidant, prebiotic, anti-inflammatory, etc.) (Messinese et al., 2023). This group includes phenolic compounds, carotenoids, alkaloids, anthocyanins, vitamins, dietary fiber, fatty acids, volatile compounds, and other pigments, which are highly represented in the by-products of fruits and vegetables, because they are generally produced by the primary and secondary metabolism of the plant cell (Messinese et al., 2023).

Phenolic compounds and dietary fiber are among the most representative bioactive components found in plant by-products and the most studied due to their great interest in food applications (Lemes et al., 2022; Sagar et al., 2018).

Polyphenols, consisting mainly of phenolic acids, flavonoids, stilbenes, quinones, tannins, and lignin, are one of the major groups of secondary metabolites produced by plants (Ganesh et al., 2022; Lemes et al., 2022). They can be used in foods as nutraceuticals and preservatives to inhibit lipid oxidation and microbial growth, due to their bioactive properties such as antihypertensive, anti-inflammatory, antiallergenic, anti-atherogenic, antithrombotic, cardioprotective, vasodilatory, anticarcinogenic,

antioxidant and antimicrobial effects (Coman et al., 2020; Lemes et al., 2022; Socas-Rodríguez et al., 2021).

Dietary fibers are carbohydrate polymers that are resistant to small intestinal enzymes digestion and are known for their multiple health benefits, including hypoglycemic and hypolipidemic properties. Based on their solubility, they are traditionally classified as insoluble fibers (cellulose, hemicellulose, lignin, etc.) and soluble fibers (pectin, beta-glucan, oligosaccharides, inulin, plant mucilage, and others) (Zou et al., 2022). Soluble fibers have been found to have stronger physiological functions and better physicochemical properties than insoluble dietary fibers, making them more promising as ingredients for food fortification. In fact, they help to regulate blood cholesterol levels, reduce the risk of cardiovascular disease, prevent gastrointestinal problems and type 2 diabetes, and act as antioxidants and anticancer agents. Soluble fibers also have a greater ability to form gels and act as emulsifiers, affecting the texture, color, sensory properties, and shelf life of foods (Zou et al., 2022).

In order to exploit and to gain the benefits of these bioactive compounds, the by-products are subjected to treatments (drying, size reduction techniques, extraction methods, fermentation etc.) to enable their up-cycling (Melini et al., 2020). The processes selected depend on the nature and economic value of the target compound(s) and the matrices from which they are derived. Due to the high water content and enzyme activity of the F&V by-products, they need to be stabilized, isolated, or purified before they can be used as functional ingredients. In general, by-products are first treated to reduce their moisture content by lyophilization, drum drying, or oven drying and then reduced to powder by crushing, grinding, milling, pulverization etc. (Amoah et

al., 2022; Melini et al., 2020). After that, they can be incorporated into food products in the form of flour or can be submitted to an extraction process.

The incorporation of F&V by-products can result in either beneficial or unfavorable alterations in the physicochemical and sensory characteristics of the final product, depending on the dosage, the food matrix in which they are integrated, and the form of the by-product (powder or extract) selected. Despite the relative simplicity and lower cost of using powders, they often exert the most significant influence on the organoleptic characteristics of foods (Trigo et al., 2019).

1.2.2 Bioactive compounds valorization

The recovery of bioactive molecules from F&V by-products necessitates the completion of several sequential steps. Following cleaning (if necessary), the material is often dehydrated due to its perishable nature. It is then subjected to a specific treatment in relation to the physicochemical characteristics of the desired bioactive compounds that are to be used. In general, when polyphenols are employed as functional ingredients, the by-products are submitted to extraction and sometimes purification/drying steps. Given the considerable diversity among these phytochemicals (e.g. solubility, polarity, stability, molecular mass, and acid-base properties) and by-product matrices, the extraction can be performed using various procedures. In the case of liquid samples, liquid-liquid extraction is used. Solid by-products undergo solid-liquid extraction, preceded by a pretreatment to obtain homogeneous and reduced-size particle samples. This approach enhances the contact between the matrix and the extractant, thereby increasing the efficiency of the extraction process. Extraction efficiency can also be

influenced by several factors, including the type of solvent utilized, sample particle size, sample/solvent ratio, extraction time, temperature, and technique employed (Mir-Cerdà et al., 2023; Pattnaik et al., 2021).

The most commonly used extraction procedures can be categorized into two main groups: conventional and non-conventional methods. The traditional extraction techniques comprise Soxhlet extraction, maceration, and hydro-distillation, which are based on the extraction power of the solvents used, the application or non-application of heat, and agitation. The polarity of the solvent and the affinity between the solvent chosen and the compound to be extracted determine the efficiency of these techniques. Furthermore, conventional methods, which are often characterized by low selectivity, require the use of long extraction times and large amounts of toxic and polluting solvents, which must then be evaporated (Azmir et al., 2013). New and promising extraction techniques are being introduced to overcome these limitations of classical methods. Some of the most promising unconventional extraction techniques include ultrasonic, enzyme, microwave, pulsed electric field, supercritical fluid, and pressurized liquid extraction (Pagano et al., 2021). In recent years, research interest has focused on optimizing these green extraction methods, allowing reduced energy consumption and using alternative and sustainable solvents. In fact, the extraction of phenolic compounds from solid matrices is conventionally conducted using hydro-organic solvents, including methanol, ethanol, acetone, and ethyl acetate. However, due to the potential toxicity and environmental concerns associated with these solvents, particularly when the extracted compounds are intended for human consumption, the use of green solvents (e.g., CO_2, water, and deep eutectic solvents) is preferred. Consequently, among organic solvents, only ethanol could be employed (Mir-Cerdà et al.,2023).

The selection of new techniques is generally the most sustainable option from an economic and environmental perspective. However, in view of the inherent variability of chemical compounds, a tailored approach involving the identification of the most suitable extraction methodology for each specific matrix is essential. Once the bioactive compounds have been extracted, it is important to consider their stability before they can be utilized as functional ingredients. Indeed, these compounds, particularly polyphenols, are susceptible to degradation reactions when exposed to light, heat, specific pH values, and high amounts of oxygen. Among the most commonly employed strategies for the protection of compounds from environmental and processing conditions is microencapsulation. This process entails the enclosing of sensitive compounds within solid matrices, which, in addition to providing protection, also allows for the masking of astringent flavors and the enhancement of bioavailability through the controlled release of the molecules into the gastrointestinal tract. Among the various encapsulation techniques, the most widely utilized for food ingredients is spray drying or atomization (Comunian et al. 2021; Pattnaik et al., 2021).

1.2.3 Fruit and vegetable by-products in food matrices: bakery foods

F&V bioactive by-products have been successfully incorporated into food products such as extruded snacks, bakery products, breakfast cereals and dairy products (More et al., 2022).

Food fortification, especially of widely consumed foods, is one of the most sustainable and cost-effective approaches to harnessing these functional compounds for the improvement of public health (Scappaticci et al., 2024; Subiria-Cueto et al., 2022). Food matrices are a complex of micro- and macro-constituents, organized into a structure capable of retaining and influencing the bioactivity of bioactive compounds. Therefore, it's important to choose a proper food carrier and a technological process to preserve their bioactivity until the time of consumption (Betoret & Rosell, 2020). Among the foods that are well suited for the valorization of F&V by-products and the incorporation of functional ingredients are bakery products. These can be both sweet and savory and include a variety of goods ranging from cakes to cookies, to crackers and breadsticks. All are appealing to consumers. In fact, they are consumed daily in large quantities, making them ideal carriers of bioactive compounds and an opportunity to provide health benefits to broad populations (Martins, Pinho, & Ferreira, 2017). Some of the most studied by-products for fortification are apple, mango, berry pomace and peel, banana flour, citrus and potato peel, grape, tomato, carrot by-products, and so on, due to their widespread consumption and processing (Gómez & Martinez, 2018). A review of the literature over the past decade shows that the inclusion of these F&V by-products (in the form of flours or extracts or powdered extracts), either as replacements or additions, can influence the sensory, technological, and nutritional properties of baked goods. Table 1 shows some cases of fortification of baked goods with F&V by-

products and their main effects. Studies on the use of these functional ingredients in wheat bread have, in all cases, shown nutritional improvements, mainly consisting of fiber and phenolic enrichment, a general change in color and, especially in the case of wheat flour replacement, a reduction in bread volume, sensory acceptability and an increase in crumb hardness (Gómez & Martinez, 2018). These effects have also been observed in studies conducted on sweet baked goods (e.g. muffins, cakes, cookies), where it was easier to incorporate F&V by-products because a strong gluten network is not required and the high content of some ingredients, such as fats and sugars, hides the bitter taste that is often characteristic of these matrices (Gómez & Martinez, 2018). To obtain healthier bakery products that can compete with traditional products in terms of sensory and technological properties, it will be necessary to find a balance with the level of addition or substitution of by-products.

Among the wide variety of by-products with functional properties derived from edible plants, those from globe artichoke are gaining more interest as a promising source of bioactive compounds for the food industry. Further details will be provided in the following discussion.

Table 1. Utilization of fruit and vegetable by-products in bakery products and their main effects. State of the art of the last decade.

Enriched Product	F&V By-product	Type of functional ingredient	Level of addition/substitution	Type of investigation	Principal effects	Reference
Cookies	Grape marc	Extract	Addition flour: grape marc ratio of 2:1 g/mL	Physicochemical, sensory, volatile analysis	↑ phenolic compounds and antioxidant capacity, volatile compounds derived from Maillard reaction and lipid oxidation; ↓ sensory scores.	Pasqualone et al., 2014
	Apple, pineapple, melon	Flour/powder	Substitution of 5, 10, and 15% (w/w)	Physicochemical, nutritional, and sensory analyses	↑ fiber, ash, and cookies diameter. Sensory accepted. Good organoleptic qualities in pineapple enriched samples.	de Toledo et al., 2017
	Orange peel	Flour/powder	Substitution of 5, 10, 15, and 20% (w/w)	Physicochemical, nutritional, antioxidant, and sensory analyses	↑ fiber, minerals, phenolic compounds, and antioxidant capacity ↓organoleptic properties but acceptable sensory quality.	Obafaye & Omoba, 2018
	Raspberry, red currant, strawberry pomace	Flour/powder	Substitution of 10, 15, and 20% (w/w)	Physicochemical, nutritional, textural and sensory analysis	↑ fiber, softness, and fragility. Good organoleptic qualities.	Tarasevičienė et al., 2021
	Chestnut shells	Extract	Water replacement	Physicochemical, nutritional, antioxidant, and sensory analyses	↑ minerals, phenolic compounds, and antioxidant capacity. High scored in terms of overall acceptability.	Pinto et al., 2023
	Coffee silverskin	Flour/powder	Addition of 11.9 %	Physicochemical, nutritional, antioxidant, and sensory analyses	↑ fiber, polyphenols, and antioxidant activity; ↓acceptability score.	Dauber et al., 2024
		Extract	Addition of 0.8 and 1.2%		↑ polyphenols and antioxidant activity. Pleasant appearance and flavor.	
			Combination of both		↑ fiber, polyphenols, and antioxidant activity; ↓acceptability score.	
Bread	Elderberry	Dried Extract	Substitution of 4 and 36% (w/w)	Dough properties, physicochemical,	↑ water absorption and dough development time; ↓dough fermentation	Martins et al., 2017

	Orange	Dried Extract	Substitution of 4 and 8% (w/w)	and textural analyses	characteristics, bread volume. Significant changes in crumb texture.	
	Pomegranate	Dried Extract	Substitution of 4 and 16% (w/w)			
Bread	Lettuce waste	Flour/powder	Substitution of 2, 4, 12 and 40% (w/w)	Physicochemical, antioxidant and sensory analyses	↑ phenolic compounds and antioxidant capacity; ↓ leavening properties. Good consumer acceptability.	Plazzotta et al., 2018
	Broccoli stems and leaves	Flour/powder	Substitution of 2% (w/w)	Physicochemical, nutritional antioxidant and sensory analyses	↑ phenolic compounds and antioxidant capacity; ↓volume. Overall acceptability and appearance were not affected.	Lafarga et al., 2019
	Artichoke stems and bracts	Flour/powder	Substitution of 5, 7.5 and 10% (w/w)	Dough properties, textural, physicochemical analyses	↑ water absorption, dough development, mixing time, stability; ↑ bread hardness ↓dough strength and bread volume. Altered color.	Canale et al., 2022
	Artichoke stems and bracts	Flour/powder	Substitution of 5, 7.5 and 10% (w/w)	Physicochemical, antioxidant, and staling rate analyses	↑ phenolic compounds, antioxidant capacity and staling resistance.	Canale et al., 2023
	Citrus pectin	Flour/powder	Substitution of 2% (w/w)	Physicochemical, nutritional, and textural analysis	↑ rheological properties	Scappaticci et al., 2024
	Grape pomace	Flour/powder	Substitution of 2% (w/w)		↑ phenolic compounds	
			Combination of both		↑ homogeneous structure, technological aspects, volume, phenolic compounds ↓simple sugar content	
GF bread	Pomegranate seed	Flour/powder	Addition of 2.5, 5, 7.5, and 10% (w/w)	Textural, physicochemical, antioxidant and sensory analyses	↑ volume and textural properties, phenolic compounds, and antioxidant capacity; ↓organoleptic aspects for the samples with >5% addition.	Bourekoua et al., 2018
Breadsticks	Grape pomace	Flour/powder	Substitution of 5 and 10% (w/w)	Shelf-life estimation, physicochemical	↑ phenolic compounds, antioxidant capacity; ↓estimated shelf-life.	Bianchi et al., 2021

				and antioxidant analysis		
	Grape pomace	Flour/powder	Substitution of 5 and 10% (w/w)	Dough properties, nutritional, textural, antioxidant and sensory analysis	↑ water absorption, dough development, degree of softening, and tenacity; ↓dough elasticity, swelling index; ↑fiber, phenolic compounds, and antioxidant capacity ↓breadsticks hardness and fracturability. Altered color, good sensorial acceptability.	Rainero et al., 2022
	Olive pomace	Flour/powder	Substitution of 5, 7.5 and 10% (w/w)	Textural, physicochemical, antioxidant and sensory analyses	↑ phenolic compounds and antioxidant capacity; ↓breadsticks hardness. Altered color, satisfactory sensorial attributes.	Simsek & Süfer, 2022
GF breadsticks	Olive leaves and mill wastewater	Dried Extract	Addition of 500 and 1000 ppm (w/w)	Shelf-life estimation, textural, physicochemical, antioxidant and sensory analyses	↑ moisture and a_w, phenolic compounds and antioxidant capacity, estimated shelf-life; ↓breadsticks hardness. Similar color and sensorial ranking score.	Conte et al., 2021
	Olive cake	Flour/powder	Substitution of 1, 2 and 3% (w/w)	Textural, nutritional, physicochemical, antioxidant, volatile and sensory analyses	↑ moisture, fiber, lipid, ash, phenolic compounds and antioxidant capacity, estimated shelf-life, overall pleasantness score; ↓breadsticks hardness, volatile compounds derived from Maillard reaction and lipid oxidation. Altered color.	de Gennaro et al., 2022
GF cake	Apple, orange, and carrot pomace	Flour/powder	Substitution of 5, 10, and 15% (w/w)	Rheology, physicochemical and sensory analyses	↑ fiber, viscosity, and sensory acceptability of cake supplemented with orange pomace.	Kırbaş et al., 2019
GF Sponge cake	Broccoli leaves	Flour/powder	Substitution of 2.5, 5, 7.5% (w/w)	Nutritional, antioxidant, and sensory analysis	↑ protein, mineral, antioxidant compounds; ↓sensory quality.	Drabińska et al., 2018

Note: ↑=higher; ↓ = lower; GF= Gluten free

1.3 Globe artichoke overview

The globe artichoke is a perennial herbaceous plant native to the Mediterranean region and widely distributed worldwide, belonging to the Asteraceae (or Compositae) family, the genus *Cynara* and the species C. *cardunculus* L. ssp.. Indeed, the *Cynara cardunculus* L. ssp. includes three botanical taxa: the globe artichoke var. scolymus (L.) Fiori; the cultivated cardoon var. sylvestris (Lamk); and the wild cardoon var. altilis DC (B. de Falco et al., 2015; Rana et al., 2023). World artichoke production has shown a stable trend over the last decade and is estimated to reach 1.58 Mt in 2022. Italy is the leading producer with approximately 0.45 Mt (28,5% of total production) followed by Spain (0.23 Mt, 14.5%) and Egypt (0.19 Mt, 12%) (FAO, 2022). Considering the Italian scenario, the regions with the highest production are Sicily (about 38% of the Italian production), followed by Apulia (about 30%) and finally Sardinia (16% c.a.) (ISTAT, 2024).

The culinary use of the globe artichoke is associated with the capitulum or head, an inflorescence that is eaten immature, before flowering, and is known for its nutritional value, due to its high content of phenolic compounds and dietary fiber (de Falco et al., 2015; Rana et al., 2023). The artichoke head consists of the fleshy peduncle (part of the floral stem), the receptacle (basal part of the flower head) and a variable number of bracts (specialized leaves with inflorescence protection function). Only the "artichoke heart", consisting of the more tender inner bracts, peduncle, and receptacle, is consumed fresh, cooked or processed. The fibrous outer bracts are usually discarded. Flower heads are produced continuously from fall to spring by the re-flowering types, while the production of the non-reflowering types is limited to the spring seasons (Rana et al., 2023). Based on the morphology of the capitulum, four groups of cultivars are

described: the Spinosi group, with spines on capitulum, bracts and leaves; the Violetti group, with violet and less spiny heads; the Romaneschi group, with spherical or subspherical heads without spines; the Catanesi, with small, elongated and spine-less capitula (B. de Falco et al., 2015). The main cultivated varieties are “Violetto di Provenza” and “Violetto di Sicilia”, “Brindisino”, “Tema”, “Catanese”, “Spinoso sardo”, “Terom”, “Romanesco” (Rana et al., 2023).

1.3.1 Artichoke by-products

Artichoke harvesting produces a large amount of waste (between 58.5 and 69% of the total biomass), consisting of roots, leaves, and most of the stems, which are usually left in the field. Considering that the edible part is represented by the heart of the capitulum (about 15-25% of its fresh weight), the artichoke processing industries (mostly canning) generate additional losses and by-products. Indeed, the remaining 75 to 85% is lost, first by removing the blackened, wrinkled, or cold damaged parts of the plant, then by sorting, calibrating and turning the heads (to preserve the "heart"), where the outer bracts, the upper part of the inner bracts and the last portion of the stem are discarded (Amoriello et al., 2022; Canale et al., 2022). In recent years, efforts have been made to find uses for the main by-products of the artichoke (bracts, leaves, and stems), especially as dietary supplements and food additives, due to their interesting composition and concentration of fatty acids, inulin, fiber, minerals, inositol and phenolic compounds (Órbenes et al., 2021).

Bioactive compounds in these residues, especially phenolics, are preferentially allocated to different parts of the plant according to their biological role (Pandino et al., 2013). Therefore, each by-product can be exploited for its specific bioactive component.

The main phenolic acids found in artichoke tissue are derivatives of caffeic acid (3,4-dihydroxycinnamic acid), particularly mono- and dicaffeoylquinic acids, which have a marked scavenging activity against reactive oxygen species and free radicals, enabling them to protect cells from oxidative damage (Ceccarelli et al., 2010; Lattanzio et al., 2009). Caffeoylquinic acid derivatives such as cynarin (1,5-O-dicaffeoylquinic acid), chlorogenic acid (3-O-caffeoylquinic acid), neochlorogenic acid (5-O-caffeoylquinic acid), 1,3-O-dicaffeoylquinic acid, 1,4-O-dicaffeoylquinic acid, and 3,5-O-dicaffeoylquinic acid have been identified on both leaves and flower heads (Lattanzio et al., 2009). These polyphenols, classified as hydroxycinnamates, are generally involved in the cross-linking of cell wall polymers because they can be used as precursors for lignin biosynthesis, making them important in plant lignification. Therefore, they are more concentrated in the lignified tissues, such as the outer bracts, which are rich in lignin because they provide physical resistance to the inner parts of the head, protecting it from biotic and abiotic stresses (Montesano et al., 2022; Negro et al., 2012). For the same reason, these compounds are highly distributed in the stems due to their role as mechanical support for immature inflorescences (Pandino et al., 2011).

Among the flavonoids, other phenolic compounds, apigenin and luteolin, both of which occur as glycosides and rutinosides, have been detected in artichoke tissues (Lattanzio et al., 2009). Luteolin derivatives are more abundant in artichoke by-products than in the edible part, where apigenin derivatives seem to be more represented (Jiménez-Moreno et al., 2020). In particular, flavonoids are concentrated in the leaves, the part of the plant most exposed to sunlight, because they also protect cells from oxidative damage caused by ultraviolet light (Negro et al., 2012; Samanta, Das, & Sanjoy Kumar, 2011).

Regarding inulin, which is naturally found in the artichoke plant as a reserve carbohydrate, as in all species of the *Asteraceae* family, it is present in the by-products, especially in the stems and outer bracts, although in slightly lower quantities than in the edible part (Lattanzio et al., 2009; Zeaiter et al., 2019).

The presence of this wide range of bioactive compounds allows the conversion of artichoke by-products into high-value products with relevant potential applications, such as the production of antioxidant extracts and functional flours. This makes them suitable to support an economically viable market serving the food and/or pharmaceutical industries and to promote up-cycling, which contributes to waste reduction, efficient use of resources and environmental benefits.

2. AIM OF THESIS

As previously stated, the current agri-food systems collectively generate considerable quantities of processing losses, commonly designated as by-products, which can have significant negative environmental, economic, and social impacts. Consequently, the food industry is reviewing its production chains adopting a circular economy approach, with a view to ensuring efficient resource use. Among the different endorsed strategies, the practice of upcycling food represents a promising solution for the reduction of food losses, whereby by-products are transformed into new food products. As by-products, especially those derived from fruit and vegetables, are still rich in bioactive substances, including dietary fiber, phenols, and vitamins, which can have health-promoting effects, the practice of upcycling involves assigning a higher value to a product than that of the original one through a process of valorization, in contrast to recycling that is also known as downcycling.

The globe artichoke (*Cynara cardunculus* var. scolymus (L.)) represents one of the most significant vegetable species in the agricultural sector of Sardinia. The local ecotype, designated "Spinoso sardo," is the most prevalent cultivar. In 2011, it was granted Protected Designation of Origin (PDO) status, and to date, it is the sole PDO artichoke in Europe. As a result, it is subject to production regulations that ensure adherence to high-quality standards, which in turn generate a considerable amount of by-products (floral stems, bracts, and leaves). These residual vegetable materials, which contain bioactive substances (especially phenolic compounds, dietary fiber, and minerals), can be processed to increase their value and utilized as functional ingredients in daily consumed foods. A study conducted to compare and identify consumer attitudes and preferences for upcycled foods in a Western country (the United States) and an

Eastern country (China) revealed that the preferred categories for upcycled foods were staple food groups. Snacks were identified as the second most preferred food category (Grasso et al., 2023). In addition, consumer acceptance of upcycled by-products depends on their taste, quality, and the consumers' dietary and cultural habits (Lu et al., 2024). In fact, it is also important to consider how common or uncommon the upcycled ingredients and foods are in their daily diet. It can be reasonably assumed that the by-products of artichoke cultivation and processing will be readily accepted by consumers, particularly as an ingredient in staple foods such as baked savory foods, given the global familiarity of the artichoke and the widespread consumption of baked products.

In light of the aforementioned considerations, this PhD thesis focused on the transformation of the by-products of the Sardinian Spinoso artichoke cultivar into upcycled ingredients with the objective of enhancing the quality profile and economic value of savory baked goods, with a particular interest on breadsticks, which are among the most popular and appreciated typical Italian baked goods, consumed in many other European countries as well (Conte et al., 2021). Given the common practice of reintroducing F&V by-products into the food chain as functional ingredients in the form of extracts and powders, the potential of adding artichoke by-products in both forms to a conventional breadstick formulation was evaluated. Specifically, polyphenol-rich extracts obtained from the main artichoke by-products (outer bracts, stems, and leaves) were added with the specific purpose of enhancing the stability to lipid oxidation that breadsticks are often subject to, thereby limiting waste production. The objective of incorporating powdered artichoke stem and bract, specifically of the Sardinian Spinoso variety, was the production of healthy and sustainable breadsticks.

The main challenges to the reintegration of these upcycled ingredients, particularly as extracts, include limitations in processing innovations and a lack of utilization of green technologies, which fail to align with the European vision set forth in the Green Deal. Consequently, in the present thesis, prior to the utilization of artichoke by-product extracts as natural antioxidants, an optimization study of the selected extraction techniques (one conventional and one innovative) was conducted using a food-grade and low-polluting solvent, with the aim of maximizing the recovery of the bioactive compounds of interest.

3. WORK PLAN

The experimental activities of this thesis work were divided into three parts:

- Study and optimization of two extraction methods, namely maceration and ultrasound assisted, to maximize the recovery of polyphenols from artichoke by-products using a food-grade solvent (ethanol-water), with the aim of producing edible extracts suitable for food fortification.
- Evaluation of the feasibility of employing antioxidant-rich extracts obtained from artichoke by-products for fortification and shelf-life extension of breadsticks.
- Study of the effect of the incorporation of freeze-dried powders obtained from artichoke by-products into breadsticks and on their nutritional, technological, and sensory properties.

The following section provides a concise overview of the experimental analyses and materials utilized in the three case studies derived from each phase of the research.

3.1 Study materials and experimental analysis

Case study 1. *Green recovery optimization of phenolic compounds from "Spinoso sardo" globe artichoke by-products using response surface methodology*

The first study was carried out on the lyophilized main by-product fractions of Spinoso sardo globe artichoke (outer bracts, leaves and stems) provided by the North Sardinia companies of consortium "Carciofo Spinoso di Sardegna D.O.P." and collected in the 2019. For each by-products the response surface methodology (RSM) was applied to optimize two selected extraction methods with the objective of maximizing the recovery of phenolic and flavonoid compounds. Indeed, RSM is a widely utilized mathematical

tool for the optimization of extractive processes, as it enables a comprehensive investigation of the diverse variables that affect the extraction performance and the identification of their combination that gives the best response. In contrast with the one-variable-at-a-time (OVAT) approach, which entailed the examination of the isolated impact of alterations in a single variable on the response, while holding constant all other variables, RSM employs experimental designs that also permit the observation of the combined effect of multiple variables through a relatively limited number of experiments (Cannavacciuolo et al., 2024).

In this case study, two extraction methods were selected for optimization: one conventional and one innovative. Among the conventional extraction methods, maceration was chosen due to its simplicity and cost-effectiveness despite its inherent limitations (Messinese et al., 2023). Ultrasound-assisted extraction (UAE) was selected as a novel technique. A food-grade solvent (ethanol-water solution) was used in both methods. The experiments were conducted according to two different Central Composite Designs (CCD), comprising 13 randomized runs with 5 replicated central points (a rotatable central composite design to optimize maceration, and a face-centered design for the UAE). CCDs were employed to examine the impact of ethanol percentage and extraction time (independent factors), at three levels (low: -1, central: 0, and high: +1), on the recovery of bioactive compounds (Table 1; Table 2). The factors and levels considered were selected based on a review of the literature and preliminary laboratory tests. The total phenolic content (TPC) and total flavonoid content (TFC) determined spectrophotometrically on each extract were used as response variables. RSM was employed to transform the experimental data into mathematical predictive models, and to optimize both extraction methods by finding the best combination of parameters for each by-product fraction. Following model validation, a characterization of the optimal

extracts was conducted in terms of TPC, TFC, antioxidant capacity (DPPH and ABTS spectrophotometric assays) and phenolic profile (HPLC-DAD analysis).

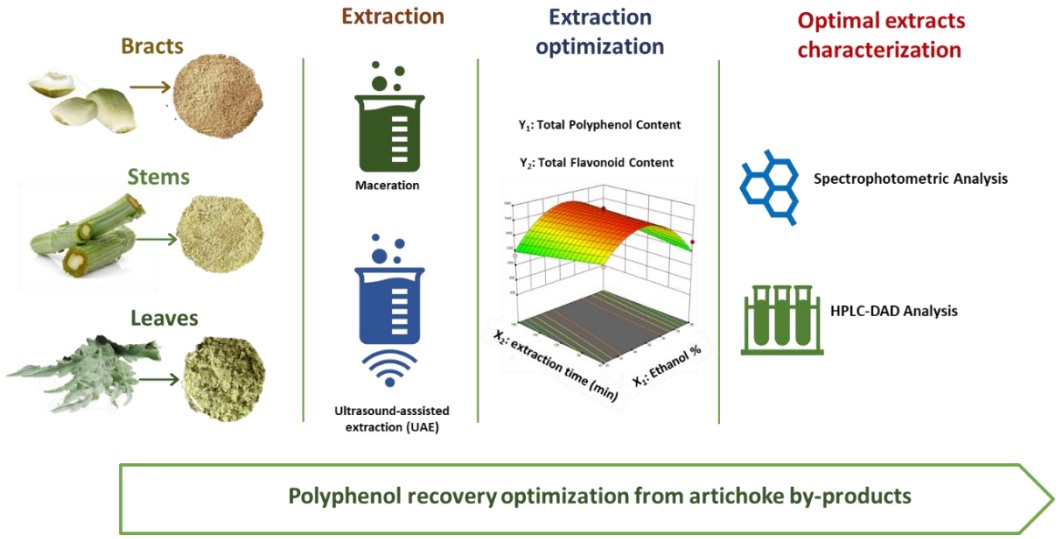

Figure 1. Graphical abstract of case study 1: Green recovery optimization of phenolic compounds from "Spinoso sardo" globe artichoke by-products using response surface methodology.

Table 1. Rotatable central composite design employed to investigate the impact of ethanol percentage and extraction time on polyphenol recovery via maceration.

Run	Coded levels*		Actual levels	
	Factor 1 Ethanol (%)	**Factor 2** Time (min)	**Factor 1** Ethanol (%)	**Factor 2** Time (min)
1	1.41	0	92	120
2	-1.41	0	8	120
3	**0**	**0**	**50**	**120**
4	**0**	**0**	**50**	**120**
5	-1	-1	20	60
6	0	1.41	50	205
7	**0**	**0**	**50**	**120**
8	1	1	80	180
9	**0**	**0**	**50**	**120**
10	0	-1.41	50	35
11	-1	1	20	180
12	**0**	**0**	**50**	**120**
13	1	-1	80	60

* The incorporation of star points (±α, where α is a function of the factors number and the value, in this case, is equal to 1.41) into the rotatable design allows for the prediction variance to remain constant, thus enhancing the overall quality of the prediction made. Central points are marked in bold.

Table 2. Central composite face-centered design employed to investigate the impact of ethanol percentage and extraction time on polyphenol recovery via ultrasound-assisted extraction.

Run*	Coded levels		Actual levels	
	Factor 1 Ethanol (%)	**Factor 2** Time (min)	**Factor 1** Ethanol (%)	**Factor 2** Time (min)
1	1	-1	80	10
2	**0**	**0**	**50**	**50**
3	-1	0	20	50
4	**0**	**0**	**50**	**50**
5	-1	-1	20	10
6	1	0	80	50
7	-1	1	20	90
8	0	1	50	90
9	**0**	**0**	**50**	**50**
10	**0**	**0**	**50**	**50**
11	**0**	**0**	**50**	**50**
12	1	1	80	90
13	0	-1	50	10

* Central points are marked in bold.

