Comprehensive Nutritional, Chemical, and Bioactive Profiling of Watercress for the Development of Functionalized Pastas

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for the Master's Degree in Chemical Engineering

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This project work does not include the criticisms and suggestions made by the Jury

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ABSTRACT

Functional foods play an essential role in promoting health by helping to reduce the risk of chronic degenerative and inflammatory diseases, as well as metabolic disorders. The inclusion of watercress (*Nasturtium officinale* R. BR.) in food products can significantly enhance their nutritional value. This vegetable is notable for its abundance of glucosinolates, carotenoids, polyphenols, and essential minerals, in addition to its traditional medicinal uses. Incorporating watercress into functional foods aligns with the trend of integrating health and nutrition, offering a unique combination of nutritional and functional benefits for the development of innovative food products.

The primary objective of this thesis was to evaluate the inclusion of watercress in food products by analyzing its physical (texture, water activity, color, weight loss, and flour viscosity), nutritional (moisture, ash, total fat, crude protein, and energetic value), and chemical (free sugars, organic acids, fatty acids, mineral elements, phenolic and glucosinolate compounds) properties. The development of new pasta enriched with watercress was conducted using either freeze-dried or fresh watercress as an ingredient in substitutions of 1.5%, 3%, and 5% of wheat flour, and the final product was analysed in both raw and cooked forms. A negative control (100% wheat flour) and a sample of fresh commercial pasta were also included in the study.

The incorporation of watercress affected the color parameters of the pasta, reduced solids loss during cooking, and altered weight gain and viscosity profiles. Texture changes were observed, influenced by water retention and gluten development. Moreover, there were modifications in the nutritional composition, enriching the pasta with proteins, lipids, and bioactive compounds. The retention of bioactive compounds, particularly *C*-glycosylated apigenins and fatty acids, was impacted by the processing methods. Thriteen glucosinolates were found in watercress extract with indicative of the large presence of gluconasturtiin. These compounds are also expected to contribute to the functional profile of the pasta developed.

The integration of watercress into pasta formulations represents a promising approach to developing functional pasta products with enhanced sensory, nutritional, and health-promoting attributes.

Keywords: Functional foods, Watercress, Pasta formulation, Bioactive compounds, Health-promoting effects

RESUMO

Os alimentos funcionais desempenham um papel essencial na promoção da saúde, ajudando a reduzir o risco de doenças crónicas degenerativas e inflamatórias, bem como distúrbios metabólicos. A inclusão de agrião (*Nasturtium officinale* R. BR.) em produtos alimentares pode aumentar significativamente o seu valor nutricional. Este vegetal é reconhecido pela sua abundância em glucosinolatos, carotenoides, polifenóis e elementos minerais, para além dos seus usos medicinais tradicionais. A incorporação de agrião em alimentos funcionais alinha-se com a tendência de integrar saúde e nutrição, oferecendo uma combinação única de benefícios nutricionais e funcionais para o desenvolvimento de produtos alimentares inovadores. O principal objetivo desta tese foi avaliar a inclusão de agrião em produtos alimentares, analisando as suas propriedades físicas (textura, atividade da água, cor, perda de peso e viscosidade da farinha), nutricionais (humidade, cinzas, gordura total, proteína bruta e valor energético) e químicas (açúcares livres, ácidos orgânicos, ácidos gordos, elementos minerais, compostos fenólicos e glucosinolatos). O desenvolvimento de uma nova massa enriquecida com agrião foi realizado usando agrião liofilizado e fresco como ingrediente, com substituições de 1,5%, 3% e 5% de farinha de trigo, e o produto final foi analisado tanto cru como cozido. Um controlo negativo (100% de farinha de trigo) e uma amostra de massa comercial fresca também foram incluídos no estudo. A incorporação de agrião afetou os parâmetros de cor da massa, reduziu a perda de sólidos durante a cozedura e alterou os perfis de ganho de peso e viscosidade. Foram observadas alterações na textura, influenciadas pela retenção de água e desenvolvimento do glúten. Além disso, houve modificações na composição nutricional, enriquecendo a massa com proteínas, lipidios e compostos bioativos. A preservação dos compostos bioativos, particularmente apigeninas *C*-glicosiladas e ácidos gordos, foi afetada pelos métodos de processamento. Treze glucosinolatos foram identificados no extrato, com indicativo da grande presença de gluconasturtiin. Espera-se que estes compostos também contribuam para o perfil funcional das pastas desenvolvidas.

A integração de agrião em formulações de massa representa uma abordagem promissora para o desenvolvimento de produtos de massa funcionais com atributos sensoriais, nutricionais e promotores de saúde aprimorados.

Palavras-chave: Alimentos funcionais, Agrião, Formulação de massa, Compostos bioativos, efeitos promotores de saúde

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1. PREFACE

This project was conducted to be submitted to the Polytechnic Institute of Bragança for the attainment of a master's degree in Chemical Engineering.

The inclusion of watercress (*Nasturtium officinale*) in food products represents a significant step forward in the search for innovative and sustainable solutions in the food industry. This vegetable, known for its nutritional properties and health benefits, is emerging as a valuable food supplement, especially due to its rich composition of bioactive compounds. In today's context of growing concern for public health and sustainability, the exploitation of plant by-products such as watercress not only contributes to the valorization of high value-added foods, but also to the mitigation of food losses and waste.