Case study 2. *Artichoke By-Product Extracts as a Viable Alternative for Shelf-Life Extension of Breadsticks*

In the second study, the extraction methods that had been optimized in the previous phase were employed to obtain polyphenol-rich extracts, from the artichoke by-product fractions. The outer bracts, stems, and leaves provided by the North Sardinia companies of the "Carciofo Spinoso di Sardegna D.O.P." consortium and collected in the 2020, were freeze-dried, powdered, and subjected to extraction. Two different concentrations (1000 ppm and 2000 ppm) of each by-product extract were separately incorporated into breadsticks prepared with a conventional formulation (type 0 wheat flour, sunflower oil, fresh compressed yeast, and salt). The selection of these two addition levels was based on the observation that the incorporation of functional ingredients in the form of extracts is typically conducted at relatively low addition levels, not exceeding 5000 ppm (Conte et al., 2021; Difonzo et al., 2018; Piechowiak et al., 2020). Additionally, preliminary laboratory tests indicated that higher addition levels, above 2000 ppm, had a detrimental impact on the dough's workability, thereby rendering the forming stage more difficult.

Firstly, the rheological properties and polyphenol content of the enriched doughs were evaluated to ascertain the effect of extract addition on dough workability and to determine whether polyphenol loss occurred during baking. In addition to polyphenol content and antioxidant capacity, chemical-physical and textural properties of breadsticks were evaluated to assess whether fortification could have an impact on the quality of the final product. The shelf-life of the breadstick samples was estimated with an accelerated novel method based on the OXITEST reactor.

Figure 2. Graphical abstract of case study 2: Are artichoke by-product extracts a viable alternative for shelf-life extension? A feasibility study on breadsticks.

Case study 3. *Effect of artichoke by-product powders on breadsticks nutritional, textural, sensorial, and volatile properties*

In the third study, freeze-dried powders derived from two fractions of artichoke by-products (outer bracts and floral stems) were employed as a functional ingredient for breadstick fortification. The by-products were provided by the consortium "Carciofo Spinoso di Sardegna D.O.P." and collected in 2021. The fortified samples were obtained through the individual addition of stem and bract powders to the basic formulation (identical to that employed in the case of study 2), at two different addition levels: low (3%) and high (5%) on a flour basis. The fortification percentages were selected based on the outcomes of preliminary trials. The use of artichoke leaf powder as a functional ingredient was not considered due to its detrimental impact on the sensory characteristics (notably the texture and flavor) of the final product, as evidenced by preliminary laboratory tests. Subsequent to the nutritional characterization of the

artichoke powders and in consideration of their fiber content, the specific water content of each sample was determined by farinographic analysis. The nutritional, textural, volatile and sensory characteristics of the breadsticks were evaluated.

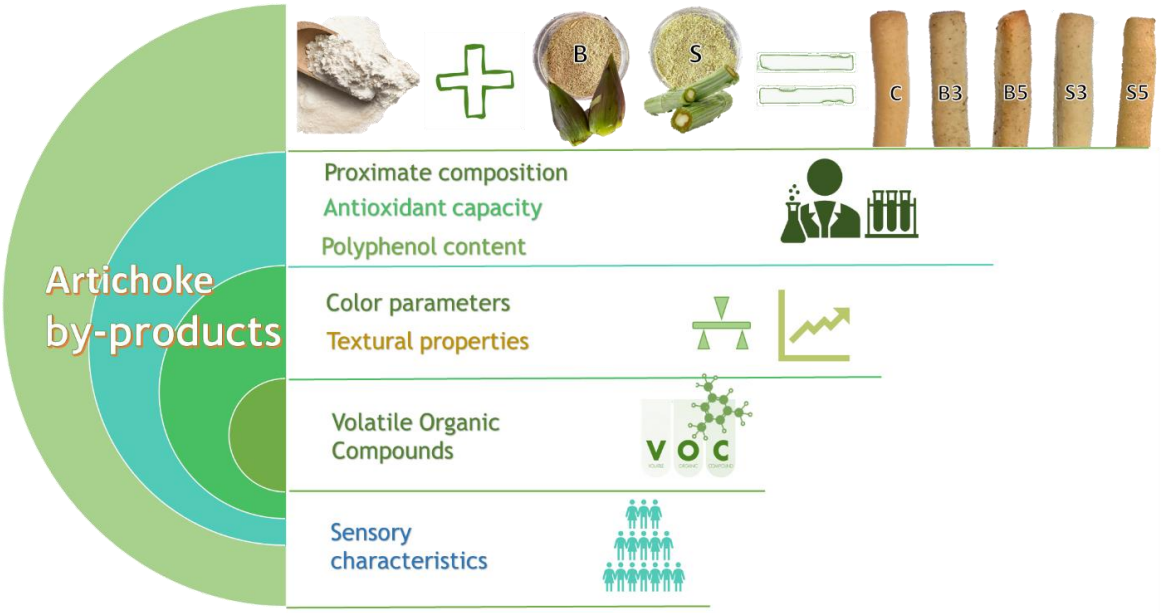

Figure 3. Graphical abstract of case study 3: Effect of artichoke by-product powders on breadsticks nutritional, textural, sensorial, and volatile properties. Abbreviations: B, bracts powder; S, stems powder; C, control breadstick; B3, B5, breadstick samples prepared with 3 and 5 % of bracts powder; S3, S5, breadstick samples prepared with 3 and 5 % of stems powder.

The methodology and statistical analyses employed for each study are thoroughly described in the subsequent chapters, along with the corresponding results.

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4. CASE STUDY 1

Green recovery optimization of phenolic compounds from "Spinoso sardo" globe artichoke by-products using response surface methodology

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Green recovery optimization of phenolic compounds from "Spinoso sardo" globe artichoke by-products using response surface methodology

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The reuse and valorization of agri-food by-products is a pivotal activity in the pursuit of a circular model that can improve sustainability and efficiency of agri-food production. During artichoke processing, 60–80% of the biomass produced by the plant consists of inedible fractions, which nevertheless represent a natural source of high value-added compounds, such as phenolics. In this study, response surface methodology was applied to investigate and optimize the amount of ethanol and the reduction of extraction time to achieve the maximum yield of polyphenols and flavonoids from artichoke stems, leaves, and bracts, by using two extraction methods, namely maceration and ultrasound-assisted extraction. Overall, phenolic compounds were most concentrated in extracts obtained from the stems, followed by those derived from the bracts and leaves, with the percentage of ethanol being the most influential factor. After applying the optimization criteria, the best factor setting to achieve maximum extraction yields and strong antioxidant capacities was: 53% ethanol for stems, 45% for leaves, and 50% for bracts and 60 min for all by-products in the case of maceration; 10 min for stems and leaves with 42 and 20% of ethanol, respectively, and 41 min and 64% ethanol for bracts in the case of ultrasound-assisted extraction. Comparison between the two techniques evidenced that maceration was significantly more efficient, but similar recoveries were obtained with ultrasound-assisted extraction in shorter extraction time and lower ethanol consumption. Therefore, using this unconventional method to convert Spinoso Sardo artichoke by-products into bioactive ingredients with interesting industrial applications could be a viable strategy to reduce food losses and mitigate related environmental impacts.

KEYWORDS
artichoke by-products, green recovery, phenolics, antioxidant activity, response surface methodology

1. Introduction

The agri-food industry generates a huge amount of by-products and waste every year, resulting in increasing disposal problems, environmental pollution, and sustainability issues. The Food and Agriculture Organization (FAO) found that around 14% of the food produced worldwide is lost between harvest and retail. Fruits and vegetables have the second highest

Green recovery optimization of phenolic compounds from "Spinoso sardo" globe artichoke by-products using response surface methodology

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Abstract

The reuse and valorization of agri-food by-products is a pivotal activity in the pursuit of a circular model that can improve sustainability and efficiency of agri-food production. During artichoke processing, 60-80% of the biomass produced by the plant consists of inedible fractions, which nevertheless represent a natural source of high value-added compounds, such as phenolics. In this study, response surface methodology was applied to investigate and optimize the amount of ethanol and the reduction of extraction time to achieve the maximum yield of polyphenols and flavonoids from artichoke stems, leaves, and bracts, by using two extraction methods, namely maceration and ultrasound-assisted extraction. Overall, phenolic compounds were most concentrated in extracts obtained from the stems, followed by those derived from the bracts and leaves, with the percentage of ethanol being the most influential factor. After applying the optimization criteria, the best factor setting to achieve maximum extraction yields and strong

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Keywords: artichoke by-products; green recovery; phenolics; antioxidant activity; response surface methodology.

1 Introduction

The agri-food industry generates a huge amount of by-products and waste every year, resulting in increasing disposal problems, environmental pollution, and sustainability issues. The Food and Agriculture Organization (FAO) found that around 14 % of the food produced worldwide is lost between harvest and retail. Fruits and vegetables have the second highest wastage rate among the different commodity groups after roots and tubers (Méndez et al., 2021). In this sense, reducing food processing wastes is one of the most important goals, but it is not the only way that can be achieved to improve and promote environmental sustainability and food security. In fact, food by-products are an extraordinary source of bioactive compounds, such as phenolics, as well as proteins, alkaloids, carbohydrates, and lipids (Fernández et al., 2018). Most agro-industrial wastes are allocated to the production of animal feed, fuel, or organic fertilizers.

However, there is growing interest in the valorization of fruit and vegetable by-products as a natural source of high value-added compounds that may be used as food and cosmetic ingredients (Taghian Dinani & van der Goot, 2022; Trigo et al., 2019).

The globe artichoke (*Cynara cardunculus* L. subsp. *scolymus* L.), which is a perennial herbaceous plant belonging to the Asteraceae family, is cultivated worldwide, and appreciated for its taste and health-promoting benefits. Its cultivation is considered an important agro-economy activity for Mediterranean region, especially for Italy, France, Spain, Egypt, and Morocco, that have an annual production of about 770,000 tons. The edible part of this plant is the core (inner bracts and receptacle) of the inflorescence called "capitula", harvested in the early stage. So, artichoke processing, which is directed to production of minimally, frozen, or canned items, generates several fractions of by-products (mainly leaves, outer bracts and stems) that represent about 60-80% of the total biomass, which amounts approximately to 460,000 tonnes of waste per year (Lattanzio et al., 2009; López-Salas et al., 2021a). This non-edible part, however, is still a source of constituents with high biological value, such as inulin and phenolic compounds, which are secondary metabolites known for their functional properties (hypocholesterolemic, antimicrobial, antioxidant, anticancer, anti-inflammatory, etc.). The main phenolics in artichoke tissues are caffeic acid derivatives, including chlorogenic acid, and a wide range of caffeoylquinic acid derivatives. Flavonoids, such as apigenin and luteolin, and several cyanidin caffeoylglucoside derivatives have also been identified (Lattanzio et al., 2009). Several studies showed that artichoke by-products are still a rich source of easily extractable phenolic compounds (Zuorro, 2014; Zuorro et al., 2014, 2016; Jiménez-Moreno et al., 2019). These bioactive compounds can be extracted by different solid-liquid conventional and non-conventional methods. The existing classical techniques, such as Soxhlet extraction, maceration and

hydrodistillation, are based on the extracting power of different solvents and on the application of heat and/or stirring. The main limitations of conventional methods are longer extraction time, usage of expensive solvents, low extraction selectivity, and thermal decomposition of thermolabile compounds. Non-conventional techniques were introduced to overcome these limitations. One of these methods is the ultrasound-assisted extraction (UAE), which causes a phenomenon called cavitation, that intensifies mass transfer and accelerates access of solvent to plant tissues (Reche et al., 2021). The benefits of UAE include a reduction of extraction time, amount of energy and solvent (Azmir et al., 2013). Organic solvents such as methanol, ethanol and acetone are generally used to extract phenolic compounds from plant matrices, often in combination with different proportions of water (Dai & Mumper, 2010). Ethanol is widely used because, in addition to being a good extraction solvent, is a food grade solvent, thus it is safe for human consumption (Dai & Mumper, 2010). Moreover, mixtures of alcohol solvents with water have been found to be much more efficient in extracting phenolics than when used individually (Garcia-Castello et al., 2022).

Besides the type of solvent used, other factors can affect the recovery of phenolic compounds, such as extraction time, temperature, and solid-solvent ratio (Živković et al., 2018). In general, shorter extraction times and smaller amounts of solvent ensure lower cost processes (Panja, 2018). With reference to artichoke by-products, other papers are present in the literature that deal with the use of UAE compared to maceration. While Reche et al. (2021, 2022) studied a mathematical model to simulate the extraction curves of total phenolic and chlorogenic acid content, as well as the effects on microstructural changes by using different temperatures and ultrasound power density in the stem fraction, Quispe et al. (2021) applied the Box-Wilson design to study the effect of ethanol concentration (40-60%), extraction time (5-15 min) and

radiation amplitude (80-100%) on the total phenolic content and antioxidant activity in the artichoke outer bracts. To the best of the authors' knowledge, however, there are no studies comparing the effect of the two extraction methods (maceration and UAE) on each individual fraction of artichoke by-products (stems, bracts, and leaves).

Therefore, in this work, the response surface methodology (RSM) with a Central Composite Design (CCD) was applied to optimize the extraction of phenolics and flavonoid compounds from three different artichoke discards – namely stems, leaves, and bracts – by finding the proper amount of ethanol and reducing the extraction time. This multivariate statistic technique is widely used for development, improvement and optimization of products and processes in which one or more responses are influenced by different variables. Furthermore, CCD, which is suitable to study factors with three to five levels, allows a large amount of information to be obtained from a limited number of experiments, but without neglecting the relationship among parameters (Yolmeh & Jafari, 2017). The experimental design was conducted using both maceration and UAE. Subsequently, the optimized extracts obtained for each fraction by both extraction methods were carried out to maximize the phenolic and flavonoid content and the antioxidant capacity. The phenolic profile was also investigated by HPLC-DAD.

The artichoke by-products used in this study belong to an important ecotype- Spinoso sardo- cultivated in Sardinia (Italy), which obtained the Protected Designation of Origin (PDO) in the year 2011 and, to date, is the only artichoke PDO in Europe. The cultivation of Spinoso sardo is currently undergoing a significant reduction due to increased irrigation volumes resulting from drought and global warming and to its seasonality. Therefore, the reduction of the huge amount of wastes produced might render more sustainable and competitive the artichoke industry.

2 Materials and Methods

2.1 Plant material and chemicals

By-products of Spinoso sardo artichoke (*Cynara cardunculus* L. var. *scolymus* Fiori) cultivar were provided by North Sardinia companies of consortium "Carciofo Spinoso di Sardegna DOP" and collected in the 2019. Outer bracts, leaves, and stems were individually stored at -20°C, freeze-dried and then finely ground with an ultracentrifugal rotor mill (WX Ultra Series, Thermo scientific, Waltham, Massachusetts, USA). The residual moisture content of artichoke samples was determined according to the official method AACC 44-15A. Dry powders were kept at -20 °C until analysis and extraction procedure.

Solvents like absolute ethanol and methanol, were purchased from VWR Chemicals BHD (Milan, Italy). The radical DPPH (2,2-diphenyl-1-picrylhydrazyl), the radical cation ABTS 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were obtained from Sigma-Aldrich (Milan, Italy). The standards used for identification and quantification of phenolic acids and flavonoids (apigenin 7-glucoside, luteolin 7-glucoside, caffeic acid, cynarin, 3,5-Di-O-caffeoylquinic acid, chlorogenic acid, neochlorogenic acid) were purchased from Extrasynthese (Genay, France). HPLC methanol was purchased from Carlo Erba (Milan, Italy).

2.2 Experimental designs

Two different Central Composite Designs (CCDs) were set with 13 randomized runs and 5 replicated central points (to evaluate the pure error) using the Design Expert software 10 (Stat-Ease Inc. Minneapolis, MN, USA). Specifically, a central composite

rotatable design was used to optimize maceration, while a face-centered design was employed for the UAE. CCDs were used to investigate the effect of two independent factors at three levels (low: -1, central: 0, and high: +1), i.e., ethanol percentage (X_1) and extraction time (X_2), on the recovery of bioactive compounds; as well as to evaluate the most influential of the 3 levels chosen for each factor. The levels of the selected factors were determined by preliminary trials. The experimental layout of the design utilized for maceration and UAE are displayed in Table 1 and Table 2, respectively.

Total phenolic content (TPC) and total flavonoid content (TFC) measured on each extract were used as outcome variables (Y_n).

2.3 Extraction and determination of total phenolic and flavonoid content

Bioactive compounds recovery from the different fractions of artichoke by-products were performed using maceration and UAE. Both extraction methods were carried out on 1 g of lyophilized sample by using 20 mL of ethanol/water food-grade solution, at different percentages (20, 50, 80%) according to the two factorial designs described above.

Maceration was conducted by shaking samples in a thermostatic water bath (model WB-MF24, FALC Instruments, Bergamo, Italy) set at 400 rpm and 38±2 °C, for a period of time varying from 60 to 180 minutes. UAE was performed using an ultrasonic bath (ARGO Lab, model DU-100, Carpi, Italy) at constant frequency of 40 kHz and a power of 144 W (parameters established by a previous experimental design), for times varying from 10 to 90 minutes. Then, the obtained mixtures were centrifuged at 9000 rpm for 10 min at 22 °C. Supernatants were collected, filtered by cellulose acetate syringe filter (0.45 µm pore-size), and stored at -20°C until analysis.

The determination of the TPC of artichoke extracts (bracts, leaves, and stems) was carried out following the Folin-Ciocalteu method proposed by Noriega-Rodríguez et al. (2020), with slight modifications. Briefly, in a test tube containing 7.5 mL of distilled water, 1 mL of sample diluted 1:10 (v/v) with the extraction solution, 0.5 mL of Folin Ciocalteau reagent (50%) and 1 mL of sodium carbonate (10%) were added. Samples were kept in the dark at room temperature for 1 h and then measured in a spectrophotometer (Agilent, model Cary 3500, Cernusco, Milan, Italy) at a wavelength of 765 nm. Results were expressed as mg of Gallic Acid Equivalent (GAE) per 100 g of dry matter (d.m.).

The TFC of the artichoke extracts was obtained by applying a spectrophotometric method (Dabbou et al., 2017a) and expressed as mg of Catechin Equivalent (CE) per 100 g of d.m.. An aliquot of 1 mL of extract, diluted 1:10 (v/v) with the extraction solution, was mixed with 5 mL distilled water and 0.3 mL of 5% $NaNO_2$ solution. Six minutes later, 0.6 mL of 10% $AlCl_3$ solution was added and allowed to react for another 5 minutes. Then, 2 mL of 1 M NaOH solution was added, and the total volume was made up to 10 mL with distilled water. The absorbance was measured at 510 nm. The analyses were conducted in triplicate.

2.4 Determination of antioxidant capacity

The antioxidant capacity was determined on the optimized artichoke extracts by both extraction methods using two different spectrophotometric assays (ABTS•+ and DPPH•) according to Prior et al. (2005), with some modifications.

DPPH• method. The DPPH• solution used was adjusted adding methanol to reach an initial absorbance of 1.0 ± 0.2. In this assay, aliquots of 70 µL of samples diluted with the extraction solution (1:10 v/v for bracts and stems and 1:4 v/v for leaves) were made

to react, for 30 minutes in darkness, with 2.03 mL of a DPPH• methanol solution (1mM). The decrease in absorbance of the radical DPPH was monitored using a spectrophotometer set at 517 nm, and results were compared to the concentration-response curve of the standard Trolox and expressed as µmol of Trolox equivalents per 1 g of d.m..

ABTS•+ method. The ABTS radical cation was produced by the reaction of 7.4 mM of ABTS•+ stock solution with 2.6 mM potassium persulfate (which were dissolved in phosphate buffer) in darkness at room temperature for 12 h. Before use, ABTS•+ was diluted with phosphate buffer to obtain a working solution with an initial absorbance of 1.0 ± 0.2 at 734 nm. After the addition of 40.8 µL of diluted sample to 2 mL of ABTS•+ solution, absorbance values were taken after 6 minutes of incubation at 22°C in the dark at 734 nm. Standard solutions of Trolox were used to calculate the antioxidant capacity and the results were expressed as µmol of Trolox equivalent (TE) per 1 g of d.m.. The assays were carried out in triplicate.

2.5 HPLC-DAD analysis

To analyze the phenolic fraction of the studied artichoke bracts, leaves, and stems, 5 mL of ethanol-water extracts that had given the best results in spectrophotometric analysis were concentrated to dryness in a rotary evaporator (Buchi, model Rotavapor R-200, Flawil, Switzerland), resuspended in 5 mL of a methanol-water solution (50:50), and filtered with 0.45 µm acetate cellulose syringe filters before HPLC analysis. The determination was performed by using an Agilent 1260 (Santa Clara, CA 95051, United States) equipped with a quaternary pump, an autosampler and a photodiode array detector (DAD). A reversed phase column Luna C18, 250 x 4.6 mm i.d., particle size 5 µm (Phenomenex, Torrance, California, USA), set at 40°C was used. The mobile phase

consisted of solvent A (methanol) and solvent B (water/acetic acid 95:5, v/v), as reported in D'Antuono et al. (2015). The following gradient was used: 85 to 60% B (0-25 min), 60% B (25-30 min), 60 to 37% B (30-45 min), 37% B (45-47 min), 37 to 0% B (47-52). The flow rate was set at 1.0 mL·min^{-1} and the injection volume at 25 μL. The photodiode array detection was performed at the absorbances of 280, 325 and 360 nm. Phenolics were identified by retention time and spectra of pure available standards. Additionally, when the standards were not available, chlorogenic acid and 3,5-*O*-dicaffeoylquinic acid were used for the quantification of mono and dicaffeoylquinic acids, respectively identified following the classification of Lattanzio et al. (2009).

2.6 Statistical analysis

Design Expert 10 Software (Stat-Ease Inc. Minneapolis, MN, USA) was used to analyze the results. To define predictive models, the experimental data were transformed, through a RSM analysis, into the following second-order polynomial model:

$$Y_n = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2$$

where Y_n are the responses, X_1 and X_2 are the actual values of the independent variables (ethanol percentage and extraction time), β_0 is the intercept, β_1, β_2 are the linear coefficients, while β_{11}, β_{22} and β_{12} are the quadratic and interaction regression coefficient terms, respectively. To obtain the most appropriate model, a backward regression technique was used to select only those independent variables and relevant interactions that showed significance ($p < 0.05$) at Analysis of Variance (ANOVA), based on the p-value ($p < 0.05$) and the Lack of Fit (LOF) test. P-values less than 0.05 indicate that model terms are significant, while a non-significant lack of fit means that the model is fitting well. Model reliability was also evaluated with the coefficients of

determination and adequate precision - which is a measure of the signal-to-noise ratio. If this ratio is greater than 4 the model can be used for the purpose of prediction and optimization (Fadjare Frempong et al., 2021). The R^2 values close to 1 are desirable; adjusted R^2 coefficients are useful when models have a great number of terms. Only significant regression coefficients should be considered in the equation and contribute to model development. Terms required to support the hierarchy are not removed from the model. Therefore, the models used in RSM are not always of second order (quadratic models), sometimes reduced models (linear, 2FI) can also be obtained (Yolmeh & Jafari, 2017). Design Expert software was also used to perform numerical optimization of parameters and model validation. The optimal levels of the studied factors were found for both extraction method via desirability function (D) that ranges from 0 to 1, where 1 is the most desirable condition for the maximization of the response variable (Garcia-Castello et al., 2022).

RSM was employed to optimize both extraction methods, by locating the best combination of parameters to minimize extraction time and maximize the responses for each by-product fraction.

The validation of the models was done by performing the UAE and maceration at the optimal parameters obtained and comparing the experimental values with those predicted.

Furthermore, the measured data on the extracts obtained by applying the optimal conditions were analyzed using the Statistica 12.0 software (StatSoft, Inc., Tulsa, OK, USA). First, the optimized extracts were subjected to the t-test and ANOVA to find statistical differences between the two extraction methods and among the by-products. Next, a two-way ANOVA was conducted to evaluate the effect of the waste fraction, the extraction method used and their interaction on the optimized data of TPC, TFC, DPPH•

and ABTS•+. Moreover, Pearson's correlation test was performed to assess the relationship between the results of antioxidant capacity, TPC, and TFC.

3 Results and Discussion

3.1 Effects of maceration parameters on TPC extraction

The results obtained for all by-product fractions from the CCD used for maceration are listed in Table 1. In general, stems showed a higher TPC, followed by bracts and leaves. In fact, TPC varied for stems from 1333 to 2441 mg GAE·100g^{-1}, for bracts from 692 to 1750 mg GAE·100g^{-1}, while for leaves ranged from 959 to 1632 mg GAE·100g^{-1}. These results are in good agreement with those reported by Fadda et al. (2018) in stems, outer bracts, and rosette and peduncle leaves of the Spinoso sardo ecotype, not only in terms of total concentration (2230, 1980, and 1830 mg GAE·100g^{-1} for stems, outer bracts, and leaves, respectively), but also in terms of distribution of these compounds in the various organs of the plant. Zuorro et al. (2014) reported similar ranges in artichoke stems (1266-2802 mg GAE·100g^{-1}) and outer bracts (1123-1944 mg GAE·100g^{-1}). Reche et al. (2021) , while observing the same distribution of polyphenols in stems and bracts of an artichoke variety grown in Spain, registered significantly higher values than those found in the present study for both fractions (4570 and 2740 mg GAE·100g^{-1} for stems and bracts, respectively). On the contrary, findings reported by Colantuono et al. (2018) in artichoke by-products of the Tondo di Paestum varietal type, while confirming the stems as the plant organs with the highest polyphenol accumulation (3470 mg GAE·100g^{-1}), revealed a higher TPC in the leaves (2160 mg GAE·100g^{-1}) than in the bracts (880 mg GAE·100g^{-1}). On the other hand, Rejeb et al. (2020), when analyzing the inedible parts of two Tunisian artichoke varieties, found that bracts (1526 mg GAE·100g^{-1}) were richer source of total polyphenols than leaves (1159 mg GAE·100g^{-}

^{1}) and floral stems (1069 mg GAE·100g^{-1}). The inconsistency on the different distribution and concentration of total polyphenols in the various organs of the globe artichoke plant that emerged in these studies, however, could be due to the genetic background, the environmental conditions, and the harvest period, as previously confirmed in the literature (Pandino et al., 2011b, 2011c).

The regression coefficients of the mathematical models obtained from the data of TPC collected for each fraction examined for maceration are reported in Table 3.

ANOVA analysis and fit statistics showed that the selected reduced quadratic models were significant ($p < 0.05$) and well fitted to the TPC for all three fractions analyzed, as evidenced by the satisfactory levels of R^2 and Adj R^2 that varied from 0.78 to 0.94 and from 0.73 to 0.91, respectively (Table 3). Moreover, the adequate precision values were greater than 4 - indicating that the model can be used to navigate the design space - and the LOF tests resulted in a non-significant F-value, denoting that the models are sufficiently accurate for predicting the TPC of the experimental by-products. As it can be seen in Table 3, the TPC of all three by-products was only affected by the negative quadratic regression coefficient of the ethanol percentage factor, while the effect of the extraction time was not significant ($p < 0.05$). This means that the extraction yield of total polyphenols increased gradually as the percentage of ethanol raised, reaching the highest values around 50% ethanol, and then decreased considerably with increasing levels of alcohol used, regardless of the extraction times chosen. This tendency can also be noted in the response surface plots displayed in Fig. 1 (A), (B), and (C), where it is evident that the extraction efficiency is reduced at small and high ethanol ratios, especially in stem and bract samples. In particular, the highest extraction efficiency values were reached at 45-55% ethanol in stems, 40-50% in leaves, and 40-60% in bracts. However, it should be noted that, although the linear regression coefficient of

extraction time (X_2) included in the model of the bracts was not statistically significant, it tended to negatively influence TPC extraction, suggesting that shorter extraction times might lead to a better extraction efficiency with lower ethanol concentrations (Fig. 1 (C)). It is known from the literature that polyphenolic compounds are present in the artichoke plant – as well as in most fruits and vegetables – mainly in the free and soluble conjugated form rather than in the insoluble-bound form (Domínguez-Fernández et al., 2021). Therefore, the lack of influence of the extraction time on the recovery of the phenolic fraction could be related to the relative solubility of the phenolics present in the plant, which, together with the solubilization capacity of the solvent and its polarity, influences their extractability and distribution coefficient (Gil-Martín et al., 2022). Probably, in a conventional extraction method, such as maceration, in which the release of polyphenols from plant matrices occurs according to their solubility (Gil-Martín et al., 2022), the equilibrium of diffusion of the solute had already been reached at the lowest level of the independent factor (within 60 minutes), making further extension of the extraction time irrelevant.

3.2 Effects of maceration parameters on TFC extraction

Flavonoids are a group of constituents that are included in the class of polyphenols, secondary metabolites found in vegetables, fruits, and some alcoholic beverages (Chávez-González et al., 2020; Panche et al., 2016). Within artichoke tissues, flavonoids are present in fewer amounts than caffeoylquinic acid derivatives, nevertheless they are important for their antioxidant properties and for their essential role in appearance of plant-based foods and, thus, in food acceptance (Lattanzio et al., 2009).

The results obtained for all by-product fractions from the CCD used for maceration are listed in Table 1. As with TPC, stems showed a higher TFC, followed by bracts and leaves. Specifically, TFC varied from 398 to 1959 mg CE·100g^{-1} for stems, from 278 to 1098 mg CE·100g^{-1} for bracts, and between 96 and 552 mg CE·100g^{-1} for leaves. Fadda et al. (2018) reported the same distribution of flavonoids in stems, outer bracts, and rosette and peduncle leaves of the Spinoso sardo ecotype and a similar TFC in the floral stems (2180 mg CE·100g^{-1}), but also higher concentration values than those found in the present study in both bract (1770 mg CE·100g^{-1}) and leaf (1220-1500 mg CE·100g^{-1}) fractions. Rejeb et al. (2020), despite a similar TFC observed in the stems of two Tunisian accessions (1113-1417 mg CE·100g^{-1}), recorded the highest concentration values in the leaves for both cultivars (5225-5823 mg CE·100g^{-1}), evidencing a wide variability in both flavonoid content and distribution depending on the plant organs and cultivar analyzed.

The regression coefficients of the mathematical models obtained from the data of TFC collected for each fraction examined for maceration are reported in Table 3. ANOVA results and fit statistics evidenced that the reduced quadratic models were significant ($p < 0.05$) and adequately accurate in predicting the TFC for stem and bract fractions only, as evidenced by the high levels of R^2 (0.85 to 0.92) and Adj R^2 (0.82 to 0.90), desirable values of adequate precision, and non-significant LOF tests. In contrast, the model selected to fit the experimental data for the leaf fraction, while significant ($p < 0.05$), was discarded because the LOF test resulted in a significant F-value, indicating that the model is not reliable and, consequently, does not allow adequate prediction of the data. As shown in Table 3, the ethanol percentage, as already observed for the recovery of the total polyphenols, was the only factor that affected the flavonoids extraction efficiency in both stems and bracts, while the effect of the extraction time was not significant ($p <$

0.05). Specifically, regarding the stem fraction, although the model analysis showed that the ethanol percentage had positive linear and negative quadratic effects on the TFC, values of regression coefficients revealed a greater influence of the linear term on the quadratic term. This means that regardless of the extraction time chosen, as the percentage of ethanol used increased, extraction yields also increased, reaching maximum efficiency at intermediate rates of ethanol concentration (40-70%). However, as ethanol percentage raised further, flavonoid yields began to decrease, although to a lesser extent than that observed at the lowest ethanol levels (Fig. 1 (D)). A similar tendency was also observed for the TFC of the bract fraction, which was found to be negatively affected only by the quadratic term of the ethanol factor. In this case, however, increasing the level of ethanol used led to an improvement in extraction efficiency, but only up to intermediate percentages, beyond which flavonoid yield strongly decreased. Although it is not possible to identify a single solvent (or aqueous formulation thereof) capable of maximizing extraction yields of phytochemicals from plant matrices of different origins, the results obtained in this study seem to confirm the high efficacy of using equivolumetric water/ethanol solutions in artichoke by-products. Similar conclusions were drawn by Zuorro et al. (2014) who reported that mixture composed of an equal proportion of low molecular weight polar compounds, such as ethanol and water, was more effective than other solvents in extracting phenolic compounds from artichoke stems and bracts. This could be due to the development of a synergistic effect between the two solvents when used in combination. Probably, water, which is a strongly polar solvent, acts as a swelling agent by increasing the contact surface area between the solvent and the plant sample, while ethanol promotes solubility and diffusion of phenolic compounds due to its lower polarity (Medina-Torres et al., 2017).