Watercress stands out for its abundant content of glucosinolates, carotenoids, polyphenols, vitamins C, A and E (α-tocopherol), folic acid, as well as essential minerals such as iron, calcium, iodine. Studies show that this vegetable is also a rich source of tannins, flavonoids, terpenoids and other phytochemicals, including lutein and zeaxanthin, which have been evidenced to have a crucial role in maintaining eye health. Traditionally, watercress leaves have been used in folk medicine for their diuretic, expectorant and hypoglycemic properties and in the treatment of numerous chronic diseases, suggesting a therapeutic and preventive value.

The growing trend of developing functional foods is a response to the need to integrate health and nutrition. These foods, which go beyond basic nutritional functions, exert significant biological activity, contributing to the reduction of the risk of chronic-degenerative diseases, inflammatory conditions, and metabolic disorders. The incorporation of proteins, fibers, and antioxidants, the reduction of fat or simple sugar content in food products or the modulation of their glyceamic index are strategies that have been explored to create functional foods. In this scenario, watercress presents itself as a promising ingredient for the formulation of such products, offering a combination of nutritional and functional benefits.

This master's thesis explores in detail the multiple beneficial impacts of watercress when incorporated into food products, providing a comprehensive overview of its nutritional properties and potential applications in the food industry. The aim is not only to highlight the health benefits associated with watercress consumption, but also to

encourage the adoption of sustainable and innovative practices in the development of new food products. We therefore hope that this research can contribute to the advancement of knowledge and inspire new initiatives in the field of functional foods.

2. STATE-OF-THE-ART

2.1. WATERCRESS AS SOURCE OF NUTRIENTS AND BIOACTIVES

2.1.1. Nasturtium officinale R. Br.

The term "Brassicas" refers to a genus of plants belonging to the Brassicaceae family, also known as Cruciferae. The consumption of vegetables from the Brassicaceae family is beneficial for human health due to their richness in sulphur-containing compounds known as glucosinolates. These compounds, which impart a pungent aroma and a spicy or bitter taste, include PEITC, which is linked to reducing cancer risk and combating oxidative stress (Yazdanparast et al., 2008). Vegetables in this genus are agriculturally significant and play a crucial role in human nutrition as the primary dietary source of glucosinolates and other bioactive molecules. Extensive research has been conducted on the content and properties of glucosinolates in these vegetables (Cabello-Hurtado, 2012).

Watercress (*N. officinale* R. Br., **Figure *1***) is a semi-aquatic plant from the Brassicaceae family that is widely used in Mediterranean cuisine. It has a sharp, peppery, and slightly spicy flavour and is eaten raw in salads and cooked, mainly in soups.

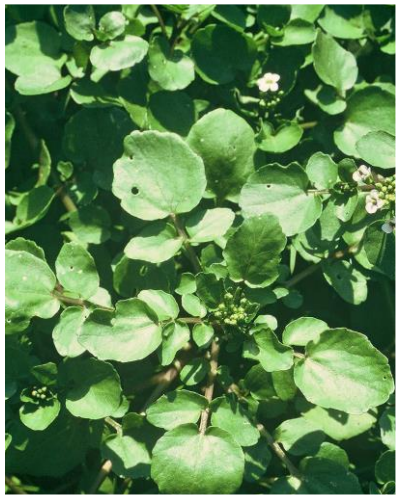


Figure 1. *Nasturtium officinale* R. Br. (Source: https://flora-on.pt/#).

Low in fat and sodium, this low-calorie food is rich in vitamin B_9 (folic acid), vitamin C (ascorbic acid) and vitamin K (phylloquinone). It also contains bioactive compounds such as flavanols, hydroxycinnamic acids, and glucosinolates, mainly phenethyl glucosinolate (Pinela et al., 2020). Watercress is currently grown in several countries and marketed as a minimally processed product. The demand for this vegetable has exponentially increased since the recognized its health benefits, parallelly to the phenomena of consuming food products with different organoleptic characteristics (Pinela et al., 2020).

2.1.2. Health benefits associated with watercress consumption

Watercress is an important food supplement, widely used in salads, juices, flavorings or garnishes in various dishes (Chaudhary, 2018).

The nutritional value of a food is determined by its proximal composition, free sugar and fatty acid profiles, as well as the amounts of vitamins and minerals, and the presence of bioactive compounds. Therefore, the presence of anti-nutrients also influences nutritional value, as these compounds can reduce the bioavailability of certain nutrients (Greenfield et al., 2003). Food constituents can therefore be classified into different categories, including macronutrients such as proteins, carbohydrates, lipids and macrominerals; micronutrients such as vitamins and trace elements; non-nutrients such as phenolic compounds and glucosinolates, and finally, antinutrients such as oxalic and phytic acids (Pinela et al., 2020).

Watercress is rich in essential nutrients such as vitamins C, A, E (α-tocopherol) and folic acid and minerals including iron, calcium, and iodine (Yazdanparast, 2008). Besides nutrients, this vegetable also contains a wide variety of phytochemicals, including the primarily hydrophilic phenolic compounds such as tannins and flavonoids, and the glucosinolate group represented by the phenylethyl isothiocyanate (PEITC), as well as lipophilic ones comprising carotenoids and other terpenoids, and chlorophylls. Those phytochemicals have been recognized as bioactive compounds. For instance, carotenoids such as lutein and zeaxanthin, have been shown to have an important role in eye health. These macular carotenoids help to protect the eyes from harmful blue light and oxidative damage, reducing the risk of age-related macular degeneration (AMD) and cataracts (Wong et al., 2022).

Watercress can be harvested in its natural habitat and used in both fresh and dried forms. Its leaves have traditionally been used in folk medicine as a diuretic, expectorant, hypoglycemic and in the treatment of various chronic diseases (Clemente et al., 2019). More recently, watercress has also been associated with heart health due to its ability to improve endothelial function and reduce blood pressure. The nitrates present in watercress can help dilate blood vessels, improving blood flow and reducing the risk of hypertension. Additionally, the antioxidants and anti-inflammatory properties attributed to watercress consumption may contribute to the prevention of atherosclerosis, a condition characterized by the buildup of plaque in the arteries (Burke 2019).

Özen (2009) demonstrated that aqueous and ethanolic solutions of watercress extract act as novel antioxidants, combating lipid peroxidation in linoleic acid homogenate, liver, brain and kidney model systems. Watercress has been shown to have significant antioxidant effects, reducing cellular lipid peroxidation, superoxide anion and free radical scavenging activities. In addition, daily intake of watercress decreased DNA damage and increase the antioxidant status of blood in healthy adults (Aires et al., 2013).

Watercress leaves have been found to contain 14 phenolic compounds, including gallic acid, coumaric acid derivatives, ferulic acid, apigenin, proanthocyanidin B1, sinapic acid, hydroxybenzoic acid, coumaric acid, caffeoyl malic acid, caftaric acid, kaempferol and quercetin glycosides. Several pharmacological studies have confirmed the antioxidant, antibacterial, anticancer, antipsoriatic, anti-inflammatory, cardioprotective, hepatoprotective and antigenotoxic effects of watercress (Zeb et al., 2018).

Watercress demonstrates antitumor activity and can interfere with several axes, including oxidative stress, apoptosis, cell cycle progression and mitogen-activated protein kinase (MAPK) pathways (Wu et al., 2009). Several studies suggest that diets rich in brassica plants can reduce the risk of lung cancer, colorectal carcinoma and prostate cancer (Kumar et al., 2009). These plants are rich in glucosinolates, which are converted into isothiocyanates (ITCs) by human intestinal microflora or in plants. ITCs are characterized as important chemopreventive factors in the control of carcinogenesis and have shown strong protective effects against tumours in experimental models (Sakao et al., 2015).

Watercress leaves are commonly used both as food and for their medicinal properties, acting as an anti-inflammatory, diuretic, expectorant, hypoglycemic, antihypertensive, as well as being used in the treatment of urinary tract infections and

cardiovascular diseases (Shahani et al., 2017). Research conducted by Sadeghi et al. (2014) demonstrated the anti-inflammatory potential of the hydroalcoholic extract of the aerial part of *Nasturtium officinale R. Br.* in different animal models of inflammation.

2.1.3. Glucosinolate composition in watercress

Glucosinolates are secondary metabolites of plant origin that contain sulfur (**Figure *2***); mainly found in the *Brassicaceae* family such as watercress, cabbage, broccoli, among others, and in a limited number of other plant families. In recent decades, the importance of these molecules has increased following the discovery of their potential as cancer prevention agents, crop protection compounds, and biofumigants in agriculture (Halkier, 2006).

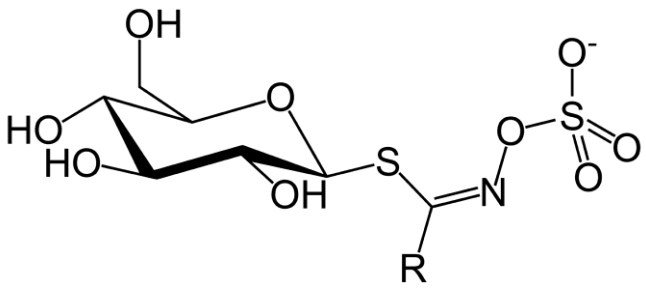


Figure 2. Base structure of glucosinolates.

Glucosinolates are characterized by a sulfated core with an isothiocyanate group, which is conjugated to thioglucose, and an additional R group. Both glucose and the central carbon of the isothiocyanate are frequently subject to further modifications, leading to a wide array of diverse glucosinolate structures. According to Clarke (2010), the reported number of glucosinolates reached approximately 200. These compounds are intrinsically linked to thioglucosidase enzymes, commonly referred to as myrosinases. When plant tissues are subjected to damage, glucosinolates undergo hydrolysis, a process catalyzed by myrosinases.

Myrosinase is a thioglucosidase stored in specialized myrosinase cells found in all plant organs, and they play a fundamental role in this process. When there is some kind of damage to plant tissue, such as that caused by chewing insects, there is contact between the glucosinolates stored in the vacuole and myrosinase. As a result of the enzymatic activity of myrosinase, glucose and sulphate are released, along with the formation of a

variety of toxic and pungent chemicals, including isothiocyanates, nitriles and oxazolidinetiones (Hopkins, 2009). These products manifest a broad spectrum of biological activities, encompassing both beneficial and detrimental nutritional properties. Moreover, they play a pivotal role in mediating interactions between plants and herbivores. (Mithen, 2001).