3.3 Effects of UAE parameters on TPC extraction

The results obtained for all by-product fractions from the CCD used for UAE are listed in Table 2. As already observed for maceration, stems showed a higher TPC, followed by bracts and finally by leaves. In this case, however, the data obtained exhibited lower variability probably in relation to the different extraction system, which, by allowing better solvent penetration even in a short time, resulted in greater leaching of phenolics. In fact, UAE relies on acoustic cavitation, which increases the permeability of the solvent within the plant matrix, particularly in the cell walls, and enhances the release of bioactive compounds (Medina-Torres et al., 2017). Specifically, in the present study, TPC ranged from 2236 to 2753 mg GAE·100g^{-1} for stems, from 1610 to 2212 mg GAE·100g^{-1} for bracts, while varied from 1309 to 1747 mg GAE·100g^{-1} for leaves. Reche et al. (2022), when performing an UAE for 35 min at 25°C in the stems of an artichoke variety grown in Spain, observed lower (1290 mg GAE·100g^{-1}) or similar (2370 mg GAE·100g^{-1}) yields of total polyphenols depending on the ultrasound power density applied. Quispe et al. (2021) extracted similar amount of total polyphenols from the outer bracts of an artichoke variety grown in Peru, using water/ethanol in different proportions as extraction solution. In contrast to the present study, Kollia et al. (2017) reported a lower total polyphenols concentration in both artichoke stems (330 mg GAE·100g^{-1}) and bracts (410 mg GAE·100g^{-1}), whereas Stumpf et al. (2020), applying the UAE and using 40% methanol as extraction solution, recovered higher yields of total polyphenols from the leaf fraction (2860 mg GAE·100g^{-1}). However, to the best of the authors' knowledge, the latter is the only study that carried out the extraction of phenolic compounds from artichoke leaves by using this unconventional extraction technique. Therefore, comparison of the data obtained with the literature is difficult.

The regression coefficients of the mathematical models obtained from the data of TPC collected for each fraction examined for UAE are reported in Table 4. As it can be seen, the relationship between the two independent variables and the selected response fitted well with the reduced quadratic model for stems and bracts and with a linear model for leaves. All regression models were found to be statistically significant ($p < 0.05$) and valid for the studied response within the selected range of factor's levels, as confirmed by the adequate R^2 and Adj R^2, the desirable values of adequate precision, and the non-significant F-value of the LOF test (Table 4). It is worth nothing that the model selected to fit the experimental data for the leaf fraction, while showing an intermediate R^2 that explained 59% of the variability in the data, was considered reliable since all the other statistical parameters, such as adequate precision and LOF test (as well as the final model validation) proved its effectiveness.

The selected fitting models revealed that polyphenols extraction efficiency was affected differently by the two independent factors depending on the by-product analyzed. Indeed, while in stem and leaf fractions TPC yield was influenced only by the change in ethanol percentage, in the bract fraction it was also affected by the extraction time. Specifically, regarding stems, model analysis showed that the ethanol percentage had positive linear and negative quadratic effects on the TPC, with values of regression coefficients revealing a greater influence of the linear term on the quadratic term. This means that the total polyphenol yields increased with increasing ethanol percentage until a maximum level of recovery is reached, beyond which additional alcohol increments led to a reduction in the total amount recovered. This behavior can be also observed in the response surface plot displayed in Fig. 2 (A), where the highest (2753 mg GAE·$100g^{-1}$) and the lowest (2236 mg GAE·$100g^{-1}$) TPC were reached at 50% and 80% ethanol, respectively. In contrast, the extraction of polyphenols from artichoke

leaves, being negatively affected only by the linear term of the ethanol percentage factor, followed a different trend. In fact, in this case, the recovery of total polyphenols decreased linearly as the level of the alcohol used increased (Fig. 2 (B)). Moreover, although the linear regression coefficient of extraction time (X_2) included in the model was not statistically significant, it tended to positively influence TPC extraction, suggesting that prolonged extraction times might lead to a better extraction efficiency. In fact, the maximum polyphenols yield, which amounted for 1747 mg GAE·100g^{-1}, was achieved at the lowest level of ethanol percentage and at intermediate to high extraction times. To better understand the different behavior observed in the two by-product fractions, it must be born in mind that, normally, free phenolic compounds are contained in cell vacuoles, whereas insoluble phenols are covalently bound to structural components of the cell and to rod-shaped structural proteins in the cell wall (Acosta-Estrada et al., 2014). Protein denaturation caused by the use of high concentrations of ethanol, may have hindered the dissolution of polyphenols, impairing their extraction efficiency (Chen et al., 2013). Probably, in this study, such effect was more pronounced in the leaves than in the stems due to the higher amount (at about 2.5-fold) of protein contained in this fraction (data not shown).

Unlike stems and leaves, model analysis for the bract fraction evidenced that the TPC extraction was positively affected by the linear term of the extraction time and negatively influenced by the quadratic term of the ethanol percentage (Table 4). This suggests that the highest TPC was achieved at intermediate ethanol percentages (40-60%), but longer extraction times (70-90 min), as also evidenced by the response surface plot depicted in Fig. 2 (C). These findings are consistent with those reported by Ghafoor et al. (2009), who observed higher yield of total polyphenols in grape seeds when UAE was done for a longer time.

3.4 Effects of UAE parameters on TFC extraction

The results obtained for all by-product fractions from the CCD used for UAE are listed in Table 2. Consistent with the results previously reported for TPC, stems showed a higher TFC, followed by bracts and leaves. Specifically, TFC varied from 928 to 1660 mg CE·100g^{-1} for stems, from 606 to 1247 mg CE·100g^{-1} for bracts, and between 337 and 593 mg CE·100g^{-1} for leaves. The polyphenols concentration observed in this study in the leaf fraction is similar to that recorded by Stumpf et al. (2020) in UAE extracts (at about 600 mg·100g^{-1}) obtained using 40% methanol as extraction solution. A more in-depth comparison of data is unfortunately difficult because, to the best of the authors' knowledge, there are no studies in the literature reporting the yield of TFC from artichoke by-products using the ultrasound-assisted technique.

The regression coefficients of the mathematical models obtained from the data of TFC collected for each fraction examined for UAE are shown in Table 4. The results of the ANOVA analysis and fit statistics evidenced that the only reliable regression model was the one obtained for the bract fraction. In fact, the models selected to fit the experimental data of both stems and leaves, while significant ($p < 0.05$), were discarded because the LOF test resulted in a significant F-value, indicating that the models do not allow adequate prediction of the data. Regarding the bract fraction, the relationship between the two independent variables and the TFC fitted well with a linear model in which only the regression coefficient of the ethanol factor was highly significant ($p < 0.01$). On the other hand, the linear coefficient of the extraction time, although not significant, was included in the model, suggesting it may have a positive influence on the extraction efficiency. This trend is clearly visible in the response surface plot shown in Fig. 2 (D), in which the yield of total flavonoids increased as the concentration of

ethanol used increased, with a more pronounced effect for extraction times from 30 min onward (Fig. 2 (D)).

3.5 Optimal parameters and their validation

The numerical optimization was performed considering the mathematical models gained, the significance of terms of the regression equations and the statistical parameters. Design Expert software, through the desirability function, allowed simultaneous optimization involving both factors and responses to achieve the desired goals. In the present study, the optimization was conducted for all by-product fractions and for both extraction methods with the aim of maximize the responses (TPC and TFC), keeping the value of X_1 factor in its range (20-80%) and specifying the value of X_2 factor as the minimum desirable. Specifically, while the former choice was made considering the greater impact exerted by the percentage of ethanol on the extraction efficiency of the bioactive compounds from all three by-products studied, the second was aimed at achieving the dual objectives of making the process more sustainable – through the reduction of energy consumption and, consequently, costs – and increasing the competitiveness of the industries. Accordingly, several combinations of optimal parameters were obtained for each fraction and extraction method under consideration. The best combination was found by using, when applicable, the combined maximum desirability of the models of the two responses (TPC and TFC). In fact, the desirability function (D) was applied to models that were able to predict well, with a not significant LOF. As a result of maceration optimization, the following parameters were obtained: 53 % of ethanol and 60 min of extraction (D = 0.91) for stems, 45 % and 60 min (D = 0.90) for leaves, and 50% and 60 min (D = 0.92) for bracts.

Whereas, with UAE optimization the succeeding combinations were reached: 42 % of ethanol and 10 min of extraction (D = 0.87) for stems, 20% and 10 min (D = 0.91) for leaves, and 64% and 41 min (D = 0.60) for bracts.

Therefore, the optimized results obtained evidenced that the maximum extraction efficiency can be achieved at intermediate ethanol concentrations for both extraction method and for all three by-products fractions – except for the sonicated leaves – probably due to the aforementioned synergistic effect established between the two polar solvents in equivolumetric solutions. The optimized extraction time, on the other hand, coincided with the lowest level of the factor-selected range for both type of extraction, except for sonicated bracts where the time required to maximize the recovery of bioactive compounds was at intermediate value.

The optimal extraction conditions predicted by the designs were then used to perform additional experiments needed to validate the models and confirm the accuracy of their predictive ability. To this end, TPC and TFC were redetermined on the extracts obtained at the optimal factor settings from both the extraction methods and for each artichoke by-product (Table 5). Specifically, the extracts produced by maceration showed the following TPC values: 2603.5 ± 10.33 mg GAE·100g^{-1} for stems, 1863.26 ± 5.81 mg GAE·100g^{-1} for leaves, and 1865 ± 4.93 mg GAE·100g^{-1} for bracts. The TFC values obtained were as follows: 1845.80 ± 72.06 mg CE·100g^{-1} in stems, 873.08 ± 4.83 mg CE·100g^{-1} in leaves, and 1464.64 ± 18.15 mg CE·100g^{-1} in bracts. On the other hand, the optimized UAE process enabled the following amount of TPC to be extracted: 2516.03 ± 4.35 mg GAE·100g^{-1} for stems, 1723.10 ± 20.03 mg GAE·100g^{-1} for leaves, 2014.40 ± 31.13 mg GAE·100g^{-1} for bracts; while the amount of TFC was of 1947.75 ± 4.67, 754.55 ± 29.07, 1380.39 ± 4.53 mg GAE·100g^{-1}, for stems, leaves, and bracts,

respectively. All the values were within the 95% prediction intervals, confirming that the models have a good fit to the experimental data and high predictive performance.

From the outputs of the two-way ANOVA shown in Table 5, it can be observed that for both TPC and TFC a highly significant effect ($p < 0.001$) of the by-product fraction was found. In fact, the highest values were obtained in the stems, regardless of the extraction method used, closely followed by the bracts and then the leaves. The gap in phenolic content among different parts of the globe artichoke plant is confirmed by the literature, as already reported above (Pandino et al., 2011b, 2011c).

The two-way ANOVA also revealed that the effect of the extraction method was significant only for TPC ($p<0.05$), with maceration being more effective than UAE, and that there was a highly significant interaction ($p < 0.01$) between the two simple effects (by-product fraction and extraction method) on both TPC and TFC.

The results revealed that significant differences were found in stems between the two extraction methods and that higher TPC were obtained with maceration, while UAE allowed better recovery of TFC. In leaves the maceration conducted to a significant higher yield extraction for both TPC and TFC. Concerning bracts, the macerations significantly increased the TFC content, but UAE was found more effective for TPC extraction ($p < 0.05$).

As it was possible to note, in most cases, contrary to what is usually found in the literature, maceration resulted in a more effective extraction of phenolic compounds. Generally, alternative extraction methods allow higher yields than conventional methods (Osorio-Tobón, 2020). In previous studies conducted on mango and kinnow peel, UAE proved to be a more efficient technique and resulted in higher phenolic content than maceration (Safdar, Kausar, & Nadeem, 2017; Safdar, Kausar, Jabbar, et al., 2017). Comparative investigations conducted on citrus peel and fresh olives also

indicated the greater efficiency of UAE in extracting phenolics, both in terms of yield and antioxidant properties, with respect to maceration (Deng et al., 2017; Saini et al., 2019). The contrasting results obtained in the present study are probably due to the different operating conditions (sample preparation, state of the raw material, solvents, extraction times, instrumentation etc.) and matrices used.

3.6 Antioxidant capacity

The complex nature of phytochemicals in plant extracts hinders accurate assessment of total antioxidant capacity by a single method, therefore two commonly used assays were employed in this study: DPPH• and ABTS•+. Both methods are based on electron transfer and reduction of colored oxidants by various antioxidant species in the extracts, which could react in different ways with the two radicals used. In fact, the values achieved with the ABTS•+ assay were always higher than those recorded with the DPPH• method (Table 5).

The following values were obtained from the DPPH• assay for maceration and UAE, respectively: in stems 84.14 ± 2.09 and 83.23 ± 2.68 µmol of TE·g^{-1}; in leaves 42.33 ± 2.00 and 22.37 ± 3.36 µmol of TE·g^{-1}; in bracts 65.41 ± 1.01 and 63.44 ± 1.34 µmol of TE·g^{-1}. Regarding the ABTS•+ assay, the results measured for maceration and UAE in artichoke by-products were as follow: in stems 105.35 ± 4.36 and 128.87 ± 5.95 µmol of TE·g^{-1}; in leaves 81.77 ± 4.42 and 61.21 ± 0.80 µmol of TE·g^{-1}; while in bracts 114.38 ± 2.33 and 116.37 ± 2.63 µmol of TE·g^{-1}.

In Table 5, the two-way ANOVA indicated a highly significant ($p<0.01$) effect of extraction method only for DPPH•. Instead, the by-product fraction effect affected significantly both the DPPH• and ABTS•+ results ($p<0.01$). In fact, the values measured on each fraction were different. The two-way ANOVA also pointed out the presence of

a significant interaction ($p<0.01$) between the effects of the extraction type and the by-product fraction. Therefore, it was noted that overall, the antioxidant capacity was highest in stems, followed by bracts and then leaves. In the DPPH• assay, the extraction method significantly influenced only the leaves fraction, where maceration yielded a higher extraction of antioxidant compounds. Besides, the ABTS•+ method put in evidence that maceration was more efficient for the leaves respect to UAE. On the contrary, in the stems the UAE led to higher results than maceration.

An additional aspect that emerged was that the antioxidant capacity values were consistent with the phenolic content of the extracts. As a matter of fact, Pearson's correlation test showed that TFC was significantly correlated with the antioxidant capacity, both in extracts obtained by maceration (DPPH• $r=0.99$ $p< 0.001$; ABTS•+ $r= 0.83$ $p<0.05$) and by UAE (DPPH• $r= 0.98$ $p<0.01$; ABTS•+ $r= 0.95$ $p<0.01$). With reference to TPC, a significant correlation with the values acquired from the DPPH• assay ($r=0.84$ $p<0.05$) was found for the extraction with maceration, while in the UAE the TPC resulted significantly correlated with both DPPH• and ABTS•+ values ($r= 0.93$ $p<0.01$ and $r= 0.88$ $p<0.05$ respectively). This correlation between the phenolic content and antioxidant capacity, in extracts obtained from artichoke residues, appears consistent with previous findings reported by other authors (Jiménez-Moreno et al., 2019; Rejeb et al., 2020). Moreover, the highest correlation values between TFC and ABTS•+ and DPPH• results, suggested that the antioxidant capacity of these extracts is closely related to their flavonoid content, probably due to the fact that flavonoids act as good hydrogen donors (Brown and Rice-Evans, 1998; Jiménez-Moreno et al., 2019).

3.7 Phenolics screening

Stems, bracts, and leaves extracts obtained by optimization of maceration and UAE were analyzed by HPLC-DAD to investigate their phenolic profiles. The concentration of the 16 identified compounds (flavones and caffeoylquinic acids) is displayed in Table 6. As it is conceivable to note, the two extraction methods allowed the extraction of the same compounds.

In both cases (UAE and maceration), stems and bracts showed similar phenolic compositions, in which predominant constituents were caffeoylquinic acid derivatives, such as chlorogenic acid, 1,5-di-*O*-caffeoylquinic acid, and 3,5-di-*O*-caffeoylquinic acid, in order of prevalence. Otherwise, in leaves there was a predominance of flavonoid compounds, with a high concentration of luteolin 7-*O*-glucoside. Apigenin was found in leaves extracts as both glucoside and rutinoside. These flavonoids were poorly represented or absent in extracts obtained from bracts and stems. In agreement with previous works, these results confirmed that phenolic compounds are distributed differently in distinct parts of the plant, probably depending on their specific biological role (Fratianni et al., 2007; Lombardo et al., 2010; Pandino et al., 2011b). In fact, as these flavonoids are also deputed to protect cells from oxidative damage by ultraviolet light, they are mainly concentrated in leaves, which are the parts of the plant most exposed to sunlight (Lombardo et al., 2010; Pandino et al., 2011b; Samanta, Das, & Das, 2011). Whereas in the stems there is a higher content of caffeoylquinic acids, probably because these compounds are involved in structural support within plant cell walls (Pandino et al., 2011b).

Overall, the total phenolics amount, obtained from the sum of all compounds quantified by HPLC-DAD, was highest in stems, followed by bracts and leaves (Table 6). Additionally, the t-test revealed that maceration was significantly more effective in stems and leaves, while for bracts UAE allowed for higher performance. The extraction

method did not affect the amount of cynarin (1,3-di-*O*-caffeoylquinic acid) taken out from all the three fractions, while it affected the quantity of the other phenolic compounds. This aspect becomes important if the aim is to obtain an extract with characteristics related to the specific phytochemicals.

4 Conclusions

Achieving the sustainable development goal of halving food losses and waste by 50% by 2030, set by the United Nations in 2015, inevitably comes through the upcycling and valorization of plants by-products, which are known to be rich in bioactive compounds with potential interest for the food industry. In the present study, the effect of maceration and ultrasound assisted extraction process variables on the green recovery of phenolic compounds from artichoke stems, leaves, and bracts were evaluated by RSM. The investigation and optimization of the independent variables influencing the extraction efficiency within the studied design space revealed that maximum polyphenol and flavonoid yields were maintained at the lowest levels of extraction time for both maceration (60 min) and UAE (10 min) – except for sonicated bracts, which took about 41 min – and at intermediate percentages of ethanol for both techniques (42%-64%) – except for sonicated leaves (20%). Under these optimal conditions, although maceration led to higher extraction efficiency, UAE resulted in very tight recoveries in shorter times and with lower ethanol consumption (except for bracts). Therefore, the use of UAE, in addition to being a competitive advantage for companies, can have a positive impact on the environment, economy, and society, allowing for a reduction in the amount of food waste generated, the energy consumption required during the recovery and valorization processes, and, consequently, process costs and the environmental impact.

Further research is needed to quantify the cost reduction associated with process optimization and the development on an industrial scale, as well as to evaluate the feasibility of incorporating these extracts into food products.

5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6 Author Contributions

MC, PC and ADC: methodology. MC: formal analysis and writing – original draft; PC, AP, and ADC: supervision and writing – review & editing; AP: funding acquisition. All authors contributed to manuscript revision, read, and approved the submitted version.

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FIGURES

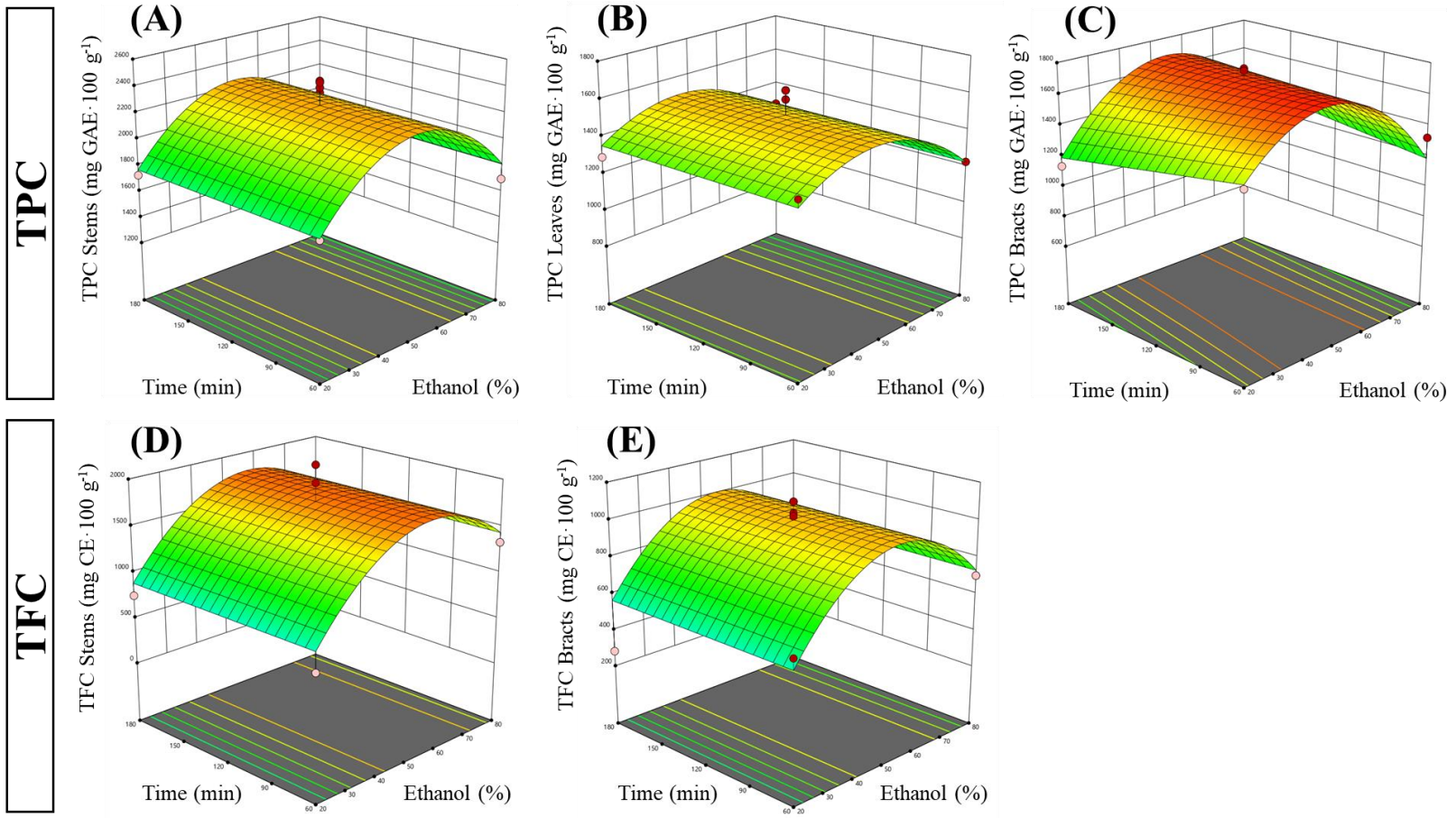

Figure 1. Response surfaces plots explaining the effect of time (X_1) and ethanol (X_2) factors during maceration on total phenolic content (TPC) in stems (A), leaves (B), bracts (C) and total flavonoid content (TFC) in stems (D) and bracts (E).

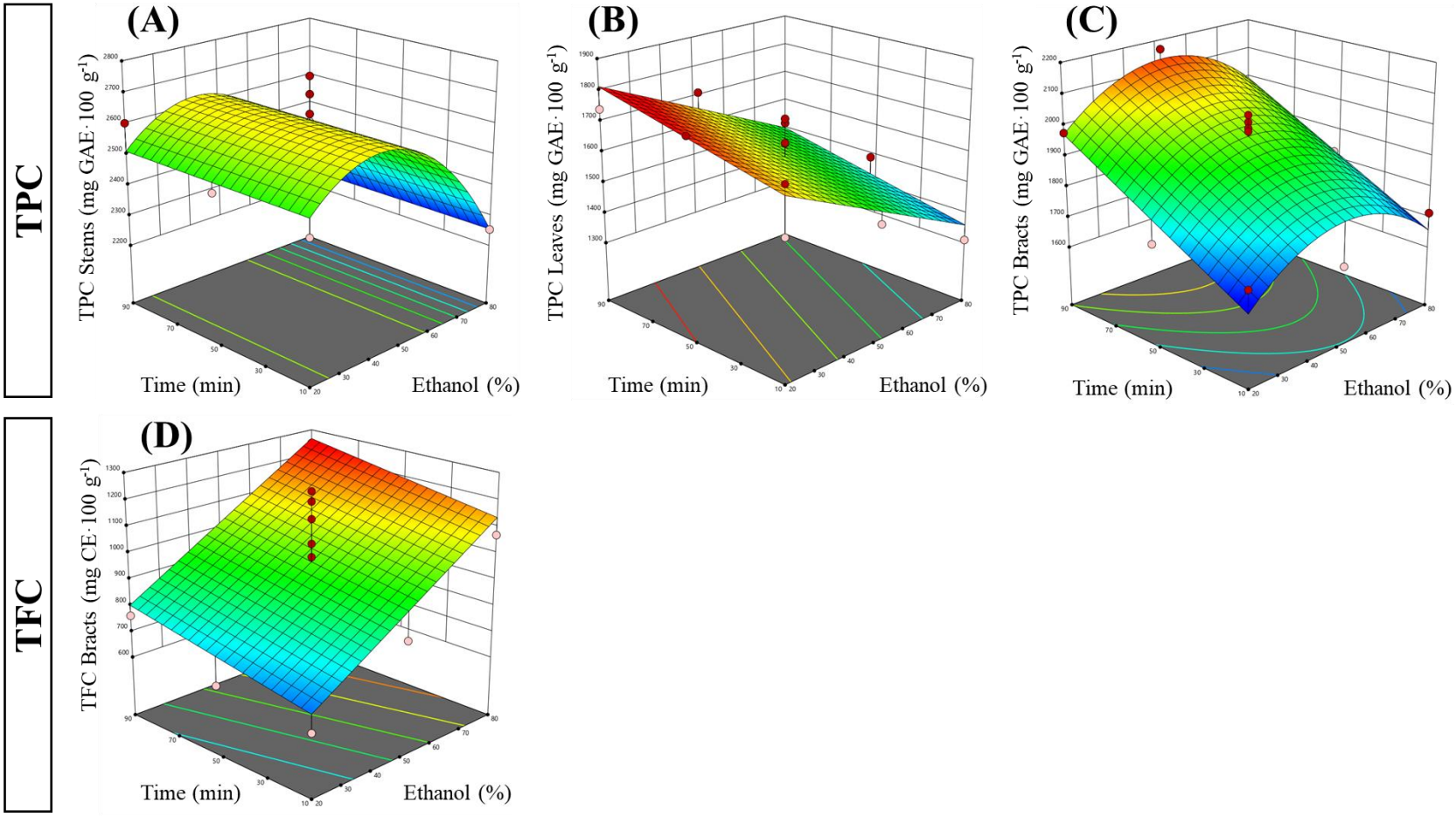

Figure 2. Response surfaces plots explaining the effect of time (X_1) and ethanol (X_2) factors during ultrasound assisted extraction on total phenolic content (TPC) in stems (A), leaves (B), bracts (C) and total flavonoid content (TFC) in bracts (D).

TABLES

Table 1. Central composite design used for maceration extraction (variable levels are presented in actual values) and experimental results of each by-product fraction.

Run order	Actual Levels		Stems		Bracts		Leaves	
	EtOH (%)	t (min)	TPC	TFC	TPC	TFC	TPC	TFC
1	92	120	1341 ± 113	838 ± 20	692 ± 25	431 ± 14	959 ± 65	231 ± 15
2	8	120	1333 ± 132	398 ± 3	1122 ± 158	307 ± 0	1189 ± 59	108 ± 12
3	**50**	**120**	**2429 ± 43**	**1744 ± 43**	**1750 ± 230**	**1098 ± 106**	**1632 ± 22**	**543 ± 6**
4	**50**	**120**	**2441 ± 66**	**1622 ± 57**	**1684 ± 234**	**861 ± 7**	**1584 ± 42**	**509 ± 10**
5	20	60	1760 ± 75	680 ± 7	1196 ± 235	278 ± 33	1396 ± 20	106 ± 1
6	50	205	2156 ± 541	1846 ± 72	1721 ± 171	1012 ± 56	1503 ± 157	537 ± 10
7	**50**	**120**	**2391 ± 234**	**1959 ± 16**	**1648 ± 171**	**968 ± 58**	**1632 ± 45**	**552 ± 5**
8	80	180	1970 ± 142	1670 ± 27	1460 ± 50	692 ± 53	1280 ± 16	215 ± 2
9	**50**	**120**	**2040 ± 77**	**1659 ± 26**	**1664 ± 187**	**1021 ± 43**	**1414 ± 131**	**532 ± 2**
10	50	35	2201 ± 15	1914 ± 3	1720 ± 65	1166 ± 39	1350 ± 36	498 ± 14
11	20	180	1729 ± 254	752 ± 39	1136 ± 146	282 ± 8	1292 ± 101	96 ± 1
12	**50**	**120**	**2164 ± 47**	**1731 ± 23**	**1598 ± 52**	**1038 ± 65**	**1372 ± 77**	**514 ± 6**
13	80	60	1701 ± 46	1333 ± 0	1323 ± 22	703 ± 28	1228 ± 84	192 ± 7

This table shows mean values ± standard deviation. The central points of the experimental design are marked in bold. Abbreviations: ethanol (EtOH); time (t); total phenolic content (TPC); total flavonoid content (TFC). The results of total phenolic content and total flavonoid content are expressed as mg of gallic acid equivalent (GAE) and catechin equivalent (CE) per 100 g of dry matter, respectively.

Table 2. Central composite design used for ultrasound assisted extraction (variable levels are presented in actual values) and experimental results of each by-product fraction.

Run order	Actual Levels		Stems		Bracts		Leaves	
	EtOH(%)	t (min)	TPC	TFC	TPC	TFC	TPC	TFC
1	80	10	2254 ± 18	1387 ± 7	1715 ± 11	1070 ± 10	1309 ± 17	507 ± 1
2	**50**	**50**	**2609 ± 17**	**1591 ± 21**	**1610 ± 11**	**1037 ± 31**	**1317 ± 11**	**550 ± 34**
3	20	50	2480 ± 4	1031 ± 40	1726 ± 2	628 ± 24	1747 ± 2	337 ± 12
4	**50**	**50**	**2633 ± 23**	**1576 ± 34**	**1923 ± 10**	**987 ± 39**	**1630 ± 10**	**520 ± 11**
5	20	10	2455 ± 20	934 ± 42	1707 ± 21	606 ± 12	1704 ± 21	340 ± 9
6	80	50	2277 ± 38	1236 ± 7	1824 ± 3	1063 ± 8	1489 ± 3	593 ± 14
7	20	90	2604 ± 5	928 ± 35	1977 ± 14	762 ± 32	1740 ± 14	364 ± 31
8	50	90	2560 ± 57	1151 ± 31	2169 ± 24	851 ± 23	1711 ± 24	381 ± 1
9	**50**	**50**	**2753 ± 26**	**1660 ± 50**	**1993 ± 20**	**1129 ± 18**	**1710 ± 20**	**565 ± 8**
10	**50**	**50**	**2696 ± 29**	**1616 ± 49**	**2012 ± 26**	**1194 ± 47**	**1696 ± 26**	**531 ± 14**
11	**50**	**50**	**2479 ± 4**	**1581 ± 51**	**2212 ± 2**	**1232 ± 14**	**1632 ± 2**	**558 ± 13**
12	80	90	2236 ± 3	1435 ± 13	1806 ± 23	1247 ± 6	1460 ± 23	553 ± 24
13	50	10	2563 ± 84	1442 ± 22	1654 ± 12	795 ± 39	1475 ± 12	410 ± 16

This table shows mean values ± standard deviation. The central points of the experimental design are marked in bold. Abbreviations: ethanol (EtOH); time (t); total phenolic content (TPC); total flavonoid content (TFC). The results of total phenolic content and total flavonoid content are expressed as mg of gallic acid equivalent (GAE) and catechin equivalent (CE) per 100 g of dry matter, respectively.