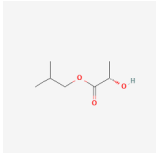
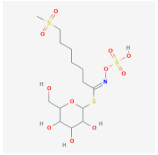
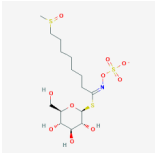
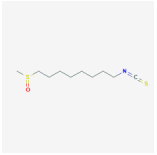
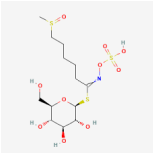
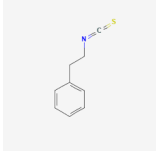
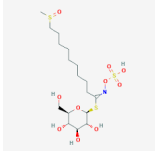
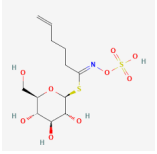
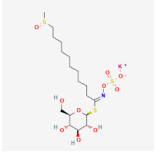
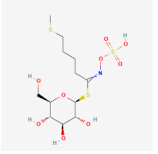
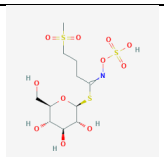
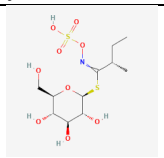
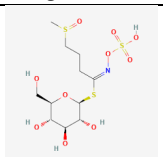
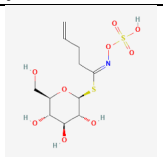
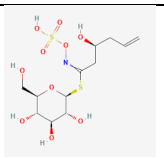
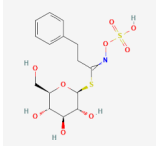
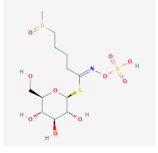
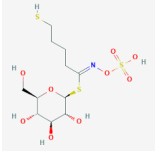
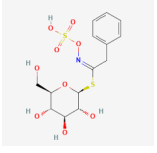
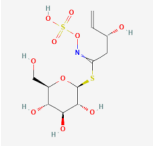
Isothiocyanates are recognized for their potent anticarcinogenic and antioxidant effects, making them valuable in cancer prevention and general health maintenance (Fimognari, Lenzi, & Hrelia, 2008). Nitriles, on the other hand, possess potential antimicrobial and pesticidal properties, with applications in agriculture and food preservation (Poveda, Eugui, & Velasco, 2020). Thiocyanates have demonstrated their role in modulating inflammation and immune response, holding promise in treating inflammatory conditions. Collectively, these bioactive compounds underscore the multifaceted nature of plant-microbe-herbivore interactions and their significant implications in both human health and ecological systems (Poveda, Eugui, & Velasco, 2020).

The profile of glucosinolates in watercress and related plants genus, has already been extensively described in the literature (Rose et al., 2000; Ji & Morris, 2003; Song et al., 2005; Kyriakou et al., 2012; Liang et al., 2018; Castellaneta et al., 2022). **Table 1** shows the most representative glucosinolates found in watercress. The abundance of glucosinolates in watercress can be attributed to the plant's adaptive response to environmental stressors, such as herbivore attacks and unfavourable growing conditions. Glucosinolates serve as a defence mechanism for the plant, deterring herbivores with their pungent taste and aroma (Winde & Wittstock, 2011).

Watercress offers a diverse array of glucosinolate compounds, with specific types including gluconasturtiin, glucoerucin, and glucotropaeolin. What sets watercress apart from other cruciferous vegetables is not just its glucosinolate content but also the balanced and synergistic combination of different types of these compounds (Ağagündüz et al., 2022). This variety enhances the potential health benefits, as different glucosinolates have varying bioactive properties. It's essential to enjoy this cruciferous green as part of a balanced diet to harness its full health potential. While watercress is indeed a glucosinolate powerhouse, it's essential to acknowledge that its health benefits are best achieved when combined with a diverse and balanced diet. A diet rich in various cruciferous vegetables can help optimize the intake of different types of glucosinolates,

enhancing their potential protective effects. Watercress's contribution to a wholesome diet underscores the importance of incorporating a wide array of vegetables to maximize the diverse array of nutrients and bioactive compounds they offer.

Table 1. Most representative glucosinolates found in watercress.

				
2-methylpropyl GSL Castellaneta et al., 2022	**6-Methylsulfinyhexyl** Rose et al., 2000	**7-Methylsulfinyheptyl** Rose et al., 2000	**8-Methylsulfinyloctyl** Rose et al., 2000	**Glucoalyssin** Song et al., 2005
				
2-phenethyl isothiocyanate Ji & Morris, 2003	**Glucoarabin** Kyriakou et al., 2012	**Glucobrassicanapin** Liang et al., 2018	**Glucocamelinin** Kyriakou et al., 2012	**Glucoerucin** Castellaneta et al., 2022
				
Glucocheirolin Liang et al., 2018	**Glucocochlearin** Castellaneta et al., 2022	**Glucoiberin** Song et al., 2005;	**Gluconapin** Castellaneta et al., 2022	**Gluconapoleiferin** Castellaneta et al., 2022
				
Gluconasturtiin Song et al., 2005	**Glucoraphanin** Song et al., 2005	**Glucosativin** Liang et al., 2018	**Glucotropaeolin** Kyriakou et al., 2012	**Progoitrin** Castellaneta et al., 2022

Watercress is the richest source of gluconasturtiin, which on hydrolysis produces phenethyl isothiocyanate (Palaniswamy et al., 2003). Gluconasturtiin (2-phenethyl glucosinolate) and its hydrolysis product, 2-phenethyl isothiocyanate, were shown to play a role in suppressing tumor growth. However, the use of *N. officinale* as a source of herbal medicines is currently limited due to insufficient genomic and physiological information (Jeon et al., 2017). The compound 2-phenethyl isothiocyanate has also been receiving attention for its role in reducing carcinogenic activation by inhibiting phase I enzymes (such as cytochrome P450s) and its potential to induce phase II enzymes. This product can effectively inhibit tumorigenesis by increasing the metabolism and increasing the excretion of the lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, as demonstrated in animal and human studies. By consuming 30 grams of raw watercress,

which contains 21.6 mg of gluconasturtiin, a study conducted on *in vivo* urinary excretion demonstrated the conversion potential to produce 2.3-5.0 mg of 2-phenethyl isothiocyanate (as observed in the research by Kopsell et al., 2007). This finding underscores the remarkable potential of glucosinolates within this plant.

Due to their unique properties, glucosinolates have been used since ancient times for many different purposes, culinary uses, traditional medicine, and even biological control:

1) **Culinary Uses:** One of the most common traditional applications of glucosinolate-rich plants is in culinary preparations. The cruciferous vegetables are integral components of many cuisines around the world (Melrose, 2019), that add depth and zest to dishes due to the pungent and often bitter flavors of glucosinolates. Mustard-flavor is one of the most representative examples of glucosinolates-induced flavour to culinary science;
2) **Traditional Medicine:** In some cultures, certain cruciferous plants have been used in traditional medicine for their potential health benefits. Ancient herbal remedies have employed these vegetables as a source of natural medicine. For example, they have been utilized to treat various ailments, including digestive issues and respiratory problems (Abbaqui et al., 2018; Miękus, et al., 2020);
3) **Biological Pest Control:** Some glucosinolates, particularly those in horseradish and mustard plants, have insecticidal properties. Historically, these plants were cultivated alongside others to protect them from insect damage. These natural pesticides discouraged herbivores and served as an early form of biological pest control (Vig et al., 2009; Chhajed et al., 2020).

While modern science has provided deeper insights into the biochemistry and potential health benefits of glucosinolates, their traditional uses highlight the long history of humans exploiting the power of these compounds in various aspects of life, from culinary arts to folk medicine and agricultural practices. Understanding their importance in nutrition and health is enriched by this historical background, leveraging their potential application in many fields of study.

Modern applications of glucosinolates include a broad spectrum of fields, highlighting their versatility and significance, but also reflect a growing understanding of their diverse properties and potential contributions to various industries, from nutrition

and health, to pharamaceuticals, agriculture, food preservation flavor and aroma, to cosmetics, and even biotechnological applications.

An example of the practical application of glucosinolates is their promising potential in preventing biofilms formation on medical devices implants and catheters by pathogenic bacteria of clinical relevance, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. They exhibit robust antimicrobial properties, effective against a variety of clinically significant bacteria and fungi. Additionally, glucosinolates have been applied to prevent bacterial and fungal spoilage of food products, especially in advanced atmospheric packaging technologies. This application contributes to extending the shelf life of these products (Melrose, 2019). These chemical compounds have also the ability to be hydrolyzed by intestinal bacteria into active metabolites such as isothiocyanates. However, it is important to note that variations in the individual gut microbiota can result in different abilities to metabolize glucosinolates. These variations in metabolic capacities can impact the role of glucosinolates and their active metabolites in human health (Wu, 2023). In addition, previous studies have also shown that the intestinal absorption of glucosinolates is significantly lower than the absorption of their metabolites. However, it is important to consider that isothiocyanates, which are the main active metabolites, tend to degrade or volatilize over time, which makes storing these substances a challenge. Therefore, these issues related to the metabolization, absorption and stability of glucosinolates and their derivatives contribute to the complexity and challenges in the practical application of these substances (Wu, 2021).

Glucosinolates have been identified as potent cancer prevention agents. They are generally classified based on the structure of their precursor amino acid, with aliphatic, indole and aromatic glucosinolates derived from methionine, tryptophan, and phenylalanine/tyrosine, respectively, being the main classes found in the *Brassica* genus. Their activation occurs by a class of hydrolytic enzymes called myrosinases that are physically separated from the glucosinolates in intact cells. After tissue rupture, the hydrolysis reaction mediated by myrosinase results in the formation of glucosinolate hydrolysis products, which are considered the bioactive component of this system (Becker et al., 2016).

Chemopreventive bioactivity generally refers to the ability of a chemical compound, or mixture of compounds, to induce phase I/phase II detoxification enzymes and/or antioxidant enzymes in the human body. Antioxidant enzymes generally act to

regulate glutathione metabolism and quench free radicals through one- and two-electron reductions, thus contributing to the reduction of oxidative stress (Becker et al., 2016). Among the compounds that have chemopreventive qualities are the hydrolysis products of glucosinolates, mainly isothiocyanates derived from aliphatic precursors of glucosinolates (Singh & Singh, 2012).

According to Becker et al. (2016) the level of the chemopreventive effect of Brassica vegetables may depend on the interaction of several variables, including the level of consumption of other dietary factors and the genotype/metabolism of the individual. In addition, the profiles of glucosinolates and subsequent hydrolysis products are important variables in determining the chemopreventive effect of consuming a particular Brassica vegetable.

The predominant method for determining glucosinolates in plants is based on EN ISO 9167-1:1995. The method is based on methanol extraction, enzymatic purification and desulphation, and determination by reverse phase chromatography. This method does not determine glucosinolates which are substituted in the glucose molecule. The method uses anion exchange columns (DEAE Sephadex A25) to retain glucosinolates from methanolic plant extracts. The intact and bound glucosinolates are enzymatically desulphated with sulphatase (H1, Helix pomatia) and the eluted desulphated derivatives are analyzed with HPLC (Förster el al., 2015).

According to Clarke (2010) the extraction of glucosinolates in *Brassicas* can be achieved using protic solvents. This has largely been restricted to the use of methanol and water. Both ethanol and water (1:1) and methanol and water (7:3) are recommended for freeze-dried green tissues. However, as methanol breaks down cell walls, a solution of methanol, water and ammonia has been used successfully, with 10% ammonia in methanol containing 5% water, in a solvent to seed ratio of 1:1, being sufficient to reduce the glucosinolate content below the detection limit. Therefore, large-scale solvent-based extraction is disadvantaged by time, energy and safety issues and are therefore not ideal.