Table 3. Estimated regression coefficients of mathematical models (final equations in terms of actual factors) obtained for each response for different by-product fractions with the maceration and statistical criteria used to assess model accuracy.

Effect	Factor	Coefficient	Response: TPC			Response: TFC		
			Stems	Leaves	Bracts	Stems	Leaves	Bracts
	Intercept	β_0	930.92	1024.57	965.80	-362.14	-200.73	-117.16
Linear	$\mathbf{X_1}$	β_1	52.30 ns	21.14 ns	37.65 ns	76.00**	26.14 ns	42.03 ns
	$\mathbf{X_2}$	β_2	-	-	-3.03 ns	-	-	-
Interactive	$\mathbf{X_1 \cdot X_2}$	β_{12}	-	-	0.055 ns	-	-	-
Quadratic	$\mathbf{X_1^2}$	β_{11}	-0.51 ***	-0.23***	-0.45 ***	-0.67***	-0.25***	-0.39***
	$\mathbf{X_2^2}$	β_{22}	-	-	-	-	ns	ns
Statistics	**LOF**		Ns	ns	ns	ns	**	ns
	$\mathbf{R^2}$		0.89	0.78	0.94	0.92	-	0.85
	Adj $\mathbf{R^2}$		0.87	0.73	0.91	0.90	-	0.82
	Adequate precision		14.48	10.70	13.91	19.00	-	13.46

Abbreviations: total phenolic content (TPC); total flavonoid content (TFC); lack of fit (LOF); not significant (ns). Asterisks indicate significance levels at ANOVA: * significant at $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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Table 4. Estimated regression coefficients of mathematical models obtained for each response for different by-product fractions with the ultrasound assisted extraction and statistical criteria used to assess model accuracy.

Effect	Factor	Coefficient	Response: TPC			Response: TFC		
			Stems	Leaves	Bracts	Stems	Leaves	Bracts
	Intercept	β_0	2191.72	1756.92	1313.84	198.11	-193.46	503.77
Linear	$\mathbf{X_1}$	β_1	21.16**	-5.18 **	17.25 ns	46.27**	3.39 ***	7.69 **
	$\mathbf{X_2}$	β_2	-	1.77 ns	4.23***	-	6.17 ns	1.62 ns
Interactive	$\mathbf{X_1 \cdot X_2}$	β_{12}	-	-	-	-	-	-
Quadratic	$\mathbf{X_1^2}$	β_{11}	-0.25***	-	-0.17 **	-0.40**	-	-
	$\mathbf{X_2^2}$	β_{22}	-	-	-	-	-0.06**	-
Statistics	**LOF**		ns	ns	ns	**	*	ns
	$\mathbf{R^2}$		0.81	0.59	0.84	-	-	0.59
	Adj $\mathbf{R^2}$		0.77	0.51	0.78	-	-	0.51
	Adequate precision		9.34	8.50	12.33	-	-	7.99

Abbreviations: total phenolic content (TPC); total flavonoid content (TFC); lack of fit (LOF); not significant (ns).
Asterisks indicate significance levels at ANOVA: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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Table 5. Results of two-way ANOVA performed on total phenolic and flavonoid content, and on DPPH and ABTS results of the optimized extracts.

Source of variation	**TPC** mg GAE/100 g of d.m.	**TFC** mg CE/100g of d.m.	**DPPH** µmol TE/1 g of d.m.	**ABTS** µmol TE/1 g of d.m.
Extraction method				
Maceration	2110.77±381.71 a	1394.51±439.65 a	63.96±18.20 a	100.50±14.96 a
UAE	2084.51±359.13 b	1360.89±533.99 a	56.35±26.98 b	102.15±31.35 a
Significance	*	ns	***	ns
By-product fraction				
Stems	2559.76±50.91 a	1896.77±72.13 a	83.68±2.21 a	100.38±13.70 a
Leaves	1793.18±81.81 c	813.82±70.51 c	32.35±11.21 c	71.49±11.61 b
Bracts	1939.98±87.84 b	1422.51±49.83 b	64.42±1.51 b	115.38±2.48 a
Significance	***	***	***	***
Extraction method*By-product fraction				
Stems* Maceration	2603.50±10.33 a	1845.80±72.06 b	84.14±2.09 a	105.35±4.36 c
Stems*UAE	2516.03±4.35 b	1947.75±4.67 a	83.23±2.68 a	128.87±5.95 a
Leaves*Maceration	1863.26±5.81 d	873.08±4.83 e	42.33±2.00 c	81.77±4.42 d
Leaves*UAE	1723.10±20.03 e	754.55±29.07 f	22.37±3.36 d	61.21±0.80 e
Bracts*Maceration	1865.56±4.93 d	1464.64±18.15 c	65.41±1.01 b	114.38±2.33 b
Bracts*UAE	2014.40±31.13 c	1380.39±4.53 d	63.44±1.34 b	116.37±2.63 b
Significance	***	**	***	***

This table shows mean values ± standard deviation. Asterisks indicate significance levels at ANOVA: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; different letters denote significant differences ($p < 0.05$) at LSD test.

Abbreviations: total phenolic content (TPC); total flavonoid content (TFC); ultrasound assisted extraction (UAE); not significant (ns).

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Table 6. Phenolic composition of the different artichoke by-products fractions optimized extracts, obtained with maceration and UAE, expressed in mg •100g^{-1} of lyophilized material.

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Compound	Maceration			UAE		
	S	L	B	S	L	B
1-*O*-caffeoylquinic acid[a]	26.48±0.11 Ba	0.42±0.06 cA	37.32±0.01 aA	24.32±0.28 bB	0.40±0.02 cA	32.36±1.13 aB
Neochlorogenic acid[b]	35.06±0.61 aA	6.05±0.04 cB	17.37±0.06 bA		7.87±0.06 cA	20.41±1.16 bA
Chlorogenic acid[a]	977.16±1.68 aA	75.26±0.01 cA	660.81±2.29 bA	954.39±0.98 aB	33.08±0.08 cB	672.80±4.62 bA
1,3-di-*O*-caffeoylquinic acid (Cynarin)[c]	4.61±0.01 aA	0.75±0.00 cA	2.82±0.49 bA	4.53±0.03 aA	0.75±0.00 bA	3.12±1.10 aA
Caffeic acid[d]	35.76±0.03 aA	14.26±0.07 bA	13.34±0.30 cA	33.48±0.03 aB	9.75±0.01 cB	15.19±0.56 bA
1,4-di-*O*-caffeoylquinic acid[e]	20.70±0.01 aA	0.63±0.03 cB	9.44±0.05 bA	20.80±0.13 aA	0.78±0.01 cA	9.40±0.06 bA
4,5-di-*O*-caffeoylquinic acid[e]	34.28±0.26 aA	0.84±0.03 cA	16.37±0.31 bA	32.73±0.14 aB	0.23±0.01 cB	16.80±0.18 bA
3,5-di-*O*-caffeoylquinic acid[e]	484.77±0.38 aA	8.22±0.02 cA	406.90±0.30 bB	442.48±0.52 aB	2.72±0.03 cB	429.69±0.07 bA
1,5-di-*O*-caffeoylquinic acid[e]	886.16±0.67 aA	-	782.40±1.47 bB	846.56±0.99 aB	-	814.68±1.15 bA
3,4-di-*O*-caffeoylquinic acid[e]	22.30±0.50 aA	-	16.32±0.24 bA	19.56±0.10 aB	-	15.49±0.02 bB

Luteolin 7-*O*-glucoside[f]	-	383.53±3.48 A	-	-	264.31±0.42 B	-
Luteolin[f]	-	4.84±0.04 A	-	-	2.61±0.06 B	-
Apigenin 7-O-rutinoside[g]	-	16.10±0.26 A	-	-	12.90±0.05 B	-
Apigenin 7-*O*-glucoside[g]	-	4.53±0.31 bA	140.30±0.73 aB	-	4.08±0.05 bA	145.72±0.73aA
Luteolin[f]	17.41±0.09 bA	155.06±0.41 aA	17.03±0.04 bA	17.44±0.31 bA	101.45±0.15 aB	16.98±0.04 bA
Luteolin[f]	10.56 ±0.03 cA	30.47±0.19 aA	28.48±0.09 bA	10.35±0.07 cA	13.78±0.09 bB	26.21±0.09 aB
Total polyphenols	**2555.26±15.48 aA**	**700.95±3.17 cA**	**2148.89±4.93 bB**	**2438.68±18.07 aB**	**454.70±0.41 cB**	**2218.83±9.08 bA**

This table shows mean values ± standard deviation. Different letters indicate significant differences ($p<0.05$) between by-product fractions (lowercase letters) and extraction method (uppercase letters) at ANOVA LSD test and t-test.

Abbreviations: Ultrasound assisted extraction (UAE); stems (S); bracts (B); leaves (L). [a] Expressed as chlorogenic acid equivalent; [b] expressed as neochlorogenic acid equivalent; [c] expressed as cynarin equivalent; [d] expressed as caffeic acid equivalent; [e] expressed as 3,5-di-*O*-caffeoylquinic acid equivalent; [f] expressed as luteolin 7-*O*-glucoside equivalent; [g] expressed as apigenin 7-*O*-glucoside equivalent.

5. CASE STUDY 2

Artichoke By-Product Extracts as a Viable Alternative for Shelf-Life Extension of Breadsticks

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Artichoke By-Product Extracts as a Viable Alternative for Shelf-Life Extension of Breadsticks

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Abstract: The upcycling of agricultural by-products and the extension of the shelf-life of staple foods represent crucial strategies for mitigating the consequences of food losses and enhancing the competitiveness of the agri-food industry, thus facilitating the attainment of higher financial revenues. This is particularly relevant for global artichoke cultivation, where 60–80% of its biomass is discarded annually. The present study investigated the potential of using non-stabilized polyphenol-rich extracts from the main artichoke by-products (bracts, leaves, and stems) to fortify and extend the shelf-life of breadsticks. The incorporation of hydroalcoholic extracts at two addition levels (1000–2000 ppm) resulted in an increased antioxidant capacity and oxidative stability of fortified breadsticks. Rheological tests revealed that the fortification did not affect the dough's workability, with the exception of the leaf extract. While a slight deterioration in texture was observed, the shelf-life of breadsticks was significantly extended, particularly at the highest levels of addition, without any visible alteration in their appearance. The stem extract demonstrated the most promising outcomes, exhibiting a maximum increase of 69% in antioxidant capacity (DPPH) and an extension of the estimated shelf-life by 62% in the resulting breadsticks, prompting the potential for utilizing them to develop nutritious and healthy snacks with extended shelf-life.

Keywords: artichoke by-product extracts; breadsticks; fortification; hydroalcoholic extracts; antioxidant capacity; estimated shelf-life; OXITEST

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1. Introduction

According to FAO estimates, one-third of the food produced for human consumption annually, which is equivalent to about 1.3 billion tons, is lost or wasted worldwide, along the entire supply chain, from agricultural production to final household consumption [1]. In recent years, one of the primary objectives has been to guarantee food security and environmental sustainability. This has entailed the production of safe and nutritious foods while reducing food losses, with the implementation of cost-effective solutions. The growing attention to this problem is reflected in the United Nations Sustainable Development Goals, which call for a reduction in per capita food waste by 50% by 2030 [2]. The fruit and vegetable sector suffers from a high incidence of food surplus and waste, and a food loss rate of about 22% from post-harvest to distribution is realistic [3]. A reduction in wastage, especially in industrialized countries, could be attained by intervening along the various stages of the supply chain, via different strategies, for example, the reuse of fruit and vegetable by-products, which are extremely rich in bioactive compounds, to obtain functional ingredients with high added value. In addition, these by-products, which are also replete with antioxidants, can be employed to delay lipid oxidation and extend the shelf-life of food products. The term "shelf-life" is not used to define the real life of the product; rather, it represents the period of time that ends when the product undergoes physical and sensory deterioration that cannot be tolerated by the consumer, which compromises its marketability [4]. In fact, a longer shelf-life should minimize domestic

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Artichoke By-Product Extracts as a Viable Alternative for Shelf-Life Extension of Breadsticks

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Abstract: The upcycling of agricultural by-products and the extension of the shelf-life of staple foods represent crucial strategies for mitigating the consequences of food losses and enhancing the competitiveness of the agri-food industry, thus facilitating the attainment of higher financial revenues. This is particularly relevant for the global artichoke cultivation, where 60-80% of its biomass is discarded annually. The present study investigated the potential of using non-stabilized polyphenol-rich extracts from the main artichoke by-products (bracts, leaves, stems) to fortify and extend the shelf-life of breadsticks. The incorporation of hydroalcoholic extracts at two addition levels (1000-2000 ppm) resulted in an increased antioxidant capacity and oxidative stability of fortified breadsticks. Rheological tests revealed that the fortification did not affect dough's workability, with the exception of the leaf extract. While a slight deterioration in texture was observed, the shelf-life of breadsticks was significantly extended, particularly at the highest levels of addition, without any visible alteration in their appearance. The stem extract demonstrated the most promising outcomes, exhibiting a maximum increase of 69% in antioxidant capacity (DPPH) and an extension of the

estimated shelf-life by 62% in the resulting breadsticks, prompting the potential for utilizing them to develop nutritious and healthy snacks with extended shelf-life.

Keywords: artichoke by-product; breadsticks; fortification; hydroalcoholic extracts; antioxidant capacity; estimated shelf-life; OXITEST.

1 Introduction

According to FAO estimates, one-third of the food produced for human consumption, which is equivalent to about 1.3 billion tons, is annually lost or wasted worldwide, along the entire supply chain, from agricultural production to final household consumption (FAO, 2011). In recent years, one of the primary objectives has been to guarantee food security and environmental sustainability. This has entailed the production of safe and nutritious foods while reducing food losses, with the implementation of cost-effective solutions. The growing attention to this problem is reflected in the United Nations Sustainable Development Goals, which call for a reduction in per capita food waste by 50% by 2030 (FAO, 2019). The fruit and vegetable sector suffers from a high incidence of food surplus and waste, and a food loss rate of about 22% from post-harvest to distribution is realistic (Bartezzaghi et al., 2022). The reduction of wastage, especially in industrialized countries, could be attained by intervening along the various stages of the supply chain, via different strategies. For example, the reuse of fruit and vegetable by-products, which are extremely rich in bioactive compounds, to obtain functional ingredients with high added value. In addition, these by-products, which are also replete of antioxidants, can be employed to delay lipid oxidation and extend the shelf-life of food products. The term “shelf-life” is not used to define the real life of the product; rather, it represents the period of time that

ends when the product undergoes physical and sensory deterioration that cannot be tolerated by the consumer, which compromises its marketability (Alamprese et al., 2017). In fact, a longer shelf-life should minimize domestic wastes, considering the careless attitude to manage food provision of most consumers, and reduce the economic and environmental impacts of the distribution logistics (Bacenetti et al., 2018).

Baked products such as breadsticks are distinguished by a low water activity, which confers them a long stability. However, they are prepared by including high amounts of lipids, such as un-saturated vegetable oils or animal fat, in their basic formulation. Consequently, lipid oxidation is one of the primary causes of the quality decay of breadsticks (Bianchi et al., 2021; Conte et al., 2021). To counteract lipid oxidation and extend their shelf life, antioxidants can be added to their formulation (Bianchi et al., 2021; Conte et al., 2021; Hammad et al., 2021). Furthermore, these cylindrical-shaped snacks, are commonly eaten all over the world as appetizers due to their convenience, crunchiness, and taste. Consequently, they can be easily fortified with functional ingredients and employed as carriers of bioactive compounds to confer additional health benefits, given that they are staple foods and are consumed on a daily basis. Only few studies concerning the impact of by-products or by-product extracts incorporation on the shelf-life and oxidative stability of breadsticks are available in the literature. Conte et al. (2021) investigating the effect of the incorporation of phenolic-rich extracts, from olive leaves and olive mill wastewater into gluten-free bread-sticks formulations, observed an increase in the content of polyphenols and a significant extension of their shelf-life. By replacing wheat flour with grape pomace powder, Bianchi et al. (2021) reported an improvement of the nutritional profile but a worsening in the oxidative stability of breadsticks. In another work, breadsticks fortified with different amount of brewer's spent grain were found to be quite stable, in terms of both texture and water

activity, during storage, and with an implemented protein and dietary fiber content (Ktenioudaki et al., 2012). To date, no research has been conducted to evaluate the effect of artichoke by-product fortification on the oxidation stability and shelf-life of breadsticks.

Indeed, globe artichoke (Cynara cardunculus L. subsp. scolymus L.), consumed worldwide, represents an important agro-economic source for the Mediterranean basin, which produces considerable amounts of leftovers (60-80% of the total biomass). The main by-products derived from the processing industry are outer bracts, leaves, and stems, which, like the edible parts, contain a great variety of natural antioxidants, such as polyphenols, particularly phenolic acids and flavonoids (Cannas et al., 2023; López-Salas et al., 2021; Lattanzio et al., 2009). In general, agri-food wastes are transformed into powders, flours, or, less frequently, extracts for incorporation into bakery products. This is done in order to improve the nutritional value of the products, particularly in terms of phenolic compound content and antioxidant capacity (Melini et al., 2020). The most straightforward approach for enhancing the nutritional profile of staple foods is the use of powders. Indeed, the same authors recently conducted a study in which they successfully fortified breadsticks by adding increasing percentages (3 and 5 %) of powdered by-products such as artichoke stems and bracts (Cannas et al., 2024). The incorporation of these by-products significantly increased the nutritional and textural properties, as well as the antioxidant capacity of the breadsticks. This evidence substantiates the potential of arti-choke by-products as a fortification strategy for this type of bakery product.

Nevertheless, the utilization of extracts may be of interest, particularly with regard to the prolongation of the shelf life of baked goods. This is due to the fact that they are more concentrated than their initial matrices in antioxidants, which can be added in

small amounts without significantly altering the appearance of the final product. Extracts are usually stabilized to protect the more labile bioactive compounds, through techniques such as spray drying and freeze drying, resulting in higher production costs (Kurek et al., 2022). In this contest, the present study aimed to evaluate the feasibility of employing polyphenol-rich extracts derived from artichoke bract, stem and leaf fractions to improve the antioxidant capacity and shelf life of breadsticks. In order to minimize the economic impact and com-ply with the principles of sustainability, the three hydroalcoholic extracts (prepared with food-grade ethanol) were added directly to the formulations without any further pro-cessing. Different phytochemical species can be extracted from each by-product fraction (Cannas et al., 2023), and consequently distinct effects could be observed in the enriched product. To accomplish this, two different levels (1000 ppm and 2000 ppm) of each by-product extract were individually incorporated into the formulation. Firstly, the rheological properties and the polyphenol content of the enriched doughs were evaluated to assess both the effect of the extract addition on the workability of the dough and if there was any loss of polyphenols as a result of the baking process. In addition to the polyphenol content and antioxidant capacity, chemical-physical and structural properties of the breadsticks were measured to determine whether fortification could affect the quality of the final product. The shelf-life of breadstick samples was estimated by using OXITEST, which was found to be an excellent and innovative tool for estimating the shelf-life of baked goods in a short time (Caruso et al., 2017).

2 Materials and Methods

2.1 Raw materials

Breadsticks ingredients (wheat flour, sunflower oil, fresh compressed yeast, and salt) were purchased from a local grocery store. “Spinoso sardo” artichoke (*Cynara scolymus* L.) by-product fractions (stems, leaves and outer bracts), from the 2020 crop season, were supplied by the North-Sardinian companies of consortium “Carciofo Spinoso di Sardegna D.O.P.”.

2.2 By-product extracts preparation

The artichoke by-products were individually freeze-dried and finely milled and extracted in accordance with procedures optimized by the same authors for each fraction in a previous study (Cannas et al., 2023). This was done with the objective of obtaining the highest polyphenol content present in each fraction. To this end, the leaf extract was obtained by macerating 1 g of freeze-dried leaves for 60 minutes at a temperature of 38±2°C using 20 mL of a 45% ethanol hydroalcoholic solution. The stems and bracts were subjected to ultrasound-assisted extraction (frequency of 40 kHz and power set at 144 W) using a hydroalcoholic solution (with a concentration of 42% ethanol for stems and 64% ethanol for bracts) at a ratio of 1/20 (w/v) for 10 and 41 minutes, respectively. The decision to proceed with ultrasonic-assisted ex-traction for the bracts and stems, rather than maceration, was made on the grounds that this approach still permitted a high level of polyphenol recovery while offering significant time savings. Subsequently, all three extracts were centrifuged for 10 minutes at 9000 rpm, filtered through cellulose acetate syringe filters (0.45 μm pore-size) and stored at -20°C be-fore use.

2.3 Total polyphenol content and antioxidant capacity of by-product extracts

Total polyphenol content of the extracts obtained from artichoke by-products (bracts, leaves and stems) was determined following the Folin-Ciocalteu method, reported by Cannas et al. (2023). Specifically, 1 mL of extract was mixed with 7.5 mL of distilled water and then 0.5 mL of Folin Ciocalteau reagent (50%) and 1 mL of sodium

carbonate (10%) were added. After 1 hour in the dark at room temperature, the absorbance was measured in a spectrophotometer (Agilent, model Cary 3500, Cernusco, Milan, Italy) at a wavelength of 765 nm and the results were expressed as mg gallic acid equivalent (GAE) per 100 g dry matter (d.m.).

Antioxidant capacity was determined using two different spectrophotometric methods (ABTS and DPPH) according to Cannas et al. (2023). In the DPPH assay, 70 μL of the extract were made react in the dark with 2.03 mL of a DPPH-methanol solution (0.1 mM), having an initial absorbance of 1.0 ± 0.2 (obtained by correcting with methanol additions). After 30 minutes under constant stirring, the drop in DPPH absorbance was measured using a spectrophotometer set at a wavelength of 517 nm. In the ABTS assay, a solution was prepared by combining equal volumes of ABTS (7.4 mM) and potassium persulfate (2.6 mM), both prepared with phosphate buffer and left to react for at least 12 hours in the dark at room temperature (20-22 °C). The resulting solution was then further diluted with phosphate buffer before use to obtain an initial absorbance of 1.0 ± 0.2 at 734 nm. Then 40.8 μL of extract were added to 2 mL of ABTS working solution. The absorbance values at 734 nm were measured after 6 min of incubation at 22°C in the dark. Con-centration-response curves produced with standard Trolox solutions were used to calculate the antioxidant capacity, and the results were expressed for both assays as μmol of Trolox equivalent (TE) per 1 g of d.m. The assays were performed in duplicate.

2.4. Doughs and Breadsticks preparation

The samples were fortified by individually adding the hydroalcoholic extracts of artichoke outer bracts (BE), leaves (LE) and stems (SE) to the base formulation, at two different addition levels: level 1 (1000 ppm) and level 2 (2000 ppm). These levels were selected based on data reported in the literature (Conte et al., 2021; Difonzo et al., 2018)

and preliminary laboratory trials, which demonstrated that higher fortification levels negatively impact dough machinability, particularly during the forming stage.

The control formulation consisted of type 0 flour, 50% water, 10% sunflower oil, 3% compressed yeast, 1.8% salt (% based on flour).

Samples for dough analysis were prepared in duplicate by kneading the ingredients provided in the formulation without the addition of yeast, using a mixer (KitchenAid Professional, Model 5KSM7990, St. Joseph, MI, USA) furnished with a dough hook, at a speed 2 for 6 minutes. The resulting doughs were immediately frozen (-20°C), lyophilized after 24 hours, finely ground, and stored at -20°C until analysis.

The breadsticks were prepared by first suspending the extracts, yeast, and salt in different aliquots of water (about 26°C), which were then added to the flour and sunflower oil and kneaded (6 minutes, speed 2). The doughs obtained were subsequently laminated (Domino S.r.l., Model SFO600, Schio, Italy) to a final thickness of 0.3 cm, cut into 18 cm long sheets and formed using a breadstick machine (Italpan, Model AFP/GR15, Schio, Italy) equipped with 1 cm diameter grooves. The shaped doughs were then proofed in a climatic chamber (Tecnomac Lev2+, Castel MAC S.r.l. Veneto, Italy) at a temperature of 30 °C and a relative humidity (RH) of 75 %. The leavening process was completed when the initial volume had doubled, which took about 35 minutes. After baking for 16 minutes in an electric oven (Europa, Malo, VI, Italy) at 200 °C, the breadsticks were cooled for 30 minutes before the analysis. Three batches for each sample were made.

Sample codes were assigned to the doughs according to the type of extract used (BE, LE, SE) and the level of supplementation (1 or 2): DCTRL (control dough sample), DBE 1, DBE 2, DLE 1, DLE 2, DSE 1, DSE 2. To distinguish breadstick samples from dough samples, codes beginning with B were assigned: BCTRL for the control

breadsticks sample and BBE 1, BBE 2, BLE 1, BLE 2, BSE 1, BSE 2 for the fortified breadsticks.

2.5 Determination of phenolic fractions of doughs and breadsticks

The methodologies previously elucidated by Conte et al. (2021) were employed to determine the soluble and insoluble phenolic fractions in doughs and breadstick samples. In detail, the soluble fraction was extracted twice from 1 g of finely ground sample using 2 mL of a HCl conc/methanol/water (1:80:10, v/v) mixture under agitation for two hours at room temperature. After filtering and recovering the supernatants, the sample residues were subjected to extraction of hydrolysable (insoluble) polyphenols with 5 mL of a methanol/ concentrated sulfuric acid solution (10/1, v/v), for twenty hours in a shaking water bath at 85°C. The extracts were then made to react with Folin-Ciocalteau reagent and a 7.5 % sodium carbonate solution and analyzed using a spectrophotometer at a wavelength of 750 nm. The analyses were repeated twice, and the results were expressed in mg of gallic acid equivalent (GAE) per 100 g of dry matter (d.m.), through calibration curves. The total polyphenol content was calculated by the sum of soluble and insoluble fractions.

2.6 Dough rheological measurements

2.6.1 Dough extensibility (Kieffer test)

As previously reported by Dahdah et al. (2024), the uniaxial extensional properties were assessed with a texture analyzer (TA-XT2i, Stable Micro System, Surrey, UK) equipped with the Kieffer extensibility rig (A/KIE, Stable Micro Systems, Surrey, UK) and a 30 kg load cell. A small portion of dough (30 g) was gently manipulated into a cylindrical shape, placed in a Teflon mold (formerly sprinkled with paraffin oil to prevent sample adhesion) and pressed with a clamp to gain uniform dough strips. The excess of dough was then removed with a spatula. The strips (still inside the press) were kept at 25°C

and 75% RH in a climate chamber, for 40 minutes to allow relaxation of the dough structure. Next, the tensile test was conducted on 6 dough strips per batch taken from the center of the mold, at the following conditions: pre-test speed 2.0 $mm \cdot s^{-1}$, trigger force 5 g, test speed 3.3 $mm \cdot s^{-1}$, post-test speed 10.0 $mm \cdot s^{-1}$. At the end of the test, a force-distance curve was generated by Texture Exponent TEE32 software (v. 6.1.10.0 Stable Micro System, Surrey, UK), from which the following parameters were determined: resistance of extension (the maximum peak force recorded), expressed in N, and extensibility expressed in mm (the distance needed to break the dough strips).

2.6.2. Dough stickiness

The measurement of dough stickiness was performed using the SMS/Chen-Hoseney dough stickiness rig (A/DSC) and a 25-mm Perspex cylinder probe (P/25P) (Stable Micro-Systems, Surrey, UK) attached to the texture analyzer. A small quantity of dough was placed within the sample chamber of the kit. After removing the excess of sample, a 1-mm-high portion of the sample was extruded through the holes of the lid by turning the screw inside the cell. This was then promptly covered with the Perspex cap to minimize moisture loss. After a 30 second rest, the chamber was placed under the cylinder probe for analysis. The dough was then removed with a spatula and extruded again, repeating the test six times per batch. Stickiness values were derived as peak positive maximum force, expressed in N, from the force-time graph generated during the test by the Texture Exponent TEE32 software (v. 6.1.10.0 Stable Micro System, Surrey, UK).

2.7 Breadsticks measurements

2.7.1 Texture analysis

Three-point bending test was used to evaluate the textural properties of 30 breadstick halves for each sample (BC, BBE 1, BBE 2, BLE 1, BLE 2, BSE 1, BSE 2), one hour

after their preparation, in accordance with the methodology previously outlined by Conte et al. (2021). For these measurements, a texturimeter (TA-XT2 Texture Analyzer, Stable Micro systems, Surrey, UK) equipped with a 30 kg load cell and a three-point bending rig (HDP/3PB) was employed. Each sample was placed on the two moveable supports of the rig base, positioned 60 mm apart, and fractured by the probe blade, which was slid downward at a pre-test speed of 1 $mm \cdot s^{-1}$ and a test speed of 3 $mm \cdot s^{-1}$. The software out-puts force-distance curves, from which the hardness and brittleness parameters were obtained. These parameters correspond to the maximum force required to snap the sample (N) and the distance travelled by the blade before the breadstick cracked (mm), respectively.

2.7.2 Moisture content and water activity

Moisture content and water activity (a_w) measurements were performed on the ground breadsticks, with a moisture analyzer, set at 105°C with a standard heating profile (KERN & SOHN GmbH, Model Kern-DAB 100-3, Balingen, Germany) and an electronic hygrometer (Rotronic, Model Aw-Win, Bassersdorf, Switzerland) paired with a Karl-Fast probe, respectively. The analyses were replicated five times.

2.7.3 Color determination

Color parameters (lightness L*, redness-greenness a* and yellowness-blueness b*) were measured on the fresh ground sample to prevent measurement inaccuracies due to the small caliber of the breadsticks. Ten measurements were made on each sample using the tristimulus colorimeter (Minolta CR-300, Konica Minolta Sensing, Osaka, Japan) equipped with a measuring head CR-300 and a granular material equipment CR-A50. Additionally, the total color difference (ΔE) was calculated using the equation below:

$$\Delta E = \left((\Delta L^2) + (\Delta a^2) + (\Delta b^2)\right)^{1/2}$$

2.7.4 Antioxidant capacity

The antioxidant capacity of the breadsticks was determined using the DPPH and ABTS spectrophotometric assays. Briefly, 3 g of finely ground sample was subjected to extraction with 10 mL of a methanol:water solution (50:50 v/v) acidified with hydrochloric acid (pH 2) for 1 h at room temperature and under constant stirring. After centrifugation (3500 rpm, 10 min) of the sample and collection of the supernatant, the residue was extracted a second time under the same conditions, but with 10 mL of an acetone:water solution (70:30 v/v). The supernatant obtained from the second extraction was mixed with the previous one and used for the determination of antioxidant capacity, performed with the two spectrophotometric assays (DPPH and ABTS) used for by-product extracts, as explained in the paragraph above (2.3). The antioxidant capacity, conducted in duplicate, was expressed as μmol of Trolox equivalent (TE) for g of d.m..

2.7.5 Oxidation stability (OXITEST)

The oxidation stability of the breadstick samples was evaluated using the Oxitest reactor (VELP Scientifica, Usmate Velate MB, Italy), according to the AOCS International Standard Procedure Cd 12c-16, which speeds up lipid oxidation reactions through two accelerating factors, temperature and oxygen pressure. Specifically, 30 g of homogeneous, finely ground sample (10 g per titanium sample holder) was placed inside each reaction chamber of the instrument. The samples were then exposed to an oxygen pressure of 6 bar and at three different temperatures (80, 90, 100°C). All measurements were performed in duplicate. At the end of the tests, the induction period (IP) at the specific temperature was automatically calculated from the pressure–time curves obtained by the dedicated OXISoftTM software, when the flex was reached. In fact, the bending curve corresponds to the end of the product's intrinsic resistance to lipid oxidation and the beginning of accelerated oxygen adsorption. After evaluating

the.repeatability of the IP data for each sample and its linear dependence on temperature, the software calculated a linear regression equation on a semi-logarithmic scale (log of the IP–temperature curve). This equation was used to estimate the shelf life of the products at the specified storage temperature (22°C) (Conte et al., 2021). Results were expressed in days, and correlation coefficients (R^2) were reported.