2.2. FUNCTIONAL FOODS

2.2.1. Definition and relevance of functional foods

Functional foods are so called because, in addition to their basic nutritional functions, they exert a significant biological activity and have been identified as important in reducing the risk of various chronic-degenerative diseases, inflammatory diseases and metabolic disorders. Modern consumers are looking for practical, easy-to-prepare foods that, in addition to nutritional quality, provide well-being and health benefits. In this context, functional food products have been developed by incorporating proteins, fibers and antioxidants, or by reducing the fat content, for example (Paucar-Menacho et al., 2008).

In recent years, scientists and producers have dedicated themselves to developing new food formulations that, in addition to providing nutrients and energy, also beneficially modulate one or more specific functions of the body, improving physiological responses and/or reducing the risk of disease (Melini et al., 2020).

Pasta is a staple food in many countries, widely accepted around the world due to its low cost, ease of preparation, versatility, sensory attributes, and long shelf life. It is mainly used as a source of energy due to its high carbohydrate content (Prabhasankar et al., 2009). Due to its ease of preparation, pasta is ideal for adding functional ingredients (Lemes et al., 2012).

2.2.2. Impact of adding watercress to food products

The inclusion of watercress (*N. officinale*) in food products has multiple beneficial impacts, attributed to its nutritional properties and health benefits. Vegetable by-products, such as watercress, are rich sources of bioactive compounds, representing a significant opportunity for the development of high value-added products in the food industry, as well as contributing to the reduction of food losses and waste (Faustino et al., 2019). The short shelf life of vegetables and fruits, associated with their seasonal nature, results in considerable losses and waste, a problem that can be mitigated through the valorization of these by-products (Neri et al., 2020).

The inclusion of watercress can significantly enrich the nutritional value of food products. These vegetables are particularly rich in vitamins (C, A and K) and minerals (calcium and iron), as well as containing bioactive compounds such as polyphenols, carotenoids and glucosinolates, which have antioxidant and anti-inflammatory properties. The bioactivity of these compounds, especially isothiocyanates, is preserved or even increased during processing and storage, ensuring continued health benefits for consumers (Araújo-Rodrigues et al., 2021).

Proper processing and storage are crucial to maintaining the nutritional quality and bioactivity of watercress. Studies indicate that methods such as freezing and drying have varying impacts on bioactive compounds. For example, freezing can preserve or increase the antioxidant activity and carotenoid profile, while drying can result in a significant reduction of these compounds. However, both methods manage to maintain relevant levels of phenolic compounds and vitamin E, demonstrating the feasibility of using watercress in processed products (Faustino et al., 2019; Neri et al., 2020).

Furthermore, the application of watercress in various food products, from soups and salads to smoothies and breads, not only enriches the nutritional value, but can also act as a natural preservative due to its antimicrobial properties, extending the shelf life of food without the need for synthetic additives (Faustino et al., 2019). Therefore, the valorization of watercress in the food industry not only enriches products in nutritional terms, but also contributes to the sustainability of the food system by taking advantage of plant by-products that would otherwise be wasted (Araújo-Rodrigues et al., 2021).

2.2.3. Processing strategies to preserve watercress' bioactive compounds

The seasonality and perishability of plant foods require effective processing and preservation strategies to extend their shelf life, ensuring the availability of quality and safe products during the off-season (Neri et al., 2020). The freezing technique is widely used by both industrial companies and families for the long-term preservation of plant foods, and is an effective processing technology (Gonçalves et al., 2009).

Minimally processed vegetables are highly perishable due to the exposure of internal tissues, the absence of protective skin or cuticle and high metabolism, which accelerates deterioration compared to intact vegetables. An effective alternative for improving the safety and extending the shelf life of food products is gamma irradiation

treatment. This method has gained commercial acceptance as it offers a safe solution for quarantines and is applicable to a wider variety of fresh products compared to alternative treatments (Hallman, 2016).

Dehydrated watercress has emerged as an innovative product for use in food recipes or as a food supplement, due to its high nutritional content. As watercress is rich in nutrients such as vitamin C, minerals and bioactive compounds, including phenolics and antioxidants, various drying methods, such as solar, convective, microwave, osmotic, vacuum and freeze-drying, have been developed to improve the characteristics of food products, increasing quality and consumer preference. However, fresh watercress leaves are highly perishable and have a short shelf life. Drying is one of the oldest and most effective processing techniques for extending the shelf life of food by reducing water content and consequently decreasing microbial and biochemical activities (Ek et al., 2018).

3. OBJECTIVES

The present work aims to study watercress (*Nasturtium officinale*) flour, for the subsequent development of a functionalized pasta product, with a complementary study of the various technological aspects of the obtained flours and final products.

3.1 Specific objectives

The specific objectives are described below, along with the implemented methodologies, and the work plan is outlined in **Figure 3.**

- Nutritional evaluation of watercress flours by AOAC methods, namely, moisture, ash, total fat, crude protein, carbohydrates, and energy value;
- Determination of the chemical composition in:
    - Sugars by HPLC coupled to a Refractive Index (RI) detector;
    - Organic acids by UFLC coupled to a Diode Array Detector (DAD);
    - Fatty acids by Gas Chromatography (GC) coupled to a Flame Ionization Detector (FID);
    - Phenolic and glucosinolate compounds by High Performance Liquid Chromatography coupled to an Electrospray Ionization Mass Spectrometer (HPLC-DAD/ESI-MSn) after preparation of hydroethanolic extracts (80:20 *v/v*) from watercress flour;
- *In vitro* evaluation of the bioactive properties of hydroethanolic extracts obtained from the studied flour:
    - Antioxidant activity: inhibition of lipid peroxidation through the thiobarbituric acid reactive substances (TBARS) assay
- Development of novel functionalized pasta products with liophilzied and fresh watercress, with 1.5%, 3%, and 5% wheat substitution. Development of a negative control with 100% wheat flour, and comparison with a commercial fresh pasta sample;
- Evaluation of the physical and organoleptic properties, namely texture, water activity (Aw), color, weight loss after baking, and specific volume;
- Evaluation of the nutritional, chemical, and bioactive properties of the obtained products, in comparison with the 100% wheat flour control and commercial ppasta, using the same analytical methodologies used for the flours.

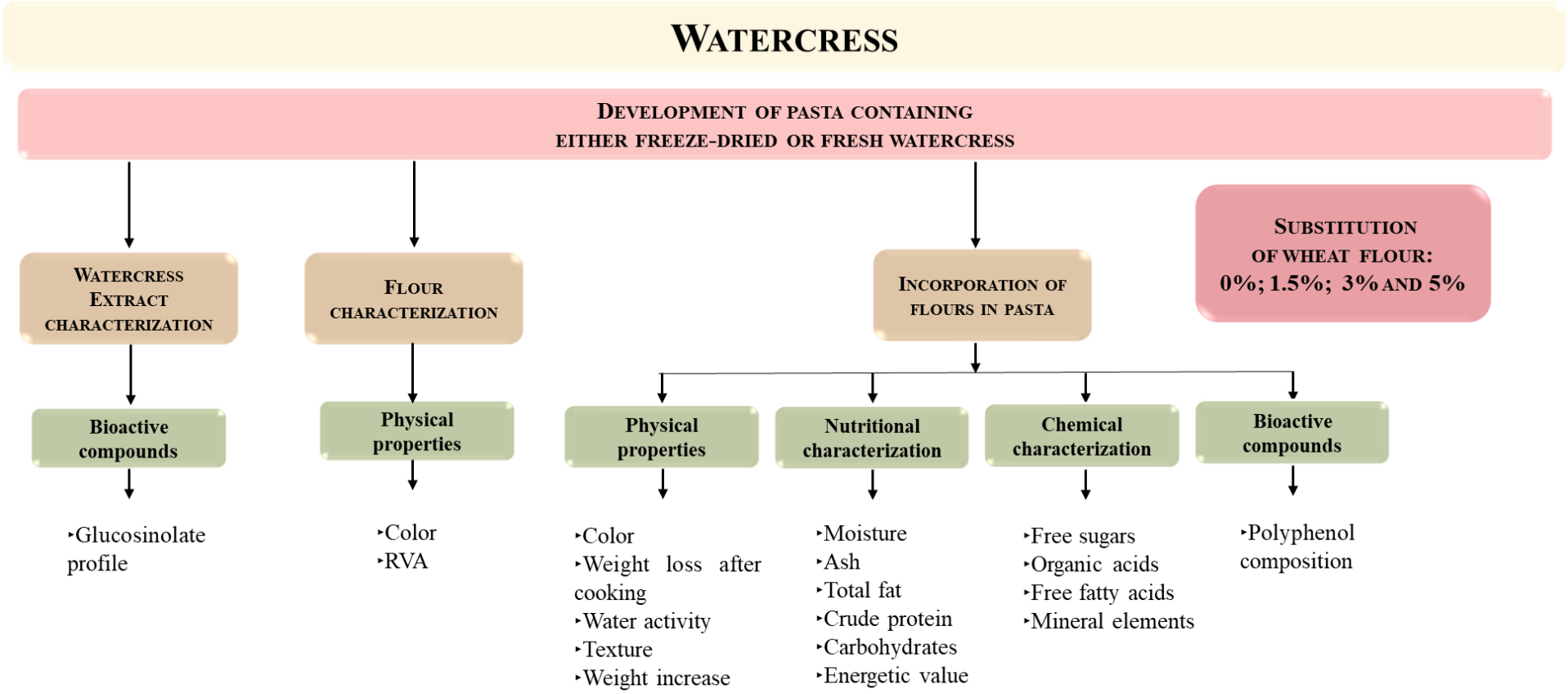


Figure 3. Schematic representation of the developed work plan (Own authorship, 2023)

4. MATERIAL AND METHODS

4.1. Samples, standards, and reagents

Samples of fresh vegetative parts of commercial watercress (Vitacress) were obtained from a local retailer, Bragança, Portugal (***Figure 4***). The samples were carefully weighed, frozen and subjected to freeze-drying to obtain a fine dry powder, which was the stored under controlled humidity and light conditions for future analysis. The non-liphilized sample was maintained under refrigeration conditions (4ºC) until further analysis.

The wheat flour, commercial fresh, and remaining ingredients used to make the pasta products were purchased in commercial areas dedicated to retail sales in the city of Bragança, Portugal.

A **B**

Figure 4. Fresh vegetative parts of commercial watercress (**A**) and respectively lyophilized sample (**B**).

The standard mixture of fatty acid methyl ester (FAME) 37 (standard 47885-U) was purchased from Sigma-Aldrich (St. Louis, MO, USA), along with the individual fatty acid isomers, sugars (D(+)-saccharose and D(+)-melezitose) and organic acid standards (oxalic acid, shikimic acid, fumaric acid and quinic acid). For the analysis of phenolic and glucosinolate compounds, acetonitrile (99.99%, LC-MS grade) was purchased from Fisher Scientific (Lisbon, Portugal) and LC-MS formic acid from Sigma-Aldrich (St. Louis, MO, USA). The phenolic compound standards (such as apigenin-7-*O*-glucoside, caffeic acid, gallic acid, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, luteolin-7-*O*-glucoside, *p*-coumaric acid, and quecetin-3-*O*-glucoside) were obtained from Extrasynthèse (Genay, France).