2.8 Statistical analysis

The experimental data were subjected to one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test to separate means with a 95% confidence interval. The t-test was used to evaluate the differences between the doughs and the resulting breadsticks in order to assess the effect of baking on the content of total polyphenols and their respective fractions. Pearson correlation analysis was also employed to investigate the relationships among the analyzed parameters. Statistical analyses were performed using Statistica 12.0 software (StatSoft, Inc., Tulsa, OK, USA).

3 Results and Discussion

3.1 Total polyphenol content and antioxidant capacity of by-product extracts

Table 1 presents the findings of the total polyphenol content and antioxidant capacity of the extracts derived from artichoke by-products (BE, LE, SE). As illustrated in the table, the SE exhibited a significant ($p < 0.05$) higher polyphenol content than the BE and the LE samples. The data obtained from the two antioxidant capacity assays reflected the findings of the total polyphenol content, with the SE exhibiting the highest antioxidant capacity in both assays. Furthermore, the BE sample demonstrated a significantly higher antioxidant capacity than the LE sample, particularly in the case of ABTS.

The results obtained were found to be lower than those previously reported for the three Spinoso sardo by-product fractions gathered during a distinct harvesting season (Cannas et al., 2023). It is known that the climatic and edaphic environment, in addition to the management of the crop, exert a considerable influence on the content of antioxidants, particularly polyphenols, which are affected by both genetic factors and external variables (Lombardo et al., 2018). Indeed, there are polyphenols that are normally synthesized by the plant during the development of plant tissues and are species-specific, while others are produced in response to biotic or abiotic stresses (Beckman, 2000).

3.2 Rheological parameters of dough samples (Dough stickiness and extensibility)

The rheological properties of a dough play a crucial role during processing steps after kneading, in particular, in the sheeting and molding operations. A proper balance of viscoelastic properties is required. If the elastic component is too dominant the dough springs back too far after sheeting and becomes difficult to give it the desired final shape; on the other hand, a too extensible dough is also undesirable for the molding phase. Furthermore, a dough that is excessively sticky can lead to major issues, resulting in significant downtime on the production line. In order to evaluate the effect of the introduction of polyphenol-rich extracts obtained from artichoke by-products on dough technological properties, the parameters of dough stickiness, extensibility and resistance to extension were analyzed and the results are summarized in Table 2.

The data revealed that the incorporation of the artichoke by-product extracts at both levels of addition did not significantly ($p > 0.05$) affect the rheological parameters of resistance to extension and extensibility. Conversely, significant differences ($p < 0.05$) were observed in the stickiness parameter, whereby only the doughs enriched with LE

differed from all other samples, displaying the higher values. Although no significant differences were found in the other samples, there was a tendency for the extracts to increase the stickiness of the doughs. It is known that gluten proteins interact through disulfide bonds, hydrophobic cross-links, and hydrogen bonds, providing the basis for network formation. However, there are factors that can affect this structure, such as phenolic compounds, which not only improve the nutritional profile of doughs but also inhibit gluten disulfide cross-linking. As a result, their incorporation results in specific rheological alterations (Czajkowska–González et al., 2021; Koh & Ng, 2009). In fact, several authors have demonstrated that different classes of phenolic compounds can differentially influence the texture of fortified doughs. For instance, phenolic acids have been documented to reduce dough mixing strength and lead to the formation of sticky doughs (Girard & Awika, 2020). The incorporation of caffeic and ferulic acids, quercetin and black rice anthocyanins resulted in a decrease of dough stability and resistance to extension (Han & Koh, 2011; Koh & Ng, 2009; Lin & Zhou, 2018; Sui et al., 2016). Conversely, the inclusion of tannic acids and oleuropein improved the properties of the doughs, making them stronger, more elastic, and less sticky (Renoldi et al., 2022; Zhang et al., 2010). Moreover, the presence of flavonoids (in both aglycone and glycoside forms) influenced gliadin conformation, particularly affecting disulfide bridges (Krekora & Nawrocka, 2024). Indeed, these proteins are involved in the adhesive behavior of doughs and are known to enhance the stickiness of doughs (Ghorbel & Launay, 2014; Ye et al., 2023).

It can thus be postulated that the significant increase in dough stickiness observed in the present study may be attributed to the different phenolic composition of the leaf extracts, in comparison to those obtained from bracts and stems. This hypothesis is corroborated by the findings of a previous study conducted by the same authors, in

which it was observed that the leaf extracts had a higher concentration of flavonoids, including luteolin 7-*O*-glucoside and apigenin 7-*O*-rutinoside, than extracts from bracts and stems (Cannas et al., 2023).

3.3 Moisture and water activity of breadsticks sample

As can be seen in Table 3, a comparison of the control and fortified breadsticks revealed differences that were not always statistically significant with regard to both moisture and aw parameters. The moisture values measured on the fortified breadsticks ranged from 10.63% of the BLE 2 sample to 13.27% of the BBE 2, compared to 9.66% for the BCTRL. These results fall within the wide range reported in the literature for moisture content for breadsticks (mean values: 6.63%-15.52%) (Simsek & Süfer, 2022; Zeppa et al., 2007). In general, the addition of the extracts resulted in a slight increase in the final moisture content of the fortified breadsticks. Particularly with the addition of bract extract (BE), this increase was significant ($p < 0.05$) with respect to the control and became more pronounced with higher addition levels. This was probably due to the presence of fiber in the extracts used for the supplementation, which could affect the absorption capacity of the resulting breadsticks. In fact, artichoke by-products, especially bracts, are a potential source of dietary fiber, mainly pectin and inulin (Borsini et al., 2021; Domingo et al., 2019). In addition, ethanol-water mixtures are not only efficient for the extraction of polyphenols but can also allow the recovery of moderate amounts of inulin from lyophilized artichoke by-products (Garcia-Castello et al., 2022; Noriega-Rodríguez et al., 2020b; Soto-Maldonado et al., 2020). Furthermore, the use of ultrasound can facilitate the extraction of pectin and other saccharides, through the disruption of cell walls by the phenomenon of cavitation (Machado et al., 2015; Xu et al., 2014).

A comparable trend was identified for the a_w parameter, which demonstrated an increase in line with the moisture content. The BCTRL recorded a value of 0.56, while in the fortified breadsticks, the values exhibited a range from a maximum of 0.73 in BBE 2 to a minimum of 0.61 in BLE 2. As in the case of moisture parameter, the addition of BE caused a significant ($p < 0.05$) increase in the available water in the finished products compared to the control, especially at the high level of supplementation. In support of this, the positive value of the correlation coefficient (r) showed that a higher moisture content corresponded to a greater aw value ($r=0.971$; $p < 0.001$).

3.4 Textural and color parameters of breadstick samples

Two of the most influential factors affecting consumer acceptance are the texture and color of the finished baked products. In this context, the values of hardness, brittleness, and colorimetric coordinates (L*, a*, b*) of the breadsticks were analyzed and reported in Table 4.

With regard to texture, a tendency towards a decrease in hardness was observed in comparison to the control sample (40.90 ± 0.94 N) when the extracts were added. However, only the samples prepared with BE exhibited a statistically significant difference ($p < 0.05$). Furthermore, the fortified breadsticks, with the exception of BLE1, had significantly higher brittleness values, resulting in greater deformation before breaking than the BCTRL sample, which exhibited a more crumbly structure with a value of 1.02 ± 0.01 mm. The most pronounced increase was measured in BBE2 sample (1.87 ± 0.17 mm). The observed differences in texture parameters were probably related to the higher moisture content of the supplemented breadsticks, particularly those prepared with bract extracts, in comparison with the control. In fact, there is

evidence that an increase in moisture content in foods with a rigid and brittle structure results in an enhanced rubbery and flexible behavior (Chang et al., 2000). This was corroborated by the significant inverse correlation between moisture and hardness parameters ($r = -0.822$; $p < 0.001$) and the significant positive correlation between the values of moisture and brittleness ($r = 0.806$; $p < 0.001$).

With regard to color, the incorporation of the extracts had a significant ($p < 0.05$) effect on the colorimetric parameters, as can be seen in Table 4. In particular, a general reduction of the a* colorimetric coordinate with respect to the control was observed in all the fortified samples, with the exception of BSE 1. This decrease was more pronounced in the samples obtained by adding BE, which exhibited negative values, indicating a tendency to green especially at higher addition levels. This was probably due to the bright green color of the bract extract. The color of BE also affected the b* coordinate. Indeed, the samples BBE 1 and BBE 2 exhibited the lowest values, denoting a reduced yellow tendency, in comparison to the other samples. Conversely, as the color of the stem extract tends towards yellow ochre, among fortified breadsticks, those prepared with SE exhibited the highest values of the a* and b* parameters, resulting in more reddish and yellowish tones. However, these color differences, as also evident from the measured values of ΔE, which range between 0.4 and 1.8, were not readily evident to the human eye, suggesting that the extracts exerted only a minimal influence on the color of the resulting breadsticks. Indeed, as previously documented (Romankiewicz et al., 2017), according to the criteria established by the International Commission on Illumination, values of ΔE within the range of 0-2.0 indicate an unrecognizable color difference. Values between 2.0 and 3.5 indicate differences that are recognizable even by an inexperienced observer. Finally, values above 3.5 indicate differences that are obvious to the human eye.

3.5 Polyphenol content of dough and breadstick samples

Typically, fruit and vegetables contain a significant proportion of phenolics in a soluble form, whereas cereals serve as an excellent source of insoluble-bound polyphenols (Zhang et al., 2020). Therefore, to more accurately assess the composition of phenolic compounds in the fortified breadsticks, both fractions of polyphenols - soluble (SF) and insoluble (IF) - were determined. Moreover, due to the inherent instability and reactive nature of these compounds, as well as the inevitable degradation that occurs as a result of heat and oxidation during the baking phase, a preliminary analysis was conducted on the doughs with the aim of assessing the impact of the thermal process on the SF and IF content.

In the present study, a comparison of the DCTRL sample with the extract-fortified doughs revealed significant differences ($p < 0.05$) in the total polyphenol content with the exception of DBE1 and DLE1 samples (Table 5). In particular, the highest values were recorded in the DSE 2 and DBE 2 samples, closely followed by the DSE 1 and DLE 2 doughs. The greater contribution of SE and BE can be justified by their higher polyphenol content in comparison to LE (see Section 3.1). A more detailed examination of the data revealed that the addition of artichoke extracts mainly affected the SF of the resulting doughs, with a more pronounced impact at higher addition levels of SE and BE (98.00 ± 7.0 mg of GAE 100 g^{-1} and 95.65 ± 5.9 mg of GAE 100 g^{-1}, -respectively). In contrast, no significant change in the concentration of SF was observed in the doughs obtained when LE was added at both levels, in comparison with the control. The IF exhibited minimal alteration, with a slight, though not statistically significant, tendency to increase observed in nearly all fortified doughs when compared to the control. The sole exception was the DLE1 sample, which exhibited the lowest IF concentration

(mean value: 155.0 ± 4.4 mg of GAE 100 g^{-1}). The limited impact of the extract addition on the IF concentration observed in all doughs is presumably attributable to the low proportion of phenolic compounds present in an insoluble form in the fresh artichoke (1.81-3.11% of the total amount) (Domínguez-Fernández et al., 2021). Moreover, the food-grade solvents used to obtain the extracts may have enabled the predominant extraction of polyphenols in soluble form over those in insoluble form, which require a more rigorous extraction procedure.

After baking, all the breadstick samples exhibited similar levels of polyphenol content with values ranging from 244 to 248 mg of GAE 100 g^{-1} (Table 5).

However, a more detailed examination of the data evidenced that the baking process exerted a different influence on soluble and insoluble polyphenols. With regard to the SF, a significant reduction was observed in all samples with respect to the dough samples, which can be attributed to the lower stability and higher susceptibility to high temperatures than the IF (Xiao, 2022). Notably, the highest decrease, amounting to approximately 37%, was observed in breadsticks fortified with LE at both levels of addition. In contrast, fortification with SE, despite a baking loss ranging from 31% to 35%, yielded the highest SF concentration even after baking (61.6 ± 1.3 mg of GAE 100 g^{-1} in BSE 1 and 64.1 ± 0.6 mg of GAE 100 g^{-1} in BSE 2). No significant differences were observed between the two samples fortified with BE and the BCTRL.

With regard to the IF, all finished products, with the exception of BBE 2, exhibited a significant increased concentration following the baking process (in comparison to the dough samples), although no significant differences were observed within the breadticks. This is probably due to the formation of heat-induced compounds resulting from the Maillard reactions (Dziki et al., 2014). It is important to highlight that the samples prepared with the incorporation of LE, despite undergoing a more pronounced

decline in the SF, also exhibited the most notable increase in the IF. This may be indicative of a reallocation of compounds within the sample, rather than a loss through degradation. Domínguez-Fernández et al. (2021), by studying the effects of different cooking techniques on the phenolic profile of artichoke, found that these compounds may undergo degradation, but also redistribution due to isomerization and hydrolysis reactions (Domínguez-Fernández et al., 2021).

In summary, the significant differences in total polyphenol content observed in the dough samples were no longer detectable in the breadsticks due to the distinct influence exerted by the baking process on the soluble and insoluble fractions of the samples.

3.6 Antioxidant capacity of breadstick samples

The estimation of the antioxidant capacity of the breadsticks using the two methods gave comparable results, as evidenced by Pearson's analysis, which demonstrated a strong positive and significant correlation ($r = 0.945$, $p < 0.001$) between DPPH and ABTS values. As illustrated in Table 6, an upward significant trend was observed with the addition of increasing quantities of the extracts with respect to the control, with the exception of BLE 1 and BLE 2 samples ($p < 0.05$). In particular, the significantly higher results were recorded in both spectrophotometric assays in the samples fortified with 2000 ppm of the extracts of both the stem (with increases of + 69 % for DPPH and +26 % for ABTS compared to the BCTRL) and the bract (with increments of +57 % for DPPH and +33 % for ABTS), immediately followed by the samples prepared with the lowest level of the same extracts. Similar increments in the antioxidant capacity were obtained in salted baked snacks fortified with the addition of olive leaf extract. However, the authors employed a lower fortification level (400 ppm), which may be

attributed to the initial higher polyphenol concentration of the olive industry by-product (Difonzo et al., 2018).

The greater efficacy of SE and BE in increasing the antioxidant power of breadsticks appeared to diverge from the findings observed in the total polyphenol content, where no significant differences among the samples were found. It is established that the antioxidant capacity is influenced not only by the total amount of phenolic compounds present, but also by their composition. Indeed, this appears to be positively influenced by the number of hydroxyl groups and their position on the aromatic ring, particularly on the ortho or para position (Wang et al., 1999). In full agreement with this observation, a study conducted by Wang et al. (2003), by analyzing the DPPH scavenging activity of individual phenolic compounds extracted and purified from artichoke leaves and flower heads, found that compounds such as cynarin (1,3-di-caffeoylquinic acid), luteolin rutinoside, cynaroside (luteolin 7-*O*-glucoside), chlorogenic acid had higher antioxidant activity than 1-caffeoylquinic acid and apigenin 7- rutinoside (Wang et al., 2003).

As previously reported by the same authors (Cannas et al., 2023), the stem and bract extracts used in the present study were found to be particularly rich in compounds, including chlorogenic acid, 1,5-di-*O*-caffeoylquinic acid, and 3,5-di-*O*-caffeoylquinic acid, which possess multiple hydroxyl groups in the ortho position. These compounds were either absent or present in low concentrations in the leaf extract. It can thus be concluded that the enhanced antioxidant capacity observed in the SE- and BE-fortified breadsticks is likely attributable to the higher concentration of the caffeoylquinic acid derivatives present in these extracts.

3.7 Estimated Shelf-life of breadsticks with OXITEST

A variety of factors related to composition and nutritional properties, as well as packaging and storage conditions, can influence the shelf-life of food products. In the case of baked goods such as breadsticks, which often require significant amounts of fat to achieve the desired texture and flavor, lipid oxidation can play a crucial role in defining their shelf-life (Caruso et al., 2017).

Indeed, lipids undergo oxidative degradation as a consequence of complex chemical chain reactions that involve fatty acids and oxygen. This degradation phenomenon is also referred to as rancidity, as it results in the formation of intermediate compounds (free radicals) and secondary compounds (such as aldehydes, ketones, and hydrocarbons) that contribute to the development of undesirable flavors, which can negatively impact the quality of the food product. The rate of lipid oxidation is influenced by different factors, including the fatty acid composition, the storage conditions (e.g., temperature, light, oxygen availability, and water activity), and the presence of prooxidants and antioxidants (Mozuraityte et al., 2015). Plant extracts, for instance, are known to contain a multitude of natural antioxidants, including phenolic acids, flavonoids, and anthocyanins. These antioxidants act as free radical scavengers by virtue of their multiple hydroxyl groups, which function as hydrogen donors, preventing the reaction of peroxyl or alkoxy radicals with new fatty acids (Mozuraityte et al., 2015). Therefore, the use of artichoke by-product extracts may represent an effective strategy for the enhancement of the oxidative stability of breadsticks and consequently their shelf-life.

The accelerated oxidation tests, conducted at three different temperatures (80, 90, and 100°C) and a constant overpressure (6 bar) in the OXITEST reactor, revealed a linear relationship between the IPs and temperatures, as evidenced by the R^2 values greater than 0.99 (Table 7). Indeed, an overall decrease in the IP was noted as operating

temperature increased across all samples. This allowed for the estimation of the shelf-life of the breadsticks at a temperature of 22°C.

In general, the lowest lipid stability to oxidation was observed in the BCTRL, which showed IPs of approximately 22 and 4 h at 80 and 100°C, respectively (data not shown) and an estimated shelf-life of 109±1 days.

The incorporation of the extracts, especially at the highest levels, allowed an improvement in the oxidative stability of the resulting breadsticks. In line with the data of antioxidant capacity, the addition of 2000 ppm of SE, BE and LE ensured a significant ($p < 0.05$) extension of the shelf-life of breadsticks by 62, 44 and 29%, respectively. The BLE 1 and BBE 1 samples did not showed significant increment with respect to the control. In contrast, the extract obtained from the stems, even at the lowest level of addition (1000 ppm), caused an improvement in shelf-life of almost twice as much (+44%). Similarly, Hammad et al. (2021) recorded a significant increase in the oxidative stability of breadsticks fortified with ginseng dried extract, with an extension recorded of up to 55 days at room temperature. Conversely, the incorporation of grape pomace was observed to have a deleterious effect on the OXITEST estimated shelf life of breadsticks, resulting in an accelerated oxidation rate. However, in this case, the fortification was conducted with the by-product in powder form, which likely also contained pro-oxidant molecules or polyunsaturated fatty acids (Bianchi et al.2021).

The highly significant ($p < 0.01$) positive correlations between antioxidant capacity and estimated shelf-life ($r = 0.787$ and $r = 0.710$ with DPPH and ABTS data, respectively) found in the present study substantiate the close association between these two parameters.

4. Conclusions

The findings of the present study indicate that the use of hydroalcoholic extracts derived from various by-products of the artichoke industry to enhance the antioxidant activity and extend the shelf-life of globally consumed snacks such as breadsticks represents a promising strategy for reducing both the environmental and economic footprint of the artichoke industry. This is the first demonstration of the capacity of these polyphenol-rich hydroalcoholic extracts to enhance oxidative stability without prior stabilization. This also offers a potential solution to reduce the impact of food waste and loss of a valuable economic resource within the Mediterranean agricultural sector, to promote a circular economy, and to increase the competitiveness of artichoke industries and of snack manufacturers. Furthermore, the exhausted by-product residues, which could be considered as lignocellulosic biomass, may have the potential to be repurposed as a sustainable source for bioethanol production or in the textile industry, thereby reinforcing the concept of circularity.

Ultimately this could result in a finished product with higher selling prices and a reduction in waste at the consumer level. Indeed, despite a slight decline in texture observed in the fortified breadsticks, the incorporation of extracts, particularly at the highest levels of SE and BE, demonstrated the capacity to enhance antioxidant activity and extend the shelf-life, without discernible alterations in the snack's final color. Additionally, the rheological data of the doughs indicated that the incorporation of the extracts did not affect the dough's workability, with the exception of the LE, which significantly increased their stickiness. Of the extracts evaluated, the one derived from artichoke stems exhibited the most promising results as a natural preservative and nutritional improver. However, given the high sensitivity of artichoke phenolic compounds, a stabilization intervention such as encapsulation would probably yield better results. Therefore, this aspect should be examined in depth to ascertain the

effectiveness of the treatment. Moreover, additional re-search is necessary to ensure the reliability and consistency of the existing findings, particularly in light of the inherent variability in the phenolic content of artichoke by-products, which is strongly influenced by soil and climatic conditions. Further investigations are required to assess the impact of these hydroalcoholic extracts on the eco-nomic aspect through a life cycle assessment, and on the sensorial properties of the finished product.

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TABLES

Table 1. Total polyphenol content and antioxidant capacity of artichoke by-product extracts.

Sample[1]	**Total polyphenols** mg GA 100 g^{-1} d.m.	**DPPH** TE μmol TE g^{-1} d.m.	**ABTS** μmol TE g^{-1} d.m.
BE	1539±64 b	39.77±2.30 b	62.18±5.19 b
LE	1256±18 c	32.37±0.60 b	55.05±3.81 c
SE	2163±206 a	86.49±16.31 a	90.19±9.20 a

[1]Mean value ± standard deviation. Different letters in the same column denote significant differences ($p < 0.05$) at Least Significant Difference (LSD) test; BE: bract extract; LE: leaves extract; SE: stem extract

Table 3. Rheological parameters of control and fortified doughs.

Samples[1]	**Resistance to Extension** (N)	**Extensibility** (mm)	**Stickiness** (N)
DCTRL	0.13±0.02 a	63.44±0.02 a	0.35±0.02 b
DBE 1	0.12±0.00 a	63.29±0.04 a	0.35±0.00 b
DBE 2	0.12±0.02 a	62.51±3.07 a	0.39±0.06 b
DLE 1	0.12±0.02 a	60.27±1.40 a	0.42±0.04 a
DLE 2	0.11±0.02 a	63.60±1.03 a	0.45±0.04 a
DSE 1	0.11±0.05 a	61.55±1.28 a	0.37±0.03 b
DSE 2	0.11±0.02 a	61.47±3.34 a	0.40±0.01 b

[1]Mean value ± standard deviation. Different letters in the same column denote significant differences ($p < 0.05$) at Least Significant Difference (LSD) test; DCTRL: control dough; DBE 1: dough with 1000 ppm of bract extract; DBE 2: dough with 2000 ppm of bract extract DLE 1 and 2: dough with 1000 and 2000 ppm leaves extract, respectively; DSE 1 and 2: dough with 1000 and 2000 ppm stem extract, respectively.

Table 3. Moisture content and water activity (a_w) of control and fortified breadsticks.

Samples[1]	Moisture content %	a_w
BCTRL	9.66±0.11 d	0.56±0.01 d
BBE 1	13.03±0.61 ab	0.73±0.01 ab
BBE 2	13.27±0.20 a	0.73±0.01 a
BLE 1	11.36±0.29 bcd	0.63±0.04 bcd
BLE 2	10.63±1.84 cd	0.61±0.08 cd
BSE 1	11.49±0.82 abcd	0.66±0.04 abcd
BSE 2	11.83±0.08 abc	0.69±0.03 abc

[1]Mean value ± standard deviation. Different letters in the same column denote significant differences ($p < 0.05$) at Least Significant Difference (LSD) test; BCTRL: control; BBE 1: breadsticks with 1000 ppm of bract extract; BBE 2: breadsticks with 2000 ppm of bract extract BLE 1 and 2: breadsticks with 1000 and 2000 ppm leaves extract, respectively; BSE 1 and 2: breadsticks with 1000 and 2000 ppm stem extract, respectively

Table 4. Textural and color properties of control and fortified breadsticks.

Samples[1]	**Hardness** (N)	**Brittleness** (mm)	**L***	**a***	**b***	**ΔE**
BCTRL	40.90±0.94 a	1.02±0.01 d	62.48±0.39 c	0.87±0.17 a	17.72±0.36 bc	-
BBE 1	35.75±2.66 bc	1.68±0.24 ab	62.93±0.14 b	-0.15±0.02 e	16.62±0.17 e	1.56
BBE 2	31.75±2.00 c	1.87±0.17 a	63.53±0.36 a	-0.39±0.06 f	17.01±0.30 e	1.79
BLE 1	39.14±1.47 ab	1.32±0.05 cd	63.31±0.07 a	0.31±0.09 d	17.02±0.44 d	1.22
BLE 2	38.90±0.34 ab	1.43±0.19 bc	62.65±0.64 bc	0.43±0.12 c	17.52±0.30 c	0.51
BSE 1	38.21±2.23 ab	1.61±0.00 abc	62.06±0.46 d	0.85±0.07 a	18.54±0.38 a	0.91
BSE 2	36.97±1.02 ab	1.46±0.15 bc	62.68±0.19 bc	0.62±0.08 b	17.96±0.50 b	0.40

[1]Mean value ± standard deviation. Different letters in the same column denote significant differences ($p < 0.05$) at Least Significant Difference (LSD) test; BCTRL: control; BBE 1: breadsticks with 1000 ppm of bract extract; BBE 2: breadsticks with 2000 ppm of bract extract BLE 1 and 2: breadsticks with 1000 and 2000 ppm leaves extract, respectively; BSE 1 and 2: breadsticks with 1000 and 2000 ppm stem extract, respectively.

Table 5. Total and polyphenol fractions content of dough and breadstick samples.

Samples[1]	Soluble Fraction mg GAE 100 g^{-1} d.m.	Insoluble Fraction mg GAE 100 g^{-1} d.m.	Total Polyphenol Content mg GAE 100 g^{-1} d.m.
Doughs			
DCTRL	83.5 ± 2.3 cA	157.7 ± 0.8 abB	241.2 ± 3.1 cA
DBE 1	86.2 ± 3.6 cA	160.1 ± 0.0 abB	246.3 ± 3.6 bcA
DBE 2	95.6 ± 5.9 abA	162.9 ± 0.3 abA	258.5 ± 5.6 aA
DLE 1	82.8 ± 0.6 cA	155.0 ± 4.4 bB	237.8 ± 3.8 cA
DLE 2	88.1 ± 1.0 bcA	163.8 ± 1.0 aB	251.9 ± 2.0 abA
DSE 1	89.9 ± 0.7 abcA	163.1 ± 0.8 aB	253.0 ± 0.1 abA
DSE 2	98.0 ± 7.0 aA	162.0 ± 0.6 aB	260.0 ± 6.5 aA
Breadsticks			
BCTRL	60.4 ± 0.0 bcB	188.0 ± 8.5 aA	248.4 ± 8.5 aA
BBE 1	59.6 ± 1.6 bcB	184.3 ± 6.1aA	243.9 ± 4.5 aA
BBE 2	61.6 ± 4.5 bcB	186.6 ± 9.7 aA	248.2 ± 5.2 aA
BLE 1	52.4 ± 1.5 dB	194.1 ± 0.3 aA	246.5 ± 1.7 aA
BLE 2	55.9 ± 0.0 cdB	192.4 ± 4.4 aA	248.3 ± 4.4 aA
BSE 1	61.6 ± 1.3 aB	186.7 ± 4.1 aA	248.4 ± 2.7 aA
BSE 2	64.1 ± 0.6 aB	181.9 ± 1.3 aA	245.9 ± 0.7 aA

[1]Mean value ± standard deviation. Different letters in the same column denote significant differences ($p < 0.05$) within the dough samples and within the breadstick samples (lowercase letters) at the Least Significant Difference (LSD) test and between the doughs and the resulting breadsticks (uppercase letters) at the t-test; DCTRL: control dough; DBE 1: dough with 1000 ppm of bract extract; DBE 2: dough with 2000 ppm of bract extract DLE 1 and 2: dough with 1000 and 2000 ppm leaves extract, respectively; DSE 1 and 2: dough with 1000 and 2000 ppm stem extract, respectively; BBE 2: breadsticks with 2000 ppm of bract extract BLE 1 and 2: breadsticks with 1000 and 2000 ppm leaves extract, respectively; BSE 1 and 2: breadsticks with 1000 and 2000 ppm stem extract, respectively.

Table 6. Antioxidant capacities of breadstick samples determined with DPPH and ABTS spectrophotometric assays.

Samples[1]	**DPPH** μmol TE g^{-1} d.m.	Δ **DPPH** (%)	**ABTS** μmol TE g^{-1} d.m.	Δ **ABTS** (%)
BCTRL	0.23±0.01 d	-	2.25±0.10 e	-
BBE 1	0.32±0.05 bc	38	2.66±0.17 bcd	18
BBE 2	0.37±0.04 ab	57	2.98±0.13 a	33
BLE 1	0.26±0.02 cd	13	2.42±0.22 de	8
BLE 2	0.29±0.01 cd	22	2.49±0.07 cde	11
BSE 1	0.35±0.00 ab	49	2.75±0.02 abc	22
BSE 2	0.39±0.02 a	69	2.83±0.05 ab	26

[1]Mean value ± standard deviation. Different letters in the same column denote significant differences ($p < 0.05$) at Least Significant Difference (LSD) test; BCTRL: control; BBE 1: breadsticks with 1000 ppm of bract extract; BBE 2: breadsticks with 2000 ppm of bract extract BLE 1 and 2: breadsticks with 1000 and 2000 ppm leaves extract, respectively; BSE 1 and 2: breadsticks with 1000 and 2000 ppm stem extract, respectively.

Table 7. Estimated shelf-life of breadstick samples based on lipid oxidation data (expressed as days at 22 ◦C) measured with OXITEST method.

Parameters	Samples[1]						
	BCTRL	**BBE 1**	**BBE 2**	**BLE 1**	**BLE 2**	**BSE 1**	**BSE 2**
Estimated shelf-life (days)	109±1 d	119±3 cd	157±4 ab	109±3 d	141±8 bc	156±10 ab	177±5 a
R^2	0.995	0.997	0.998	0.996	0.998	0.997	0.998
Shelf-life extension (%)	−	9	44	0	29	43	62

[1]Mean value ± standard deviation. Different letters in the same row denote significant differences ($p < 0.05$) at Least Significant Difference (LSD) test; BCTRL: control; BBE 1: breadsticks with 1000 ppm of bract extract; BBE 2: breadsticks with 2000 ppm of bract extract BLE 1 and 2: breadsticks with 1000 and 2000 ppm leaves extract, respectively; BSE 1 and 2: breadsticks with 1000 and 2000 ppm stem extract, respectively.

6. CASE STUDY 3

Artichoke by-products: Promising ingredients for breadstick fortification

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Artichoke by-products: Promising ingredients for breadstick fortification

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ABSTRACT

The globe artichoke industry produces significant losses, amounting to 60–80% of the biomass. These losses represent a natural source of high-value compounds. To mitigate the environmental impact of these wastes, this study evaluated the effect of adding different concentrations (3 and 5%) of powders obtained from two main artichoke by-products, stems and outer bracts, on the nutritional, bioactive, textural, aromatic and organoleptic properties of conventional breadsticks. Compared to the control, all fortified samples showed increased contents of dietary fiber, ash, flavonoids, and soluble polyphenols, especially at high addition levels. Stem powder supplementation resulted in the highest antioxidant capacity, with values of +527% and +114% in DPPH and ABTS assays, respectively. Fortification reduced the moisture and water activity of the breadsticks and improved their brittleness but did not significantly affect the color of the finished product. Sensory investigation found that consumers perceived fortified samples to be more bitter, astringent, and herbaceous, but also healthier and more sustainable. The volatile component of the fortified breadsticks was richer than the control, mostly in terms of some terpenes, acids and aldehydes. In conclusion, the fortification of breadsticks with artichoke by-products is promising for the obtaining of a functional and sustainable bakery product.

1. Introduction

Waste management is a global challenge that involves reducing food losses and minimizing their adverse economic, social, and environmental impacts through the effective valorization of agro-industrial wastes and/or by-products. The United Nations established the Sustainable Development Goals with the 2030 Agenda in response to the magnitude of food losses in the world, which amounts to 1.3 billion tons per year. The goal is to reduce food waste per capita by 50% and limit food losses along the entire supply chains, through "prevention, reduction, recycling, and reuse" (Bartezzaghi, Cattani, Garrone, Melacini, & Perego, 2022). According to FAO investigations, the fruit and vegetable sector is one of the least virtuous in terms of waste, with inefficient use of resources such as land, water, energy, and economic inputs, resulting in the production of unconsumed food and unnecessary CO_2 emissions (FAO, 2019). By-products generated during the harvesting, transportation, and processing of fruits and vegetables pose a significant problem due to their volume and high moisture content, which makes them more prone to microbial contamination and hence difficult to manage. Furthermore, it is important to consider the loss of nutrients that have great potential, as these by-products are rich in bioactive compounds with high biological importance (Jiménez-Moreno, Esparza, Bimbela, Gandía, & Ancín-Azpilicueta, 2020; Oliveira, Caleja, Oliveira, Pereira, & Barros, 2023). The utilization of by-products from fruit and vegetable is becoming increasingly significant through various approaches, including their incorporation as functional ingredients in the food industry. This contributes to the transition towards a circular economy (Ye, Sridhar, & Tsai, 2023).

Globe artichoke (*Cynara scolymus* L.) is a vegetable that is consumed globally due to its flavor and nutritional properties. It belongs to the Asteraceae family and is rich in phenolic compounds, fibers, vitamins, and minerals (Frutos, Ruiz-Cano, Valero-Cases, Zamora, & Pérez-Llamas, 2018; Soares Mateus et al., 2023). Artichoke is known for its antioxidant, hypocholesterolemic, hepatoprotective, antimicrobial, antitumor, and anti-inflammatory properties, associated with the presence of mono- and dicaffeoylquinic acids, such as chlorogenic acid and cynarin, and flavonoids, like luteolin, apigenin and related glucosides and rutinosides (Cannas, Conte, Piga, & Del Caro, 2023; Lattanzio, Kroon, Linsalata, & Cardinali, 2009; Rondanelli, Monteferraio, Perna, Faliva, & Opizzi, 2013). The plant is known for containing inulin, an

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Artichoke by-products: promising ingredients for breadstick fortification

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Abstract

The globe artichoke industry produces significant losses, amounting to 60-80% of the biomass. These losses represent a natural source of high-value compounds. To mitigate the environmental impact of these wastes, this study evaluated the effect of adding different concentrations (3 and 5%) of powders obtained from two main artichoke by-products, stems and outer bracts, on the nutritional, bioactive, textural, aromatic and organoleptic properties of conventional breadsticks. Compared to the control, all fortified samples showed increased contents of dietary fiber, ash, flavonoids, and soluble polyphenols, especially at high addition levels. Stem powder supplementation

resulted in the highest antioxidant capacity, with values of +527% and +114% in DPPH and ABTS assays, respectively. Fortification reduced the moisture and water activity of the breadsticks and improved their brittleness but did not significantly affect the color of the finished product. Sensory investigation found that consumers perceived fortified samples to be more bitter, astringent, and herbaceous, but also healthier and more sustainable. The volatile component of the fortified breadsticks was richer than the control, mostly in terms of some terpenes, acids and aldehydes. In conclusion, the fortification of breadsticks with artichoke by-products is promising for the obtaining of a functional and sustainable bakery product.

Keywords: artichoke by-products, breadsticks, bioactive compounds, aroma, CATA

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Waste management is a global challenge that involves reducing food losses and minimizing their adverse economic, social, and environmental impacts through the effective valorization of agro-industrial wastes and/or by-products. The United Nations established the Sustainable Development Goals with the 2030 Agenda in response to the magnitude of food losses in the world, which amounts to 1.3 billion tons per year. The goal is to reduce food waste per capita by 50% and limit food losses along the entire supply chains, through "prevention, reduction, recycling, and reuse" (Bartezzaghi et al., 2022). According to FAO investigations, the fruit and vegetable sector is one of the least virtuous in terms of waste, with inefficient use of resources such as land, water, energy, and economic inputs, resulting in the production of unconsumed food and unnecessary CO_2 emissions (FAO, 2019b). By-products generated during the harvesting, transportation, and processing of fruits and vegetables pose a significant problem due to their volume and high moisture content, which makes them more prone

to microbial contamination and hence difficult to manage. Furthermore, it is important to consider the loss of nutrients that have great potential, as these by-products are rich in bioactive compounds with high biological importance (Jiménez-Moreno et al., 2020; Oliveira et al., 2023). The utilization of by-products from fruit and vegetable is becoming increasingly significant through various approaches, including their incorporation as functional ingredients in the food industry. This contributes to the transition towards a circular economy (Q. M. Ye et al., 2023).

Globe artichoke (*Cynara scolymus* L.) is a vegetable that is consumed globally due to its flavor and nutritional properties. It belongs to the *Asteraceae* family and is rich in phenolic compounds, fibers, vitamins, and minerals (Frutos et al., 2018; Soares Mateus et al., 2023). Artichoke is known for its antioxidant, hypocholesterolemic, hepatoprotective, antimicrobial, antitumor, and anti-inflammatory properties, associated with the presence of mono- and dicaffeoylquinic acids, such as chlorogenic acid and cynarin, and flavonoids, like luteolin, apigenin and related glucosides and rutinosides (Cannas et al., 2023; Lattanzio et al., 2009; Rondanelli et al., 2013). The plant is known for containing inulin, an oligosaccharide with a very low glycemic response, making it suitable for diabetics; in addition, it is recognized for its positive effects on the intestinal microbiota (Canale et al., 2023; Meyer & Blaauwhoed, 2009). However, this crop typically has a low harvest index, resulting in a significant amount of residues generated during collection. When combined with processing by-products, these residues account for approximately 80-85% of the total plant biomass (De Falco et al., 2022). The edible portion of the artichoke head, which includes the receptacle of the immature inflorescence and the inner bracts, is relatively small. Therefore, the outer bracts and stems are considered waste (Soares Mateus et al., 2023). Nevertheless, these by-products can provide nutrients and bioactive compounds that offer health benefits and

can still be utilized (Órbenes et al., 2021 Ruiz-Cano et al., 2014; Soares Mateus et al., 2023). As humans cannot synthesize these substances, such as fibers and polyphenols, they must be obtained through the diet (Canale et al., 2023). Therefore, artichoke by-products have the potential to be used as dietary supplements and functional food ingredients.

In recent years, consumers have become more conscious and prefer food products that focus on quality, high nutritional value, and sustainability. Bakery goods are consumed daily worldwide, and there is a constant demand for innovative and healthier products, making them suitable for delivering functional ingredients, including those derived from agro-industrial by-products. Artichoke waste has been used in several studies to enhance the functional properties of baked products, including biscuits (Eman et al., 2018) and wheat bread (Boubaker, Damergi, et al., 2016; Boubaker, Omri, et al., 2016; Canale et al., 2022; Frutos et al., 2008). However, the prevalence of busy lifestyles has led to a notable shift in consumer eating habits, with an increased inclination towards the consumption of convenient and easily-eatable baked goods as a substitute for bread (Nicolosi et al., 2023). Among these, breadsticks, which are crusty sticks of bread with a brittle and airy texture and a long shelf-life, could capture the attention of modern consumers (Sattar et al., 2018). Moreover, studies have demonstrated that breadsticks are among the most suitable grain-based snacks for fortification with agri-food by-products. This is because the product has the potential to provide health benefits and to promote a circular economy system (Rainero et al., 2022). Indeed, previous research has utilized by-products from the olive oil industry to fortify both traditional and gluten-free breadsticks, either in their original form or as polyphenol-rich extracts. In all cases, the use of olive cake powder resulted in improvements in nutritional and bioactive profiles, as well as an enhanced sensory profile. Additionally, extracts from olive leaves and

olive mill wastewaters were found to increase shelf-life, with only minor changes in texture (Conte et al., 2021; de Gennaro et al., 2022; Simsek & Süfer, 2022). Breadsticks were also fortified with brewer's spent grains and grape pomace, by-product of winemaking, to attain nutritional benefits (Bianchi et al., 2021; Ktenioudaki et al., 2012). However, to the best of our knowledge, no studies have been conducted on the use of artichoke by-products to enhance the functional properties of these cereal-based snacks. Interestingly, Skouloudis et al. (2023) examined consumer preferences for bio-based breadsticks and the factors that determine purchase intentions, such as innovativeness, trust in science and technology, and environmental concern. They found that 47% of respondents would be willing to purchase a package of breadsticks fortified with phenolic extracts from olive mill wastewater at a premium price than conventional breadsticks (average values: 5.51€ *versus* 3.91€).

In this context, fortifying breadsticks with artichoke by-products could have significant implications for snack manufacturers seeking to develop new healthy and sustainable products with enhanced nutritional properties and a high-fiber claim, particularly given the growing market for these products. Moreover, the economic significance of artichoke cultivation in the Mediterranean agricultural sector, coupled with the substantial losses incurred, suggests that the reuse of by-products could improve the sustainability and competitiveness of the artichoke industry.

The aim of this study is to assess the impact of fortifying breadsticks with powders derived from primary by-products of artichoke, specifically the external bracts and stems, at two different concentrations (3% and 5%), on their nutritional, textural, volatile, and sensory characteristics.

2. Materials and Methods

2.1 Raw materials

The breadsticks were made using wheat flour (type 0 with 14 g·100 g^{-1} moisture, 71 g 100 g^{-1} carbohydrates, 11 g 100 g^{-1} protein, 2 g 100 g^{-1} fiber, 1.0 g 100 g^{-1} crude fat), sunflower oil, fresh compressed yeast, and salt, all purchased from a local supermarket. By-products of the 'Spinoso sardo' artichoke (*Cynara scolymus*, L.), specifically outer bracts and stems, were collected in the 2021 by the North-Sardinian companies of consortium "Carciofo Spinoso di Sardegna D.O.P.".

2.2 Preparation of Artichoke By-Products Powders

The stems and bracts samples were cleaned, washed with tap water, cut into small and uniform pieces, and placed in a freezer for 24 h. Subsequently, they were lyophilized using a freeze-dryer (Labconco Corporation, mod. FreeZone Freez Dryer, Kansas City, MO, USA) set at -40 °C and 0.1 mbar pressure for 72-48 h. Finally, the freeze-dried samples were finely ground (Moulinex, Model A320R1, Écully, France) and stored under vacuum in polyamide/polyethylene bags at -18°C until use.

2.3 Breadsticks Preparation

The formulation of each sample is reported in Table 1. Fortified samples were obtained by individually adding freeze-dried stem (S) and bract (B) powders to the basic formulation at two addition levels: low (3%) and high (5%) on flour basis. The percentages were selected on the basis of preliminary laboratory trials, which evidenced that higher supplementation levels negatively impact the machinability of the doughs and the sensory quality of the final products. The amount of water used to prepare the samples was determined using a farinograph (Farinograph-TS, Model 827507, Brabender, Duisburg, Germany), following the standard AACC method with slight

modifications. Since breadsticks require a stiffer and less sticky dough than standard bread consistency (500 UB), the water absorption capacity for each supplementation level was evaluated at an optimum consistency of 750 UB.

The breadstick doughs (2 batches) were prepared by kneading the ingredients in a mixer (KitchenAid Professional, Model 5KSM7990, St. Joseph, MI, USA) for 6 min at speed 2. The resulting doughs were laminated using a professional sheeter (Domino S.r.l., Model SFO600, Schio, Italy) until they reached a final thickness of 0.3 cm. The doughs were then cut into 18 cm long sheets and shaped using a breadstick machine (Italpan, Model AFP/GR15, Schio, Italy) with 1 cm diameter grooves. After shaping, the breadsticks were proofed in a climate chamber for 30 min (30°C-75% RH) and successively baked in an electric oven (Europa, Malo, VI, Italy) at 200°C for 20 min. A total of 40 breadsticks were obtained per batch.

2.4 Proximate Composition

The protein content was determined using the Kjeldahl method with a nitrogen conversion factor of 6.25. Total dietary fibers were measured using the K-TDFR analytical kit (Megazyme International Ireland ltd., Ireland). The determination of crude fats was conducted in an extraction apparatus (VELP Scientifica, Model SER 158, Usmate Velate, Italy) employing the Randall Technique (a modified Soxhlet extraction method), in accordance with a method delineated by the manufacturer and in compliance with the AOAC International Standard Procedure 2003.05. The ash content was determined by incinerating 3 g of the sample in a muffle furnace at 600 °C for 6 h in accordance with the Official Standard Method AACC 08-01.01 (AACC, 2005). Moisture content was evaluated using a moisture analyzer (KERN & SOHN GmbH, Model Kern-DAB 100-3, Balingen, Germany) with a standard heating profile at 105°C.

Digestible carbohydrates were calculated by indirect determination as 100 minus the sum of moisture, protein, total dietary fibers, crude fat, and ash. Additionally, water activity (a_w) was measured using an electronic hygrometer (Rotronic, Model Aw-Win and Karl-Fast probe, Bassersdorf, Switzerland). The aforementioned assays were performed on both artichoke by-product powders and ground breadstick samples. The data were reported as the mean of five repetitions per batch for a_w, three for moisture and total dietary fiber, and two for protein, crude fat, and ash.

2.5 Color Analysis

Color of by-product powders and breadstick samples was determined using a tristimulus colorimeter (Minolta CR-300, Konica Minolta Sensing, Osaka, Japan) with D65 illuminant and standard observer of 2° and expressed in CIE L* a* b* color space. The lightness L* and chromaticity coordinates a∗ (green to red) and b∗ (blue to yellow) were measured on B and S powders, as well as on 30 finely ground breadsticks per batch, using the granular material attachment (CR-A50, Konica Minolta Sensing, Osaka, Japan) of the colorimeter. A total of five repetitions were conducted for each batch. The total color difference (ΔE) for the breadsticks was also calculated using the following equation:

$$\Delta E = \left((\Delta L^2) + (\Delta a^2) + (\Delta b^2)\right)^{0.5}$$

2.6 Breadsticks Textural Properties

The textural properties of the samples were evaluated using a three-point bending test on 30 half-cut breadsticks per sample batch. The test was performed using a texture analyzer (Stable Microsystems, Model TA-XT2 Texture Analyzer, Surrey, UK) equipped with a three-point bending rig (HDP/3PB) and a 30 kg load cell. The half

breadsticks were placed in the center of the rig base support, 60 mm apart, and cracked by moving the blade probe downward at a pre-test speed of 1 mm s^{-1} and a test speed of 3 mm s^{-1}. For each sample, Texture Exponent TEE32 software (Stable Micro System, v. 6.1.10.0, Surrey, UK) was used to calculate the peak maximum force (hardness, N) and the distance to fracture (brittleness, mm).

2.7 Polyphenols analysis

To determine the total polyphenol and flavonoid content, 1 g of S and B powders were macerated with 20 mL of a 50% ethanol:water solution for 60 min at 38±2°C with constant agitation (140 rpm). Later, the mixture was centrifuged at 6,500×*g* for 10 min at room temperature (Cannas et al., 2023). The supernatant was then collected, filtered (cellulose acetate syringe filter 0.45µm pore-size), and stored at -20°C until analysis. After extraction, total polyphenol content (TPC) was assessed by the Folin-Ciocalteau spectrophotometric method reported by Noriega-Rodríguez et al. (2020). Total flavonoid content (TFC) was measured with the aluminum chloride colorimetric method previously described by Dabbou et al. (2017).

The soluble (SF) and insoluble (IF) polyphenol fractions were determined on the breadstick samples according to Conte et al. (2021). The TPC was calculated as the sum of the two fractions.

To evaluate the TFC of breadsticks, 2 g of finely ground breadsticks were extracted with 10 mL of ethanol:water solution (40:60 v/v) for 30 min at room temperature. The mixture was then centrifuged at 8,000×*g* for 20 min, and the supernatants were collected. The spectrophotometric procedure used to determine TFC on by-product powders was also applied to the breadsticks. The polyphenol results for both artichoke powders and breadstick samples were expressed as mg of gallic acid equivalents (GAE)

per 100 g of dry matter (d.m.), whereas the flavonoid data were reported as mg catechin equivalents (CE) per 100 g of d. m.. Both analyses were performed in duplicate for each production batch.

2.8 Antioxidant capacity

The antioxidant capacity (AOC) was evaluated on both artichoke by-product powders and breadstick samples. Ground samples (1 g for by-product powders, 3 g for breadsticks) were extracted for 1 h at room temperature with stirring in a 20 mL (10 mL for breadsticks) solution of methanol-water (50:50) acidified with hydrochloric acid to a pH of 2. The supernatant was recovered after centrifugation at 1,000×*g* for 10 min. The residue was subjected to a second extraction using 20 mL (10 mL for breadsticks) of a 70:30 v/v acetone:water solution under the same conditions. The resulting supernatants were combined and used to determine the AOC through DPPH and ABTS spectrophotometric assays, as reported by Cannas et al. (2023). The results (mean of two repetitions per batch) were expressed as μmol of Trolox equivalent (TE) per 1 g of d.m..

2.9 Sensory Evaluation

A Check-All-That-Apply (CATA) test was conducted to identify the attributes that best emphasize the differences among breadsticks produced with artichoke by-products. Furthermore, a liking test was performed to determine consumer acceptance of these products. The CATA attributes were selected based on terms from the literature and a preliminary laboratory test with a small group of assessors.

A group of 80 assessors, comprising 56% men and 44% women and aged between 23 and 60 years, were selected from the students and staff of the Department of Agricultural Sciences (University of Sassari) for their experience in performing sensory

tests. Informed consent was obtained from all participants. The sensory tests were conducted using the Smart Sensory Box software (Smart Sensory Solutions S.r.l, Sassari). Consumers evaluated the breadsticks using a 9-point hedonic scale to rate overall liking, with 1 indicating “dislike very much” and 9 “like very much” (Giménez et al., 2015). They were then asked to select the attributes that best described the samples from a provided list.

2.10 Volatile organic compounds (VOCs) Analysis

The volatile fraction analysis was conducted using Headspace Solid-Phase Microextraction (HSPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS) on an Agilent 7890 GC equipped with a Gerstel MPS autosampler, coupled with an Agilent 7000C MS detector. A 3g aliquot of crushed breadstick sample (1.5 g by-products powder) was weighed in a 20 mL sample vial and allowed to equilibrate for 10 min at 60 °C, 2h after baking. A 1cm 50/30µm DVB/CAR/PDMS Stableflex SPME fiber (Chromline, Prato, Italy) was then exposed for 60 min to the headspace. The extraction time was chosen based on previous optimization. The fiber was then desorbed for 2 min in a PTV injector operating at 250°C in splitless mode. Chromatographic separation was performed following the method reported by Conte et al. (2020). The VOCs were identified by comparing their retention times and spectra with those of pure standards, when available, and by matching the MS spectra and the experimental linear retention indexes with those available in the literature or in the commercial libraries (NIST/EPA/NIH 2008; HP1607 from Agilent technologies).

2.11 Statistical Analysis

Statistica 12.0 software (StatSoft, Inc., Tulsa, OK, USA) was used to perform one-way analysis of variance (ANOVA) on the measured data. The means were separated using

Fisher's LSD test (95% confidence interval). Pearson's correlation test was used to investigate the relationship between the results of AOC, TPC (SF, IF), and TFC. To identify significant differences in volatile composition among breadstick samples and between by-products ($p<0.05$), one-way ANOVA and t-tests were used, respectively ($p < 0.05$). Afterwards, a Principal Component Analysis (PCA) was applied.

The sensory data were analyzed using XLSTAT for Windows (Version 2020.1.2, Addinsoft, Paris, France). Cochran's Q Test ($p \leq 0.05$) was conducted, followed by a multiple pairwise comparison test using the Critical difference (Sheskin) procedure, to identify which attributes differed significantly among the samples. Correspondence Analysis (CA) was then performed on the contingency table to determine the relationship among the samples and the selected terms from the CATA questionnaire. Overall liking scores were analyzed using one-way ANOVA, with samples as the fixed source of variation and consumers as randoms, followed by Fisher's LSD test ($p \leq 0.05$).

3. Results and Discussion

3.1. Proximate composition of by-products powders and breadsticks

Table 2 reports the proximate composition of B and S powders, as well as control (CTRL) and fortified breadstick samples.

Moisture values of 5.61 and 6.12 g·100 g^{-1} were measured in B and S, respectively. The a_w values were quite low due to the freeze-drying process: 0.03 in B and 0.14 in S. The protein contents determined in B (9.48±0.18 g·100 g^{-1} d.m.) and S (6.83±0.03 g·100 g^{-1} d.m.) were lower than the literature values. Boubaker, Damergi, et al. (2016) detected a protein content of 11.53 g·100 g^{-1} d.m. in a Tunisian cultivar of artichoke stem powder. Similarly, Ruiz-Cano et al. (2014) reported a crude protein level of 10.5 to 15.2 g·100 g^{-}

[1] d.m. in bracts of the Spanish cultivar ‘Blanca de Tudela’, coming from different stages of industrial canning processing. Amoriello et al. (2022) found that the by-products (mixture of bracts and stems) powders obtained from artichoke cultivar ‘Campagnano’ contained 19.34 g·100 g^{-1} d.m. protein. It should be noted that the protein content of artichokes varies depending on the variety tested due to genetic differences (Melilli et al., 2014). Currently, there are no studies reporting the protein content of the Spinoso Sardo cultivar. The results for total dietary fiber were consistent with those found in other studies, reporting values ranging from 44.23 to 67.00 g·100 g^{-1} d.m. in bracts and mixtures of artichoke by-products (Boubaker, Damergi, et al., 2016; Eman et al., 2018; Frutos et al., 2008; Ruiz-Cano et al., 2014). In this study, B (68.57 g·100 g^{-1}, d.m.) had a significantly ($p<0.01$) higher fiber content than S (43.63 g·100 g^{-1}, d.m). The outer bracts to likely serve to physically protect the inner parts of the inflorescence from abiotic and biotic stresses (Pandino et al., 2011d). The proportion of fat in both by-product fractions was relatively low, with the B fraction exhibiting a significantly ($p < 0.05$) higher concentration (0.63±0.05 g·100^{-1} d.m) than the S fraction (0.39±0.03 g·100 g^{-1} d.m.). These values were found to be lower than those reported by other authors in bracts (Ruiz-Cano et al., 2014; Umaña et al., 2021) and stems (Boubaker, Damergi, et al., 2016), despite also representing a minor component in these studies.

The ash content of B (6.16±0.01 g·100 g^{-1}, d.m.) aligned with previous research on bracts (Lutz et al., 2011; Ruiz-Cano et al., 2014). The concentration of ash in S (7.19±0.04 g·100 g^{-1}, d.m.) was higher than that found by Francavilla et al. (2021) (6.4±0.31 g·100 g^{-1}, d.m.), while Amoriello et al. (2022) measured an ash content of 10.31±0.05 g·100 g^{-1} d.m. in artichoke by-products (bract and stalks mix). In line with the results of the present study, Eman et al. (2018) found a higher ash content in stems compared to bracts, while Francavilla et al. (2021) reported higher values in artichoke

heads than in stalks. However, biomass composition is highly variable and is strongly influenced by genotype, environmental conditions, and cultivation practices (Lombardo et al., 2018; Melilli et al., 2014).

The moisture and a_w values of the breadstick samples ranged from 6.16 to 7.06 g·100 g^{-1} d.m. and from 0.31 to 0.38, respectively (Table 2). Both values were significantly lower in the fortified breadsticks than in the CTRL. These outcomes, especially the moisture values, suggest that the addition of by-product powders in the formulation resulted in greater water loss during cooking, which contradicts the findings of other studies. Canale et al. (2022) and Frutos et al. (2008) found that the addition of the artichoke residues increased the moisture content of the fortified bread compared to the control. However, previous studies evaluating the influence of the addition of different fibers on bread quality have shown that adding inulin (a prevalent fiber in artichoke) to the formulation reduces the moisture of the final product compared to conventional bread (Wang et al., 2002). Similarly, Ktenioudaki et al. (2012) observed a reduction in the moisture content of breadsticks as a result of the inclusion of spent grain, although there were no significant differences from control breadsticks. The incorporation of fiber-rich by-products may reduce the water retention capacity, possibly due to the leaching of wheat flour constituents and the interference with the structure of starch granules (Rainero et al., 2022; Simsek & Süfer, 2022). The protein content of the CTRL was significantly ($p<0.001$) higher than the other samples, likely due to the wheat flour used in the formulation, which had a higher protein content (11.0 g·100 g^{-1}according to the nutrition label) compared to the artichoke bract (9.48 g·100 g^{-1} d.m.) and stem (6.83 g·100 g^{-1} d.m.) powders. Therefore, the addition of these by-products at both levels likely led to a dilution of the protein content in the final product. Of the two by-products used, B had a higher protein content, resulting in a higher protein concentration in

samples B3 and B5 compared to S3 and S5. Considering the high fiber content of B and S, significant increases ($p<0.001$) in dietary fiber content, proportional to the type and level of by-product added, were also observed in the enriched breadsticks. B5 had the highest fiber content, followed by S5 and B3, S3, and finally CTRL. It should be noted that all fortified samples exceeded the minimum required level of fiber (6 g·100 g^{-1}) to be labeled as "high fiber" according to the current regulation 1924/2006 (European Commission, 2006). As expected, the incorporation of both powdered by-products did not result in a significant change in the fat content of the breadsticks, which showed no statistically different values from each other ($p<0.05$). The ash content of the fortified breadsticks significantly ($p<0.05$) increased with the addition of artichoke by-products compared to the CTRL. The increase was more pronounced in sample S5, where the addition level was higher.

3.2. Color analysis

Table 3 presents the color data for the freeze-dried powders and breadstick samples. The addition of both powders (B and S) had a highly significant effect ($p < 0.001$) on all three colorimetric parameters measured in the enriched samples. The fortified breadsticks showed a significant decrease in brightness L* compared to CTRL, especially at higher levels of addition. This is a common occurrence in baked products with added fiber (Ktenioudaki et al., 2012). In particular, the B-supplemented samples showed a tendency towards a more greenish coloration than the CTRL, as evidenced by the significant decrease in the a* colorimetric coordinate. On the other hand, the breadsticks fortified with S had the highest values of a* and b* coordinates at both supplementation levels, showing a significant increase toward red and yellow. However, the observed changes only partially reflected the natural hue of the added powders,

especially for S3 and S5. Both B and S were powders with a greenish-brownish coloration, but S appeared brighter, more greenish-yellow than B. These findings suggest that the high temperatures reached during baking may have degraded the pigments naturally present in the by-products. Furthermore, a previous study on artichoke by-products reported that S had a higher content of reducing sugars (20.7 g/100 g d.m.) compared to bracts (10.4 g/100 g d.m.) (Zeaiter et al., 2019). Consequently, the colorimetric coordinate a* of S3 and S5 increased, likely due to enhanced Maillard reactions during baking in the S-fortified samples, resulting in a more reddish color compared to B3 and B5 (Canale et al., 2022).

However, the ΔE -calculated to assess any color differences perceptible to the human eye between the CTRL and enriched samples- were less than 1 in all cases. Therefore, it can be concluded that the addition of the two by-product powders had no effect on the final color of the breadsticks. Significant outcomes were found by Conte et al. (2021) in gluten-free breadsticks enriched with olive leaves and olive mill wastewater extracts.

3.3. Breadsticks textural properties

Table 3 shows that the inclusion of S and B powders had a significant ($p<0.001$) effect on both textural parameters, although in different ways. The addition of S powder, irrespective of the supplementation level, resulted in a significant increase in the force at break (34.97±1.35 N in S3 and 34.97±1.54 N in S5) compared to the CTRL sample, which had an intermediate hardness value (32.71± 1.39 N). Boubaker, Damergi, et al., (2016) found that increasing the amount of artichoke stem powder resulted in a progressive increase in bread crumb hardness, which is consistent with the findings of Wang et al. (2002) on the effects of different fibers on dough performance and bread quality. The study also revealed that inulin increased crumb hardness compared to the

control sample. Zeaiter et al. (2019) characterized dried artichoke waste and found that stems contained a higher amount of inulin (27.97±4.58 g·100 g^{-1} d.m.) than the bracts (15.96±1.75 g·100 g^{-1}d.m.). This suggests that the high inulin content in the S may have contributed to the increased firmness of the breadsticks. Conversely, breadsticks fortified with B had the lowest hardness values (Table 3). A comparable pattern was noted in breadsticks enriched with other fiber-rich by-products. For instance, Simsek & Süfer (2022) reported a decrease in breadsticks hardness after fortifying them with olive pomace. Similarly, Rainero et al. (2022) found that the addition of grape pomace powder resulted in a decrease in the hardness and fracturability of breadsticks in comparison to the control. Ktenioudaki et al. (2012) discovered that the breadsticks containing brewer's spent grains were less hard than the control sample. The addition of fibers typically alters the structure of baked goods. However, Uysal et al. (2007) found that the use of different fiber sources, such as apple, lemon, wheat, and wheat bran, in cookie enrichment can either decrease or increase their hardness, depending on the type and quantity of fiber used.

The values of brittleness, a parameter which describes how much a sample can be deformed before fracture, was significantly ($p<0.001$) higher in the CTRL compared to the other samples. This observation could be explained by the difference in water content and a_w between the CTRL and the other samples. Indeed, water acts as a plasticizer in food matrices, increasing flexibility as its concentration increases (Chang et al., 2000b). Therefore, the CTRL sample showed a greater degree of deformation than the fortified breadsticks, exhibiting a more rubbery behavior. This response was also observed by Rainero et al. (2022) when they fortified breadsticks with grape pomace powder. However, a reduction in plasticity in favor of brittleness is desirable as consumers prefer crispy breadsticks.

3.4. Polyphenols analysis and Antioxidant capacity (AOC)

Table 4 reports the results for TPC, TFC and AOC (DPPH and ABTS assays) of artichoke by-products. The data shows that S had significantly higher ($p<0.001$) TPC (1258.87±7.58 mg GAE·100 g^{-1} d.m.) and TFC (1233.9±18.71 mg CE·100 g^{-1}, d.m.) than B (951.52±2.01 mg GAE·100 g^{-1} d.m. and 564.31 mg CE·100 g^{-1} d.m.). Similar results were obtained for AOC, with S exhibiting higher values in both assays compared to B (Table 4). The results obtained are consistent with those found by Cannas et al. (2023) in the same fractions of artichoke by-products of the Spinoso sardo variety, recovered from the identical companies but in the earlier year (2019). It is important to note that the polyphenol content, and consequently the AOC, can vary depending on the environmental conditions to which the plant was exposed (Lombardo et al., 2018). Moreover, the values obtained from the DPPH assay were lower than those recorded with the ABTS assay. As previous studies have shown, most of the analyzed vegetables exhibited higher AOCs when measured by the ABTS assay compared to the DPPH assay (Floegel et al., 2011). It is recommended to use at least to two types of assays to detect distinct pools of antioxidants in complex food matrices. These two assays use different radicals and measure at different wavelengths, allowing detection of different antioxidant pools (Sadowska-Bartosz & Bartosz, 2022).

The results of the analysis of the breadstick samples for TFC, TPC and the corresponding SF and IF fractions, are summarized in Table 4. The CTRL sample had significantly ($p<0.001$) lower values for both TPC (272.32 ±4.41 GAE mg·100 g^{-1}, d.m.) and TFC (7.09±0.45 CE mg·100 g^{-1}, d.m.). As per data obtained from the by-products, the addition of S resulted in the most effective increase in both TPC (S3: +30%, S5: +54%) and TFC (S3: +327%, S5: +497%), which was directly proportional to the level of addition. The next most effective increase was observed with B-

supplemented breadsticks, with TPC increasing by 30% and 43% for B3 and B5, respectively. For TFC, the increase reached 163% and 341% for B3 and B5, respectively. Canale et al. (2023) found that incorporating different proportions of artichoke bract and stem flours in bread resulted in significant improvements in polyphenol content. However, they measured lower phenolic concentrations than those reported in this study and observed more efficient increments with higher percentages of added bracts (the by-product with higher content of phenolic compounds). In this case, the S powder had a higher concentration of polyphenols, resulting in greater enrichment of TPC when added as by-product.

Table 4 shows that the inclusion of the by-products resulted in a greater increase ($p<0.001$) in the SF of polyphenols in the fortified breadsticks compared to the control, with a more pronounced effect in samples enriched with S. The IF also increased moderately, but still significantly ($p<0.01$). Only at the higher incorporation rates was there a less pronounced effect on samples fortified with B. The significant increase in SF could be attributed to the prevalence of free or soluble conjugated forms in artichoke phenolic compounds compared to bound forms (Amoriello et al., 2022). Soluble polyphenols are not bound to cell wall components, making them more digestible in the gastrointestinal tract. Strengthening this phenolic fraction can effectively enrich breadsticks, as observed by Chan et al. (2016) and Conte et al. (2021). In a study by Conte et al. (2021) the addition of phenolic-rich extracts from olive leaves and olive mill wastewater resulted in a significant increase in SF in gluten-free breadsticks, while no difference in IF was found.

In the current investigation the effectiveness of CTRL and the fortified breadsticks in reducing ABTS·+ and DPPH· radicals was also evaluated, and the results are presented in Table 4. Both tests showed significant ($p<0.001$) increase in the AOC of the enriched

breadsticks compared to CTRL, especially at high addition levels of B and S. Sample S5 exhibited a more effective increase in AOC (+527% and +114% in DPPH and ABTS assays, respectively), possibly due to its greater AOC compared to B. Increasing the AOC not only reduces the negative effects of reactive species but may also improve the stability of breadsticks against lipid oxidation, to which they are often subjected. This antiradical activity is generally associated with the presence of specific compounds such as polyphenols. In fact, the antioxidant capacity of the breadstick samples was aligned with their TPC and TFC, as evidenced by the highly significant positive correlations ($p < 0.001$) found between AOC and TPC ($r=0.922$ for DPPH, $r=0.979$ for ABTS assays), SF ($r=0.937$; $r=0.992$ for DPPH and ABTS respectively) and TFC ($r=0.9645$ for DPPH, $r=0.9598$ for ABTS). However, there was no correlation found between AOC and IF. This may be due to the limited effect of adding S and B powders on this polyphenolic fraction. These results suggest that the antioxidant power of breadsticks increases with higher SF and TFC.

3.5. Sensory evaluation

Cochran's Q analysis, followed by the Sheskin test, revealed significant differences between the enriched breadsticks samples and the control ($p\leq 0.05$) (Table 5). Furthermore, differences were observed among fortified with B and S, with B5 perceived as more bitter than the other samples. Additionally, B5 exhibited a higher citation rate for the attribute "taste of artichoke", which was not significantly different from all the fortified breadsticks, but significantly different from the CTRL. All fortified breadsticks had a higher citation rate for "herbaceous odor" and "rich in fiber" compared to the CTRL sample. However, the CTRL sample received high ratings from

the consumers for the following attributes: “less bitter”, “excellent as snack”, “pleasant in mouth”, “good as appetizer” and “more friable”.

The results of the CA are presented in Figure 1, where the two dimensions accounted for a total inertia of 95.63%. It can be observed that breadsticks fortified with S were associated with attributes such as bitter, astringent, taste like artichoke, and herbaceous odor, whereas breadsticks with bracts were characterized by attributes such as rich in fiber, healthy, sustainable, and grainy. The One-Way Anova analysis of the overall liking data showed that the CTRL was significantly more appreciated than the fortified breadsticks ($p \leq 0.05$), with a score of 6.90 on a hedonic scale from 1 to 9. Conversely, B3 was the least preferred with a score of 5.60 (Figure 2). The obtained data confirmed what was reported in the literature (Frutos et al., 2008), where the control bread sample obtained a higher score than the breads added with artichoke by-products. No significant differences were found between the CTRL and fortified breadsticks for the hard attribute, which contradicts the findings reported in other papers (Boubaker, Damergi, et al., 2016; Frutos et al., 2008). However, the panel cited the CTRL sample as being more friable. As shown in Figure 3, which displays the results of the PCA applied to the correlation coefficients, the liking attribute was correlated with the following attributes: “pleasant in the mouth”, “good appearance”, “good as appetizer” and “excellent as snack”. In conclusion, although phenol-rich foods, due to their bitter and astringent characteristics, may be unappealing to a large part of the population (Spinelli et al., 2021), they are known to have beneficial effects on health. In the present study, although the fortified samples scored slightly lower than the conventional product, consumers still perceived them as higher in fiber, healthy and sustainable. However, effective strategies need to be developed to increase their acceptability.

3.6 VOCs results

The VOCs data of the artichoke by-products powders are presented in Table 6. As reported in previous literature (Dabbou et al., 2016, 2017b), the sesquiterpene hydrocarbons were the most represented volatile compounds in both bracts and stems, followed by carbonyl compounds and hydrocarbons. β-selinene was the most abundant compound, with a larger peak area ($x10^6$) in bracts compared to stems (188 vs 26.2), followed by caryophyllene and its oxide (Dabbou et al., 2017b). Table 7 and Figure 3 reported the data for the fortified breadsticks and CTRL. The first two principal components accounted for the 75.24% of the explained variance. The first component separated the breadsticks fortified with B from those fortified with S, while the second component distinguished the CTRL and the samples with the lowest percentage of fortification, S3 and B3, from the others, which were richer in aromatic compounds. Specifically, all the fortified samples contained the terpene β-selinene, which is also present in the artichoke powders, mainly in the B, as previously reported. B5 had a high content of aldehydes, including 2-hexenal and hexanal, which are also present in the B used to fortify breadsticks. Among the acids, nonanoic acid was significantly higher in the fortified samples compared to the CTRL, as expected. This is in line with the presence of nonanoic acid in the B and S artichoke powders. Additionally, benzaldehyde was found to be significantly higher in S5 and B5 compared to the CTRL, while 2-pentylfuran was significantly higher in all the fortified samples. Although not found in the artichoke by-products, the terpene β-ocimene is a component of many vegetable oils. The presence of this compound in both the CTRL and fortified samples is likely due to the use of sunflower oil in the preparation of the breadsticks. It is well known that the aroma of bread plays a crucial role in consumer perception. Bread includes a variety of compounds, including alcohols, aldehydes, acids, hydrocarbons,

ketones, pyrazines and nitrogen or sulfur compounds, which may be present in the crust, crumb or both (Pico et al., 2015). These compounds are formed during fermentation, lipid oxidation, and Maillard reactions. Breadsticks, due to the short fermentation time, are not as rich in volatile compounds as other types of bread (Pico et al., 2015). However, the analysis revealed the presence of several compounds, including those derived from lipid oxidation, Maillard reactions, flour, and artichoke by-products due to the enrichment of breadstick. As shown in Figure 4, the CTRL sample had lower level of aldehydes, especially 2-(Z)-heptenal, 2-(E)-octenal, furfural, benzaldehyde and phenylacetaldehyde compared to the other samples. Additionally, S5 and B5 had lower acid contents than CTRL, B3, and S3. Although acetoin was found in higher amounts in the S-enriched breadsticks, the difference was not statistically significant. This compound is known to impart a pleasant aroma to bread, as well as phenylacetaldehyde (honey, floral) and furfural, which gives toasted notes. Furthermore, breadsticks, especially in those supplemented with artichoke powder, contained several aldehydes such as 2,4-decadienal, (E,E)-, nonanal, octanal, 2-(E)-octenal, and heptanal, which may be the result of lipid oxidation. Indeed, lipoxygenase is known to produce unstable hydroperoxides that degrade during baking to form hexanal, 2-hexenal and 1-pentanol, all of which were found in the breadstick samples. In addition, during the baking process, thermal reactions resulted in the production of furans, pyrazines, and sulfur compounds. Thermal degradation of sugars leads to the release of compounds such as furfural and acetic acid. It should not be overlooked that flour also contains compounds such as hexanal, heptanal, and pentanal. However, the aroma of bread is much more complex than that of flour due to the various reactions that occur during kneading, fermentation, and baking.

4. Conclusions

This study has shown that the use of artichoke by-product powders in the production of breadsticks offers snack manufacturers a promising avenue for developing a finished product with enhanced technological and nutritional characteristics, which can be labeled as “high fiber”. Furthermore, it represents a successful strategy for pursuing a circular economy. The results obtained demonstrated that the addition of stem and bract powders, at both addition levels, led to an increase in dietary fiber content, ash, phenolic compounds and consequently antioxidant activity, without significantly affecting the color, but improving the breadsticks brittleness. Consistent improvements were achieved with the highest addition rates and particularly with artichoke stems. On the other hand, from a sensory point of view, consumers gave the control sample a higher overall liking score. Therefore, further investigations are needed to achieve improved sensory characteristics. However, although the fortified samples scored slightly lower than the conventional product, being considered more grainy, bitter, herbaceous, and astringent, consumers perceived them as higher in fiber, healthy and sustainable.

In view of the growing consumer awareness and preference for healthy products, artichoke by-products represent a promising ingredient for the development of novel functional bakery products. Nevertheless, a more comprehensive investigation is required to ascertain the impact of fortification on the shelf-life of the finished product, particularly in terms of oxidative stability, as well as the actual *in vivo* antioxidant effect. In addition, further studies on artichoke by-products derived from harvests of different years are necessary to confirm the above promising results, due to the inherent variability in the annual composition of the plant raw material by-products (especially with regard to the amount of accumulated and preserved bioactive compounds), which is particularly influenced by soil and climate conditions.

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Michela Cannas: Formal analysis, Data curation, Writing - original draft. **Paola Conte:** Investigation, Methodology, Data curation, Writing - review & editing. **Pietro Paolo Urgeghe:** Formal analysis, Methodology. **Antonio Piga:** Funding acquisition. **M. Elena Alañón:** Writing - review & editing**. Alessandra Del Caro:** Investigation, Methodology, Conceptualization, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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FIGURES

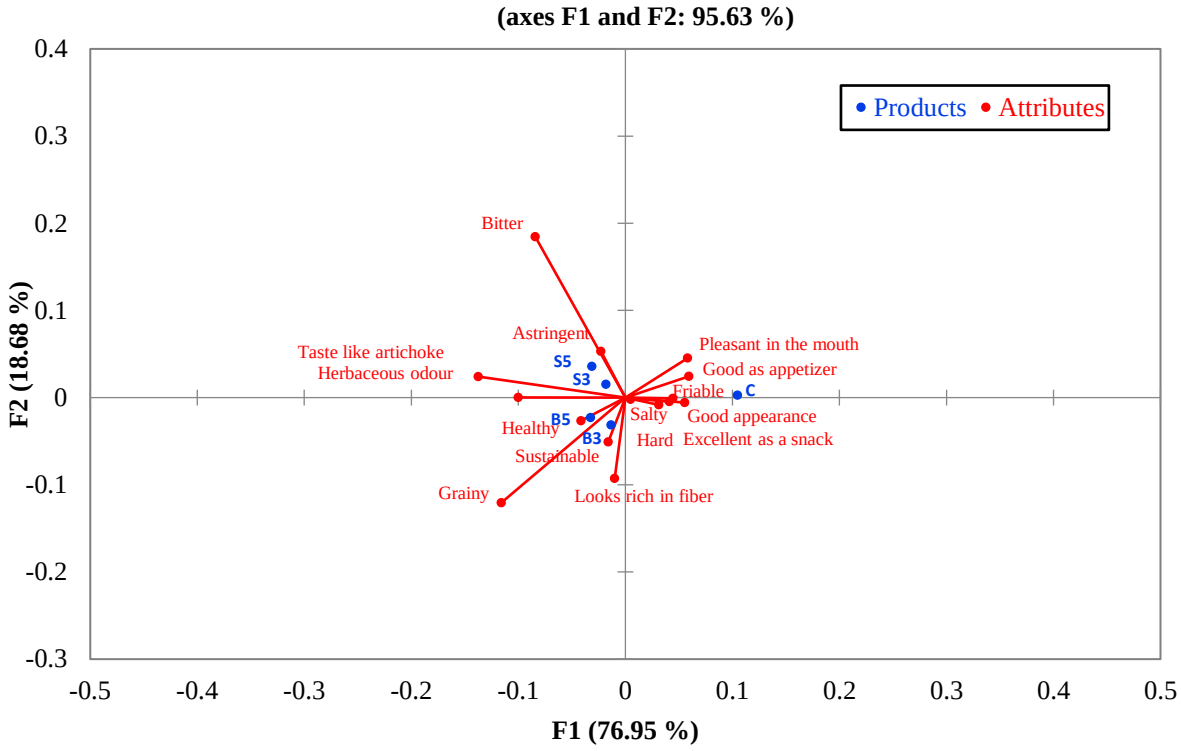

Figure 1. Biplot of the Correspondence Analysis applied to the contingency table obtained from CATA questionnaire.

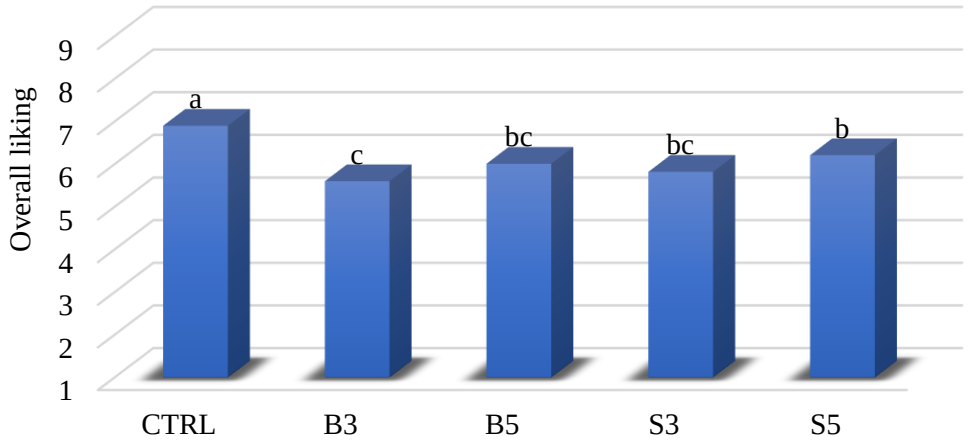

Figure 2. Overall liking mean scores results ($p < 0.05$) of the control and fortified breadsticks. Values with the same letter do not differ significantly from each other according to LSD test ($p < 0.05$).

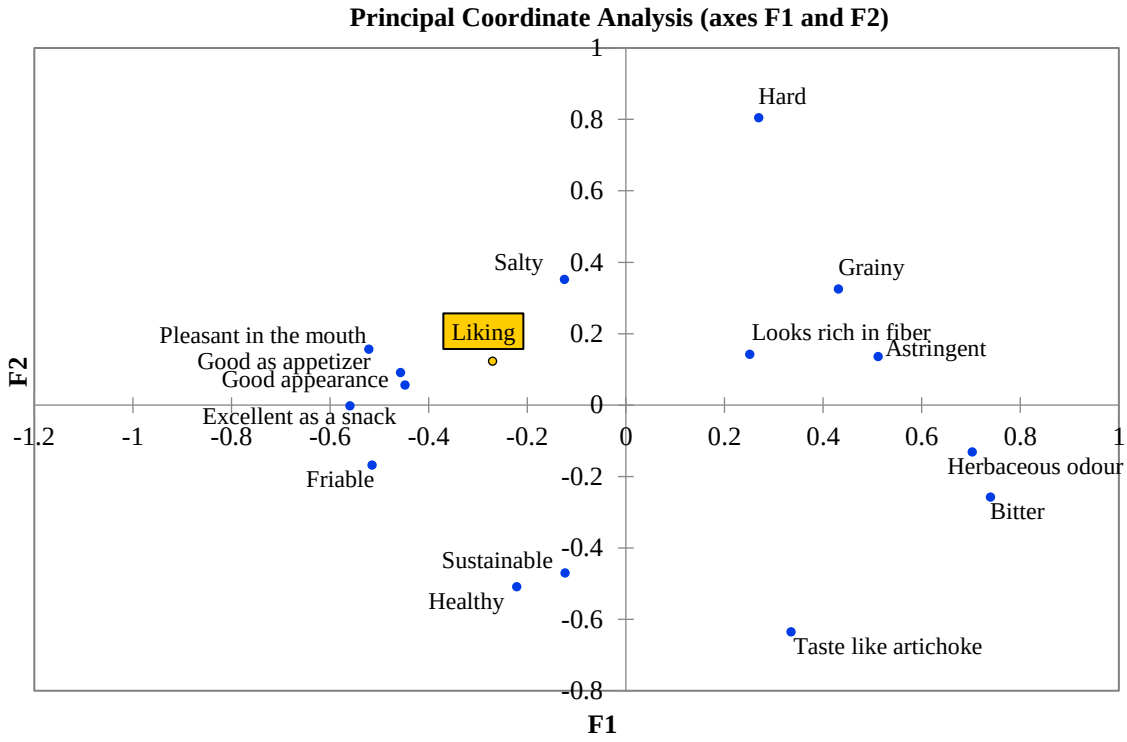

Figure 3. Graph obtained from the Principal Coordinate Analysis obtained by the correlation coefficients between the overall liking and CATA attributes.

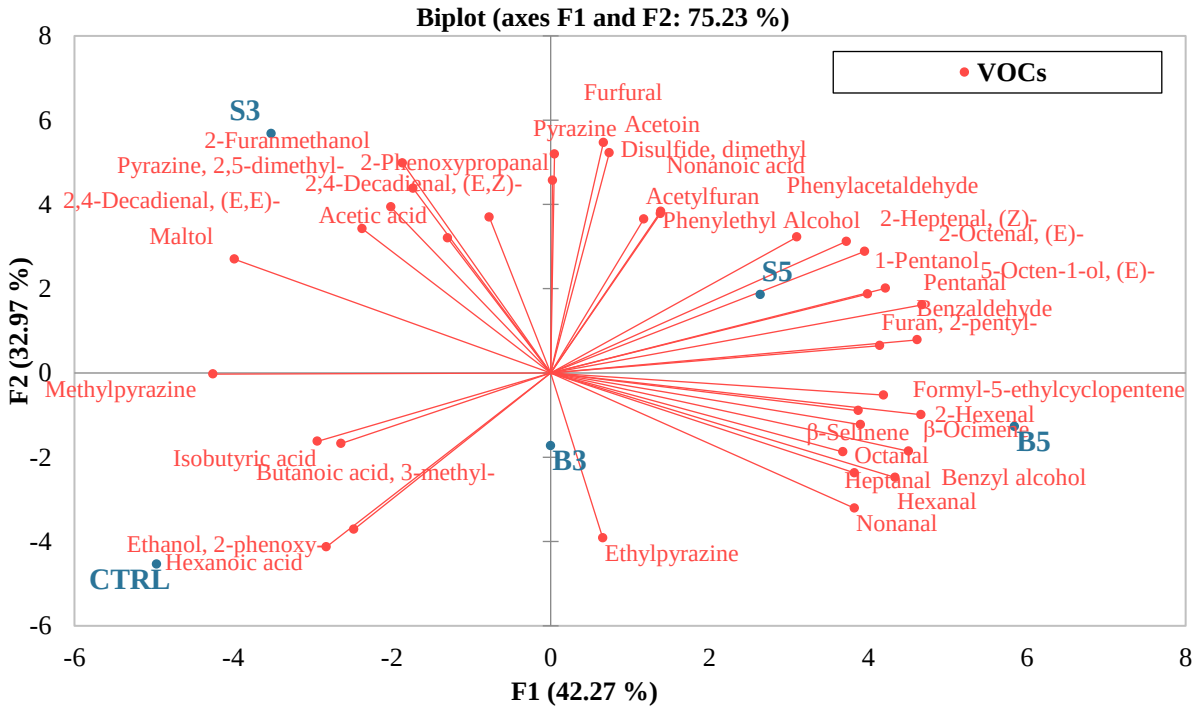

Figure 4. PCA biplot for VOCs of breadstick fortified samples and control (CTRL, B3, B5, S3 and S5) detected by GC-MS.

TABLES

Table 1. Formulation of the experimental breadsticks (g for 100g of wheat flour).

Ingredients	Samples code				
	CTRL	**B3**	**B5**	**S3**	**S5**
Basic formulation					
Wheat flour (type 0)	100	100	100	100	100
Water	50	53.3	54.7	52	52
Sunflower oil	10	10	10	10	10
Yeast	3	3	3	3	3
Salt	1.8	1.8	1.8	1.8	1.8
Supplementary ingredients					
Bracts powder (B)	-	3	5	-	-
Stems powder (S)	-	-	-	3	5

Water amount corresponding to the farinograph water absorption at 750 U.B..
Abbreviations: CTRL, control breadstick; B3, B5, breadstick samples prepared with 3 and 5 % of bracts powder; S3, S5, breadstick samples prepared with 3 and 5 % of stems powder.

Table 2 Proximate composition of artichoke by-products and breadstick samples.

Sample	Moisture g/100 g	a_w	Protein g/100 g d.m.	Total dietary fiber mg/100g d.m.	Crude fat mg/100g d.m.	Ash g/100 g d.m.	Digestible Carbohydrates* mg/100g d.m.
By-products							
B	5.61 ± 0.14^{b}	0.03 ± 0.00^{b}	9.48 ± 0.18^{a}	68.57 ± 0.27^{a}	0.63 ± 0.05^{a}	6.16 ± 0.01^{b}	9.54 ± 0.54^{b}
S	6.12 ± 0.05^{a}	0.14 ± 0.00^{a}	6.83 ± 0.03^{b}	43.63 ± 0.10^{b}	0.39 ± 0.03^{b}	7.19 ± 0.04^{a}	35.85 ± 0.05^{a}
Breadsticks							
CTRL	7.06 ± 0.19^{a}	0.38 ± 0.02^{a}	12.33 ± 0.02^{a}	5.00 ± 0.11^{d}	9.92 ± 0.17^{a}	1.82 ± 0.08^{c}	63.88 ± 0.18^{c}
B3	6.22 ± 0.08^{bc}	0.31 ± 0.01^{b}	11.45 ± 0.04^{bc}	6.42 ± 0.31^{b}	9.54 ± 0.15^{a}	2.03 ± 0.04^{b}	64.35 ± 0.11^{b}
B5	6.33 ± 0.09^{b}	0.32 ± 0.02^{b}	11.48 ± 0.07^{b}	7.59 ± 0.15^{a}	9.48 ± 0.22^{a}	2.17 ± 0.02^{ab}	62.96 ± 0.15^{d}
S3	6.16 ± 0.23^{c}	0.32 ± 0.03^{b}	11.34 ± 0.04^{cd}	6.04 ± 0.16^{c}	9.59 ± 0.03^{a}	2.17 ± 0.04^{ab}	64.71 ± 0.01^{a}
S5	6.17 ± 0.08^{bc}	0.32 ± 0.00^{b}	11.25 ± 0.04^{d}	6.52 ± 0.19^{b}	9.56 ± 0.08^{a}	2.24 ± 0.09^{a}	64.25 ± 0.12^{b}

This table shows mean values ± standard deviation (for each batch: n = 5 for water activity; n = 3 for moisture and total dietary fiber; n = 2 for protein, crude fat, and ash). Different letters in the same column denote significant differences ($p < 0.05$) at LSD test.

*Digestible carbohydrates calculated by indirect determination: 100 – (moisture+protein+total dietary fibers+crude fat+ash). Abbreviations: B, bracts powder; S, stems powder; CTRL, control breadstick; B3, B5, breadstick samples prepared with 3 and 5 % of bracts powder; S3, S5, breadstick samples prepared with 3 and 5 % of stems powder.

Table 3 Color and textural parameters of artichoke by-products and breadstick samples.

Sample	**L**			**a**			**b**			**ΔE**	**Hardness** (N)			**Brittleness** (mm)		
By-products																
B	43.13	±	0.56^{b}	-0.19	±	0.42^{a}	14.12	±	0.25^{b}	-		-			-	
S	46.56	±	1.35^{a}	-3.79	±	0.85^{b}	15.15	±	0.64^{a}	-		-			-	
Breadsticks																
CTRL	60.87	±	0.34^{a}	1.92	±	0.34^{c}	18.67	±	0.24^{b}	-	32.71	±	1.39^{b}	0.93	±	0.12^{a}
B3	55.12	±	0.19^{c}	1.63	±	0.05^{cd}	18.83	±	0.52^{b}	0.18	29.03	±	1.85^{d}	0.82	±	0.09^{c}
B5	52.73	±	0.39^{e}	1.45	±	0.10 ^{d}	18.29	±	0.63^{b}	0.28	31.43	±	1.10^{c}	0.81	±	0.09^{c}
S3	56.14	±	0.38^{b}	2.44	±	0.18^{b}	20.21	±	0.36^{a}	0.29	34.97	±	1.35^{a}	0.86	±	0.11^{b}
S5	54.50	±	0.79^{d}	2.79	±	0.43^{a}	20.27	±	0.40^{a}	0.47	34.84	±	1.54^{a}	0.81	±	0.08^{c}

This table shows mean values ± standard deviation (for each batch: n = 5 for color parameters; n= 30 for textural properties). Different letters in the same column denote significant differences ($p < 0.05$) at LSD test. Abbreviations: B, bracts powder; S, stems powder; CTRL, control breadstick; B3, B5, breadstick samples prepared with 3 and 5 % of bracts powder; S3, S5, breadstick samples prepared with 3 and 5 % of stems powder.

Table 4 Results of polyphenols analysis and antioxidant capacities of artichoke by-products and breadstick samples.

Sample	Soluble fraction mg GA/100 g d.m.	Insoluble fraction mg GA/100 g d.m.	Total polyphenols mg GA/100 g d.m.	Flavonoids mg CE/100 g d.m.	DPPH TE μmol TE/ g d.m.	ABTS μmol TE/ g d.m.
By-products						
B	-	-	951.58 ± 22.01^{b}	564.31 ± 1.20^{b}	51.38 ± 0.57^{b}	95.22 ± 0.35^{b}
S	-	-	1258.87 ± 7.58^{a}	1233.90 ± 18.71^{a}	68.86 ± 1.04^{a}	108.97 ± 0.27^{a}
Breadsticks						
CTRL	83.27 ± 0.65^{e}	189.04 ± 3.76^{cd}	272.32 ± 4.41^{d}	7.09 ± 0.45^{d}	0.42 ± 0.01^{e}	4.31 ± 0.06^{e}
B3	158.34 ± 0.00^{d}	196.34 ± 5.57^{bc}	354.68 ± 5.57^{c}	18.64 ± 6.56^{c}	1.11 ± 0.03^{d}	7.17 ± 0.08^{d}
B5	183.23 ± 0.71^{b}	205.04 ± 0.64^{a}	388.27 ± 1.35^{b}	31.30 ± 6.97^{b}	1.50 ± 0.02^{c}	8.19 ± 0.03^{b}
S3	166.14 ± 0.58^{c}	188.09 ± 0.34^{d}	354.22 ± 0.23^{c}	30.30 ± 1.35^{b}	1.54 ± 0.03^{b}	7.91 ± 0.05^{c}
S5	217.47 ± 1.46^{a}	202.54 ± 0.94^{ab}	420.01 ± 2.40^{a}	42.35 ± 8.08^{a}	2.66 ± 0.01^{a}	9.24 ± 0.03^{a}

This table shows mean values ± standard deviation (for each batch: n = 2). Different letters in the same column denote significant differences ($p < 0.05$) at LSD test. Abbreviations: B, bracts powder; S, stems powder; CTRL, control breadstick; B3, B5, breadstick samples prepared with 3 and 5 % of bracts powder; S3, S5, breadstick samples prepared with 3 and 5 % of stems powder.

Table 5 Pairwise multiple comparison data obtained by the Critical difference (Sheskin) procedure.

Attributes	**CTRL**	**B3**	**B5**	**S3**	**S5**
Good appearance	0.603^{a}	0.411^{a}	0.452^{a}	0.425^{a}	0.411^{a}
Hard	0.260^{a}	0.123^{a}	0.233^{a}	0.096^{a}	0.178^{a}
Bitter*	**0.014^{c}**	**0.055^{bc}**	**0.110^{bc}**	**0.192^{b}**	**0.466^{a}**
Excellent as a snack*	**0.603^{a}**	**0.356^{b}**	**0.370^{b}**	**0.384^{b}**	**0.329^{b}**
Astringent	0.014^{a}	0.027^{a}	0.027^{a}	0.041^{a}	0.082^{a}
Grainy	0.137^{a}	0.274^{a}	0.247^{a}	0.137^{a}	0.123^{a}
Looks rich in fiber*	**0.027^{d}**	**0.493^{ab}**	**0.562^{a}**	**0.233^{cd}**	**0.315^{bc}**
Sustainable	0.096^{a}	0.219^{a}	0.164^{a}	0.110^{a}	0.137^{a}
Pleasant in mouth*	**0.658^{a}**	**0.370^{b}**	**0.356^{b}**	**0.370^{b}**	**0.425^{b}**
Healthy$^{\#}$	**0.096^{b}**	0.205^{ab}	**0.301^{a}**	0.192^{ab}	0.219^{ab}
Herbaceous odour*	**0.014^{b}**	**0.219^{a}**	**0.288^{a}**	**0.260^{a}**	**0.274^{a}**
Good as appetizer*	**0.521^{a}**	**0.301^{b}**	**0.205^{b}**	**0.288^{b}**	0.356^{ab}
Friable$^{\#}$	**0.493^{a}**	0.329^{ab}	0.315^{ab}	**0.288^{b}**	0.329^{ab}
Taste like artichoke*	**0.014^{b}**	**0.301^{b}**	**0.479^{a}**	**0.397^{a}**	**0.493^{a}**
Salty	0.055^{a}	0.068^{a}	0.041^{a}	0.055^{a}	0.055^{a}

Significant attributes are shown in bold (* $p < 0.0001$; # $p < 0.05$). Different letters in the same row denote significant differences. Abbreviations: CTRL, control breadstick; B3, B5, breadstick samples prepared with 3 and 5 % of bracts powder; S3, S5, breadstick samples prepared with 3 and 5 % of stems powder.

Table 6. Volatile organic compounds tentatively identified in lyophilized bracts and stems by HS-SPME-GC/MS. Results are expressed as chromatogram peak area [x 10^6].

Volatile compounds	LRI*	Bracts	Stems
Acids			
Acetic acid	1468	10.28 ± 1.77[b]	17.96 ± 1.77[a]
Hexanoic acid	1851	22.66 ± 4.18[a]	14.84 ± 2.12[a]
Nonanoic acid	2098	8.05 ± 3.81[a]	11.17 ± 0.51[a]
Alchols			
1-Penten-3-ol	1160	9.27 ± 0.30[a]	4.25 ± 0.28[b]
2-(Z)-Penten-1-ol	1326	4.04 ± 0.35[a]	1.64 ± 0.10[b]
1-Hexanol	1358	16.60 ± 1.45[a]	2.57 ± 0.42[b]
3-(Z)-Hexen-1-ol	1394	8.74 ± 0.79[a]	ND
2-Butoxy-ethanol	1418	2.39 ± 0.05[a]	ND
3,5-Octadien-2-ol	1431	11.34 ± 1.07[a]	16.38 ± 2.27[a]
Benzyl alcohol	1888	6.60 ± 0.51[a]	1.19 ± 0.09[b]
Phenylethyl Alcohol	1925	3.05 ± 0.34[a]	1.19 ± 0.09[b]
Carbonyl compounds			
Acetaldehyde	699	8.05 ± 0.30[b]	15.08 ± 0.04[a]
Pentanal	990	10.38 ± 0.81[a]	7.98 ± 1.87[a]
1-Penten-3-one	1028	5.68 ± 0.08[a]	1.70 ± 0.26[b]
2-(Z)-Butenal	1051	3.71 ± 0.03[a]	3.49 ± 0.19[a]
Hexanal	1092	141.92 ± 4.22[a]	113.01 ± 13.65[a]
2-(E)-Pentenal	1143	4.18 ± 0.74[a]	ND
2-Hexenal	1236	23.71 ± 2.43[a]	7.22 ± 0.49[b]
Nonanal	1409	5.26 ± 0.03[a]	3.87 ± 0.38[b]
2,4-(E,E)-Heptadienal	1494	5.75 ± 0.91[a]	8.01 ± 1.17[a]
3,5-Octadien-2-one	1550	36.64 ± 4.64[b]	59.10 ± 3.74[a]
Benzaldehyde	1562	16.96 ± 0.22[a]	15.26 ± 0.02[b]
2-Butyl-2-octenal	1700	6.77 ± 0.86[a]	4.16 ± 0.24[a]
2,4-(E,E)-Nonadienal	1747	3.05 ± 0.22[a]	5.87 ± 1.42[a]
Esters			
Ethyl acetate	888	11.94 ± 0.35[a]	10.60 ± 2.05[a]
Ethyl octanoate	1447	7.39 ± 0.89[a]	3.32 ± 0.98[b]
Ethyl nonanoate	1554	1.77 ± 0.52[a]	2.19 ± 0.91[a]
Methyl decanoate	1617	2.31 ± 0.48[a]	1.00 ± 0.21[a]
Ethyl decanoate	1660	4.44 ± 0.74[a]	2.53 ± 0.27[a]
Methyl salicylate	1834	2.61 ± 0.77[a]	2.30 ± 0.19[a]
Ethyl palmitate	2094	17.02 ± 1.85[a]	9.10 ± 1.47[b]
Methyl palmitate	2095	10.31 ± 1.80[a]	4.58 ± 1.10[a]
Hydrocarbons			
Decane	993	4.62 ± 0.10[a]	2.10 ± 0.26[b]
2,2,4,4-Tetramethyloctane	1004	13.45 ± 0.37[a]	10.44 ± 0.91[b]
Dodecane	1197	40.55 ± 2.94[a]	22.41 ± 0.29[b]

Tridecane	1299	35.54 ± 0.88[a]	23.11 ± 0.96[b]
1,8,11-(Z,Z)-Heptadecatriene	1831	5.27 ± 0.33[b]	9.73 ± 0.28[a]
1,8,11,14-(Z,Z,Z)-Heptadecatetraene	1885	35.81 ± 0.63[b]	71.63 ± 0.65[a]
Terpenes			
Caryophyllene	1649	40.51 ± 1.54[a]	11.45 ± 0.23[b]
β-Cyclocitral	1670	2.64 ± 0.44[a]	2.97 ± 0.03[a]
Humulene	1725	3.27 ± 0.39[a]	0.86 ± 0.07[b]
β-Selinene	1769	188.08 ± 5.60[a]	26.21 ± 0.09[b]
β-Ionone	1978	8.69 ± 0.97[a]	8.28 ± 0.18[a]
β-Ionone epoxide	2037	6.29 ± 4.36[a]	2.92 ± 0.24[a]
Caryophyllene oxide	2050	12.41 ± 10.52[a]	1.08 ± 0.27[b]
Other compounds			
Ethanethiol	743	2.07 ± 0.19[a]	1.76 ± 0.93[a]
2-Ethylfuran	963	3.53 ± 0.04[a]	1.70 ± 0.28[b]
2-Pentylfuran	1240	19.34 ± 2.25[a]	12.90 ± 2.50[a]

Values are expressed as means ± standard deviation (for each batch: n = 3). Different letters within the same row mean significant differences among the samples at *t* test ($p<0.05$)
*LRI: linear retention indexes obtained on a 60m VF-WAX capillary column. ND: not detected.

Table 7. Volatile organic compounds tentatively identified in breadsticks by HS-SPME-GC/MS. Results are expressed as chromatogram peak area [x 10^6].

Volatile compounds		Samples					
	LRI*	CTRL	S3	S5	B3	B5	p-value
Acids							
Acetic acid	1465	2.13 ± 0.40^{ab}	2.40 ± 0.07^{ab}	2.63 ± 0.20^{a}	2.20 ± 0.01^{ab}	1.82 ± 0.06^{b}	0.0523
Isobutyric acid	1584	0.53 ± 0.13^{a}	0.50 ± 0.04^{a}	0.42 ± 0.01^{a}	0.64 ± 0.01^{a}	0.42 ± 0.05^{a}	NS
3-Methyl-butanoic acid	1686	2.21 ± 0.55^{a}	2.16 ± 0.16^{a}	1.69 ± 0.14^{a}	2.23 ± 0.02^{a}	2.00 ± 0.15^{a}	NS
Hexanoic acid	1851	2.10 ± 1.26^{a}	1.11 ± 0.21^{a}	0.95 ± 0.02^{a}	1.26 ± 0.03^{a}	1.24 ± 0.06^{a}	NS
Nonanoic acid	2098	1.50 ± 0.08^{b}	2.40 ± 0.08^{a}	2.26 ± 0.03^{a}	2.47 ± 0.11^{a}	2.04 ± 0.20^{a}	0.0019
Alchools							
1-Pentanol	1253	0.28 ± 0.02^{a}	0.30 ± 0.00^{a}	0.33 ± 0.02^{a}	0.28 ± 0.01^{a}	0.33 ± 0.00^{a}	NS
5-(E)-Octen-1-ol	1625	0.14 ± 0.02^{c}	0.26 ± 0.02^{b}	0.45 ± 0.02^{a}	0.30 ± 0.02^{b}	0.38 ± 0.02^{a}	0.0001
2-Furanmethanol	1677	0.55 ± 0.03^{c}	1.49 ± 0.02^{a}	0.78 ± 0.04^{b}	0.74 ± 0.02^{b}	0.57 ± 0.02^{c}	<0.0001
Benzyl alcohol	1888	0.26 ± 0.00^{bc}	0.22 ± 0.01^{c}	0.26 ± 0.05^{bc}	0.34 ± 0.00^{b}	0.46 ± 0.02^{a}	0.0015
Phenylethyl alcohol	1925	3.57 ± 0.07^{d}	4.17 ± 0.02^{b}	4.39 ± 0.02^{a}	3.23 ± 0.06^{e}	3.96 ± 0.01^{c}	<0.0001
2-Phenoxy-ethanol	2098	0.65 ± 0.03^{a}	0.32 ± 0.02^{b}	0.42 ± 0.06^{b}	0.35 ± 0.03^{b}	0.35 ± 0.02^{b}	0.0015
Aldheydes and ketones							
Pentanal	990	0.11 ± 0.01^{d}	0.18 ± 0.01^{c}	0.27 ± 0.01^{ab}	0.20 ± 0.02^{bc}	0.30 ± 0.03^{a}	0.0009
Hexanal	1092	3.00 ± 0.26^{c}	2.33 ± 0.15^{d}	3.62 ± 0.02^{b}	3.70 ± 0.08^{b}	4.37 ± 0.01^{a}	0.0002
Heptanal	1196	0.14 ± 0.01^{c}	0.14 ± 0.02^{c}	0.16 ± 0.01^{bc}	0.26 ± 0.01^{ab}	0.29 ± 0.05^{a}	0.0047
2-Hexenal	1236	0.17 ± 0.01^{a}	0.22 ± 0.01^{a}	0.23 ± 0.02^{a}	0.34 ± 0.00^{a}	0.41 ± 0.13^{a}	NS
Octanal	1302	0.13 ± 0.03^{a}	0.12 ± 0.03^{a}	0.14 ± 0.01^{a}	0.13 ± 0.02^{a}	0.14 ± 0.02^{a}	NS
Acetoin	1307	0.24 ± 0.02^{c}	0.74 ± 0.17^{a}	0.63 ± 0.06^{ab}	0.30 ± 0.10^{bc}	0.46 ± 0.06^{abc}	0.0124
2-(Z)-Heptenal	1345	0.81 ± 0.02^{e}	1.79 ± 0.03^{c}	2.63 ± 0.06^{a}	1.56 ± 0.08^{d}	2.14 ± 0.03^{b}	<0.0001
Nonanal	1409	2.51 ± 0.38^{ab}	2.02 ± 0.16^{b}	2.62 ± 0.26^{ab}	2.93 ± 0.23^{ab}	3.29 ± 0.20^{a}	0.0287
2-(E)-Octenal	1451	0.85 ± 0.02^{d}	1.33 ± 0.01^{c}	1.75 ± 0.01^{a}	1.22 ± 0.06^{c}	1.58 ± 0.02^{b}	<0.0001

Furfural	1489	0.36 ± 0.02^{d}	2.49 ± 0.09^{a}	1.77 ± 0.01^{b}	1.14 ± 0.14^{c}	1.33 ± 0.15^{c}	<0.0001
Benzaldehyde	1562	1.74 ± 0.08^{b}	1.85 ± 0.06^{b}	2.34 ± 0.12^{a}	1.92 ± 0.12^{ab}	2.36 ± 0.16^{a}	0.0072
Phenylacetaldehyde	1682	0.23 ± 0.04^{b}	0.52 ± 0.09^{a}	0.61 ± 0.04^{a}	0.56 ± 0.03^{a}	0.51 ± 0.05^{a}	0.0056
2,4-Decadienal, (E,Z)-	1794	0.39 ± 0.00^{c}	0.60 ± 0.01^{a}	0.41 ± 0.07^{bc}	0.53 ± 0.01^{ab}	0.47 ± 0.02^{bc}	0.0070
2,4-Decadienal, (E,E)-	1838	1.10 ± 0.00^{c}	1.65 ± 0.01^{a}	0.98 ± 0.00^{d}	1.23 ± 0.05^{b}	1.10 ± 0.00^{c}	<0.0001
Heterocyclic compounds							
Maltol	1988	1.28 ± 0.07^{bc}	1.72 ± 0.06^{a}	1.09 ± 0.07^{c}	1.35 ± 0.06^{b}	0.76 ± 0.05^{d}	<0.0002
Pyrazine	1231	0.05 ± 0.00^{b}	0.14 ± 0.01^{a}	0.07 ± 0.01^{b}	0.07 ± 0.01^{b}	0.10 ± 0.03^{ab}	0.0044
2-Methylpyrazine	1286	0.27 ± 0.03^{c}	0.88 ± 0.08^{a}	0.37 ± 0.01^{bc}	0.42 ± 0.00^{bc}	0.43 ± 0.01^{b}	0.0001
2,5-Dimethyl-pyrazine	1343	0.14 ± 0.02^{b}	0.25 ± 0.03^{a}	0.12 ± 0.00^{b}	0.13 ± 0.00^{b}	0.15 ± 0.02^{b}	0.0039
2-Ethylpyrazine	1356	1.62 ± 0.12^{a}	1.53 ± 0.13^{a}	1.50 ± 0.08^{a}	1.68 ± 0.06^{a}	1.65 ± 0.05^{a}	NS
Terpenes							
β-Ocimene	1261	0.08 ± 0.01^{bc}	0.07 ± 0.03^{c}	0.13 ± 0.01^{ab}	0.09 ± 0.01^{bc}	0.17 ± 0.02^{a}	0.0064
β-Selinene	1769	ND	0.11 ± 0.01^{c}	0.17 ± 0.03^{c}	0.67 ± 0.05^{b}	1.26 ± 0.08^{a}	<0.0001
Others							
Dimethyl-disulfide	1087	0.33 ± 0.02^{bc}	0.80 ± 0.16^{a}	0.70 ± 0.12^{ab}	0.30 ± 0.02^{c}	0.46 ± 0.06^{abc}	0.0110
2-Pentylfuran	1240	0.98 ± 0.03^{c}	1.57 ± 0.00^{b}	1.58 ± 0.05^{b}	1.66 ± 0.06^{b}	2.63 ± 0.07^{a}	<0.0001
Formyl-5-ethylcyclopentene	1446	0.36 ± 0.05^{b}	0.38 ± 0.01^{ab}	0.38 ± 0.02^{ab}	0.40 ± 0.00^{ab}	0.47 ± 0.02^{a}	0.0450
2-Acetylfuran	1534	0.13 ± 0.02^{c}	0.31 ± 0.03^{a}	0.17 ± 0.01^{c}	0.20 ± 0.02^{bc}	0.28 ± 0.01^{ab}	0.0013
Unknown	1876	0.35 ± 0.00^{b}	0.43 ± 0.02^{a}	0.36 ± 0.01^{b}	0.38 ± 0.01^{ab}	0.36 ± 0.03^{b}	0.0113

Values are expressed as means ± standard deviation (for each batch: n = 3). Different letters within the same row mean significant differences among the samples according to Tukey HSD test ($p<0.05$)

*LRI: linear retention indexes obtained on a 60m VF-WAX capillary column. NS: not significant; ND: not detected.

7. GENERAL CONCLUSIONS

The case studies included in this research thesis have demonstrated the considerable potential of artichoke by-products (outer bracts, floral stems, and leaves) for the creation of functional ingredients and sustainable upcycled foods that could occupy an unfulfilled food market while addressing waste disposal concerns. Indeed, it was found that these by-products, often regarded as inedible, represent a low-cost raw material that is rich in biologically active compounds. The first case study – *Green recovery optimization of phenolic compounds from "Spinoso sardo" globe artichoke by-products using response surface methodology* - demonstrated that "Spinoso sardo" artichoke by-products, particularly stems and bracts, contain a considerable amount of polyphenols. The predominant constituents identified in these by-products were caffeoylquinic acid derivatives, including chlorogenic acid, 1,5-di-O-caffeoylquinic acid, and 3,5-di-O-caffeoylquinic acid, in descending order. In contrast, the leaves exhibited a prevalence of flavonoid compounds, with a notable concentration of luteolin 7-O-glucoside. However, this was due to the fact that the occurrence of compounds in different parts of the plant depends on their biological role. In order to valorize these by-products and, in particular, the phenolic compounds contained in these matrices, it is necessary to apply efficient extraction methods. These methods should take into account the requirements for environmental friendliness and the fact that these phenolic substances vary and are not uniformly distributed in the plant material. Consequently, in this study, the impact of maceration and ultrasound-assisted extraction (UAE) process variables, including the percentage of ethanol in the extracting solution and extraction time, on the green recovery of phenolic compounds from each artichoke by-product fraction was evaluated using

Response Surface Methodology. From the investigation and optimization of the independent variables within the selected design space affecting extraction efficiency, it was found that the maximum yields of polyphenols and flavonoids were maintained at the lowest extraction time for both maceration (60 min) and ultrasound-assisted extraction (10 min). The UAE (10 min) extraction time was found to be optimal for all samples, except for the sonicated bracts, which required approximately 41 min. The intermediate ethanol percentages (42%-64%) were also found to be optimal for both techniques, except for sonicated leaves, which required a lower percentage (20%). Under optimum conditions, although maceration gave higher extraction efficiencies, UAE provided comparable results, often reducing the time required and the ethanol consumption, as well as energy consumption and the environmental impact. It was also found that the two extraction methods were able to extract the same compounds. Therefore, in order to assess the feasibility of incorporating these extracts into food matrices, the most cost-effective and environmentally sustainable extraction methods with the highest polyphenol recovery for each fraction were identified.

In fact, with this rationale, in the second case study - *Are artichoke by-product extracts a viable alternative for shelf-life extension? A feasibility study on breadsticks* - the bract and stem extracts obtained by UAE and leaf extracts prepared via maceration were selected for breadsticks fortification. Two different amounts (1000 and 2000 ppm) of extracts obtained from each by-product fraction were added to increase the shelf-life of a this widely consumed bakery product, in line with the main objective of this thesis work, which was to reduce food waste and fruit and vegetable loss. Indeed, the results showed that the extracts added as they were, without any stabilization, improved the oxidative stability of breadsticks, which are normally subject to the oxidation of the lipids that are highly concentrated in them. Fortified breadsticks, especially at high levels of addition and with

stem extract, showed a longer estimated shelf-life and higher antioxidant capacities, with slight deterioration in texture and no apparent changes in color. The rheological data of the doughs demonstrated that the extracts of bracts and stems did not impact the stickiness, extensibility, and resistance to extension of the doughs, thereby confirming the feasibility of incorporating these artichoke extracts into these snacks. Conversely, the addition of the leaf extract at both levels resulted in an increase in dough stickiness, which could potentially lead to challenges during the production process. Critical issues observed in this second case study included not only the loss of breadstick friability associated with an increase in the moisture content of the final product, but also the limited stability of the phenolic compounds in these extracts after the baking process. In fact, these extracts would require stabilization interventions such as lyophilization or microencapsulation to achieve more promising results, but the production costs would be higher.

On the basis of this, in the third case study - *Artichoke by-products: Promising ingredients for breadstick fortification* - the breadsticks were fortified with two different concentrations (3 and 5 %) of freeze-dried powders of artichoke outer bracts and stems. The decision not to use leaf powders was based on the outcomes of preliminary laboratory tests, which showed a notable decline in the sensory properties of the breadsticks, largely attributed to their high concentration of bitter compounds. The use of powders derived from by-products, which are an even more cost-effective raw material, has not only improved the nutritional profile, but also the texture of the final product, especially when stems are added at higher fortification levels. Both by-products utilized for fortification exhibited high polyphenol, dietary fiber, and ash contents. Stems, in accordance with previous case studies, exhibited the highest polyphenol and flavonoid contents, while bracts demonstrated the highest concentration of dietary fiber. Moreover, a notable

increase in antioxidant capacity, ash, and dietary fiber contents was observed in all fortified samples in comparison to the control. It is noteworthy that all supplemented breadsticks could be labeled as "high fiber," as they exceed the minimum fiber level (6 g/100 g) required by the European Commission. The fortification did not result in a consistent alteration of the breadsticks color; however, it did affect the texture of the final product, reducing the plasticity in favor of a brittleness enhancement, which is a desirable property in this type of snacks. Conversely, from a sensory perspective, consumers identified a grainy texture, a bitter taste, herbaceous flavour, and astringent sensations in the fortified samples than in the conventional product. Nevertheless, despite a slight decline in the overall liking score, breadsticks supplemented with artichoke by-products were perceived as beneficial to health and sustainability. This is encouraging, particularly in light of the growing awareness of food waste and consumer preference for products with these characteristics. Therefore, artichoke by-products represent a promising upcycled ingredient for the development of novel functional bakery products that could meet a growing market.

In conclusion, the results of this thesis indicate that the upcycling of by-products and the valorization strategies analyzed have proven to be of significant interest, not merely from an environmentally conscious standpoint but also in terms of social and economic considerations. Indeed, the utilization of these raw materials (especially outer bracts and floral stems), which would otherwise be discarded, as innovative ingredients within food intended for human consumption would not only enhance the competitiveness of the artichoke supply chain, but also benefit snack producers, who would be able to offer a more durable product with a superior nutritional profile, capable of meeting the demands of an ever-widening segment of consumers.

However, given the complexity of plant matrices, the food supply chain, and consumer behavior, further studies are necessary to validate the results obtained and to assess whether these different enhancement strategies are effectively economically and environmentally sustainable.

8. POSTER COMMUNICATION

Use of bioactive and functional substances obtained from food industry waste, such as artichoke and myrtle by-products for food products fortification

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Tutor: Prof. Alessandra Del Caro

The aim of this PhD project is to optimize the recovery of bioactive compounds from artichoke (stems, leaves and bracts) and myrtle liqueur by-products (spent myrtle berries), to develop functional ingredients and to enrich food products. For this purpose, both matrices were subjected to conventional (maceration) and ultrasound assisted extraction (UAE), with a food-grade solvent (ethanol:water). Response surface methodology (RSM) was used to design the experiments and to reduce the number of trials for specific factor and its level. The effects of independent variables at different levels, like time, ethanol-water ratio and ultrasound power on total polyphenol (TPC) and flavonoid content (TFC) were studied. Finally, RSM was used to predict the optimal conditions for achieving highest yield, while minimising extraction time.

Materials & methods

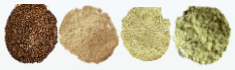

Figure 1. Powders of spent myrtle berries (MB), artichoke stems (S), leaves (L), and bracts (B).

Dry powders

Solid-liquid extraction

UAE
40 kHz; Room Temperature; 1:20 (g/mL)

Maceration
38 ± 2°C; 140 rpm; 1:20 (g/mL)

Optimization

Artichoke and spent myrtle berries dry powders were subjected to conventional (maceration) and ultrasound assisted extraction (UAE) according to three different 2^3 factorial Central Composite Designs (CCDs):

1. Maceration was conducted to investigate the effects of extraction time (60; 120; 180 min) and ethanol (EtOH) concentration (20; 50; 80 %) on TPC and TFC content;
2. UAE was performed with 50% EtOH:H_2O, which resulted the best percentage for TPC and TFC extraction during maceration, varying the sonication time (10; 50; 90 min) and ultrasonic power (48; 144; 240 W);
3. The third CCD was carried out only in the artichoke by-products (given the lack of significance achieved with the previous UAE CCD), varying the sonication time (10; 50; 90 min) and EtOH:H_2O ratio (20; 50; 80 %) at a constant power of 144 W.

The TPC and TFC were expressed as mg equivalents of Gallic Acid (GAE) per 100 g of d.m. and as mg of Catechin Equivalent (CE) per 100 g of d.m. respectively.

Results & discussion

The regression equations of the models used for optimisation that achieved significance (p-value < 0.05) at the ANOVA and how each factor (X1 and X2) affects the response (Y) examined are reported in Table 1. From the coefficients of the equations obtained, it can be seen that on artichoke by-products, ethanol content affects the response more than extraction time, except for TPC bracts in the UAE.

Table 1. Regression equations of significant models used for process optimization

Extraction method	Y	By-product fraction	X_1	X_2	Regression Equation	R^2
Maceration	TPC	Stems	% Ethanol	Time	$Y= +2258.58 + 24.16\ X_1 - 436.46\ X_1^2$	0.88
Maceration	TFC	Stems	% Ethanol	Time	$Y= +1766.10 + 274.02\ X_1 - 601.75\ X_1^2$	0.92
Maceration	TPC	Leaves	% Ethanol	Time	$Y= +1500.47 - 63.19\ X_1 - 209.24\ X_1^2$	0.78
Maceration	TPC	Bracts	% Ethanol	Time	$Y= +1694.58 - 17.61\ X_1 - 15.66\ X_2 + 99.85\ X_1 X_2 - 404.02\ X_1^2$	0.94
Maceration	TFC	Bracts	% Ethanol	Time	$Y= +1002.57 - 82.74\ X_1 - 353.46\ X_1^2$	0.85
UAE	TPC	Stems	% Ethanol	Time	$Y= +2613.34 - 128.87\ X_1 - 229.11\ X_1^2$	0.81
UAE	TPC	Leaves	% Ethanol	Time	$Y= +1586.28 - 155.38\ X_1 + 70.66 X_2$	0.49
UAE	TPC	Bracts	% Ethanol	Time	$Y= +1966.85 + 12.24\ X_1 + 169.34\ X_2 - 151.54\ X_1^2$	0.84
UAE	TFC	Bracts	% Ethanol	Time	$Y= +969.30 + 230.76\ X_1 + 64.74\ X_2$	0.59
Maceration	TPC	Myrtle Berries	% Ethanol	Time	$Y= +4595.43 + 186.99\ X_1 + 18.51\ X_2 - 46.62\ X_1X_2 - 399.15\ X_1^2 - 60.73\ X_2^2$	0.94
UAE	TPC	Myrtle Berries	Power	Time	$Y= +1110.89 - 30.36\ X_1 + 10.80\ X_2 - 54.57\ X_1X_2 - 75.69\ X_1^2 - 30.25\ X_2^2$	0.75

The regression equations showed a significant effect of factors on the Y response, a not significant Lack of fit and an adequate predictive accuracy.
The optimal parameters to maximize responses and minimize extraction time in artichoke by-products were:
- 60 min and 53% of EtOH for Stems, 60 min and 45% for Leaves, and 60 min and 50% for Bracts, during maceration;
- 10 min and 42% of EtOH for Stems , 10 min and 20% for Leaves, and 41 min and 64% of EtOH for Bracts, during UAE.

A t-test carried out to find significant differences between maceration and UAE TPC and TFC data showed that:

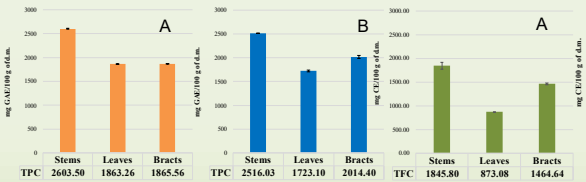

Figure 2. TPC obtained by maceration (A) and UAE (B)

Figure 3. TFC obtained by maceration (A) and UAE (B)

- in leaves the maceration conducted to a significant higher yield extraction for both TPC and TFC;
- in bracts the macerations significantly increased the TFC content, while UAE was found more efficient for TPC extraction ($p < 0.05$);
- in stems no significant differences between the two extraction methods were found for the TFC, while the TPC was significantly higher when maceration was used.

Conclusion: Overall, under our operating conditions, although the significance achieved by the t-test for the maceration in the majority of by-products, the choice to use UAE might be more convenient in terms of both time and ethanol percentage used.

Activities to be achieved:
- Optimization of extractions on spent myrtle berries;
- Characterization of extracts obtained with optimal conditions (antioxidant activity, HPLC polyphenol identification and quant ification);
- Physico-chemical, technological, nutritional and sensory characterization of food products enriched with the optimal extracts.

Aknowledgment : study performed within the project GOOD-BY-WASTE (Obtain GOOD products-reduce WASTE) - PRIN 2017.

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9. OTHER WORKS

During the course of my doctoral studies, I was afforded the opportunity to engage in other research projects that served to enhance my knowledge of baked goods, with a specific focus on gluten-free product innovation. As a result of these research activities, two scientific papers were produced.

foods

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Article

Effect of Substitution of Rice Flour with Quinoa Flour on the Chemical-Physical, Nutritional, Volatile and Sensory Parameters of Gluten-Free Ladyfinger Biscuits

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Abstract: The present study investigates the effect of partial or total substitution of rice flour (RF) with quinoa flour (QF) (at 25%, 50%, 75% and 100%) on the chemical-physical, nutritional, and sensory characteristics, as well as the volatile compounds, of ladyfinger biscuits. All quinoa-based formulations positively affected the crust colour, endowing it with lower 'lightness' and higher 'redness' values, giving the biscuits a more appealing crust colour. Biscuits with higher percentages of QF also had better structure, as they were softer. The substitution of RF with QF significantly improved the nutritional profile of the biscuits, as a result of the increase in protein, lipid, ash, total soluble (SP) and insoluble polyphenol (IP), flavonoid, and antioxidant activity levels, which increased linearly with the substitution rate. Quinoa supplementation led to an increase in volatile compounds that were nearly always characterised by positive olfactory attributes. Sensory analysis revealed that the maximal substitution rate of QF able to maintain an adequate consumer acceptability rating is probably 50%, as higher percentages impaired acceptability due to the presence of herbaceous and bitter tastes, even if the consumers also rated these samples as healthier and softer to touch.

Keywords: volatile compounds; gluten-free biscuits; nutritional value; polyphenols; quinoa flour

1. Introduction

A strict gluten-free (GF) diet is followed by many people who do not suffer from celiac disease nowadays. The exclusion of gluten is considered, by many, to be a healthy habit or a way to prevent the onset of celiac disease [1]. Thus, the food industry is continuously increasing the number of new cereal-based GF foods to offer consumers, whilst continuing to face the well-known technological, nutritional, and sensory problems characteristic of these products [2–5].

Of the different types of GF bakery foods, biscuits are of great importance from a commercial standpoint [6] due to their ease of use, adequate nutritional content, the wide selection of biscuit types available, their long shelf-life, and their relatively low selling price [7]. Moreover, fewer technological problems are encountered in biscuit production compared with bread or pasta production, as the build-up of gluten is not of fundamental importance, and thus the replacement of gluten-containing flours with GF counterparts is simpler [8]. Nonetheless, at least two major problems hamper the production of GF bakery products: inadequate sensorial acceptability; and an unbalanced nutritional profile resulting from the lower content of several important nutrients, such as minerals (iron, zinc, magnesium and calcium), dietary fibre, and vitamins (folate and B12) [3–5]. Moreover, GF baked

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Honey as a Sugar Substitute in Gluten-Free Bread Production

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Abstract: In recent years, there has been a significant focus on enhancing the overall quality of gluten-free breads by incorporating natural and healthy compounds to meet consumer expectations regarding texture, flavor, and nutritional value. Considering the high glycemic index associated with gluten-free products, the use of honey, renowned for its numerous health benefits, may serve as an optimal alternative to sucrose. This study investigates the impact of substituting sucrose, either partially (50%) or entirely (100%), with five Sardinian honeys (commercial multifloral honey, cardoon, eucalyptus, and strawberry tree unifloral honeys, and eucalyptus honeydew honey), on the rheological properties of the doughs and the physico-chemical and technological properties of the resulting gluten-free breads. The results demonstrated that an optimal balance was achieved between the leavening and viscoelastic properties of the doughs and the physical and textural attributes of the resulting breads in gluten-free samples prepared with a partial substitution of cardoon and multifloral honeys. Conversely, the least favorable outcomes were observed in samples prepared with strawberry tree honey and eucalyptus honeydew honey at both substitution levels. Therefore, the different behavior observed among all honey-enriched gluten-free breads was likely attributable to the distinct botanical origins of honey rather than to the substitution percentages employed.

Keywords: gluten-free bread; honey; physical properties; rheological properties; sucrose

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1. Introduction

Gluten represents a protein ingredient of paramount importance for the production of different items due to its viscoelastic properties, which are particularly relevant for bakery foods and specifically in bread, where it is responsible for the final porous structure [1]. The specific composition of gluten, which is characterized by a high content of prolamines and glutenins, facilitates the formation of a three-dimensional network, resulting in dough with unique properties of elasticity and resistance [2]. However, this same composition can also elicit allergic reactions or autoimmune disorders, collectively known as gluten-related disorders (GRDs). The most prevalent of these is celiac disease [3]. Intolerance to gluten can be avoided only with a long-life gluten-free (GF) diet. Consequently, it is necessary to eliminate from the diet all foods containing wheat, rye, barley, and oats, as well as any products that may contain gluten. The market for GF products is constantly upsurging, driven by an increase in the number of individuals diagnosed with GRDs and the number of consumers adopting a GF diet [4].

However, despite the extensive academic and industrial research for the development of GF products, especially bread, the quality of the commercially available products is lower than that of their gluten-containing counterparts. Indeed, GF bread is characterized by its lack of volume and texture, as well as a diminished nutritional value and an accelerated rate of staling [5]. The basic flours used in the production of GF breads include rice, corn, sorghum, and other GF cereals and starches, as well as pseudocereal flours, such as amaranth [6,7] and buckwheat [8], and legumes, such as chickpea [9] and soybean [10]. Moreover, in order to obtain a sufficient volume, crumb softness, and

10.ACKNOWLEDGEMENTS

This thesis represents the result of a portion of the research activities conducted as part of the MUR-PRIN research project entitled "GOOD-BY-WASTE, Obtain Good Products, Exploit By-Products, Reduce Waste". The principal goal of this research project was the creation of innovative circular economy models to enhance the sustainability of the supply chains of selected high-quality agri-food products originating from the southern regions of Italy.