The distilled water used was treated in a MilliQ purification system (Millipore, model A10, Billerica, MA, USA).

4.2. Development of novel functionalized pasta products with watercress

Six formulations of pasta dough were prepared using wheat flour, eggs, and lyophilized or fresh watercress at different wheat substitution percentages (1.5%, 3%, and 5%). The proportions used are indicated in **Table 2**. A negative control dough was also prepared using 100% wheat flour. The incorporation of freeze-dried watercress into the pasta was simple by mixing the dry powder with the the wheat flour.

For the incorporation of fresh watercress into the pasta, the fresh vegetative parts were grinded with the wheat flour until a homogeneous texture was achieved.

Table 2. Quantity of flour (g), watercress (g), and eggs (unit) used to formulate the different pasta doughs

	Negative control (NC)	**Pasta with freeze-dried watercress (PFDW)**			**Pasta with fresh watercress (PFW)**		
	-	1.5%	3%	5%	1.5%	3%	5%
Flour (g)	100	98.5	97	95	107.575	85.15	55.25
Watercress (g)	0	1.5	3	5	22.425	44.85	74.75
Egg (unit)	1	1	1	1	1	1	1

Once the pasta was homogenized, it was left to rest for 1 hour. A 2 mm wide rolling pin was then used to shape the pasta into Fettuccine. After cutting, the pasta was left to rest for 10 minutes. A portion of 50 g of each fresh pasta was then cooked in 500 mL of boiling distilled water for 8 minutes, reaching the desired *al dente* point. Subsequently, the raw and cooked pasta samples were carefully weighed, frozen and subjected to freeze-drying to obtain a fine dry powder, which was stored under controlled humidity and light conditions for future analysis. The commercial sample also underwent the same cutting and lyophilization process. **Table 3** shows all the formulated pastas, before and after cooking, as well as their coding (to facilitate the reading of the presente work).

Table 3. Representation and coding of the studied pastas, raw (R) and after cooking (C).

	Watercress percentage (%)	Code name	Raw (R)	Cooked (C)
Negative control	0	**NC**		
Commercial sample	0	**CS**		
Pasta with freeze-dried watercress	1.5%	**PFDW1.5**		
	3%	**PFDW3**		
	5%	**PFDW5**		

Pasta with fresh watercress	1.5%	**PFW1.5**		
	3%	**PFW3**		
	5%	**PFW5**		

4.2.1. Assessment of the physical properties of the developed pasta products

4.2.1.1. Texture

The texture profile of the pasta was assessed using the Texture Profile Analysis (TPA) method, widely used in solid and semi-solid foods, adapted from the guidelines of AACC method 74-09, with adjustments (AACC, 2000). For this analysis, a texturometer (model TA HD plus, Stable Micro System, Godalming, UK) was used, connected to a computer and operated using Exponent Lite 2016 software, version 6.1.16 Lite. Texture analysis was carried out on 2 mm thick dough samples prepared in the Fettuccine format, as illustrated in **Figure 5.** A cylindrical aluminum probe with a diameter of 75 mm (P/100) was used in a double compression test during TPA, penetrating up to 50% of the sample depth at a test speed of 2 mm/s, with a 30 s interval between the two compressions.

From the TPA curve, parameters such as hardness (N) and strength to cut (S) were calculated, providing a detailed characterization of the texture of the pasta developed.

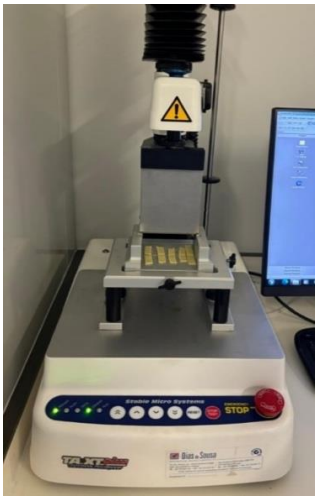


Figure 5. Texture analysis of the pasta developed using a texturometer equipment.

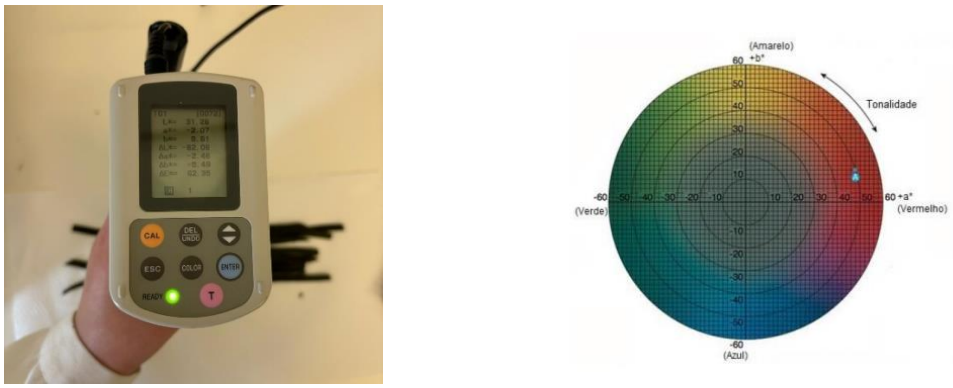
4.2.1.2. Water activity (Wa)

The Official Analysis Method no. 978.18 (AOAC, 1999) was used to determine the water activity of the developed pastas. This method involves creating a controlled atmosphere for the sample, followed by manometric measurement of the free water present in the food. The water activity analysis was conducted using the AquaLab - 4TE water activity meter, manufactured in Munich, Germany, as shown in **Figure *6***. The measurements were carried out in duplicate, maintaining a constant temperature of 24 ± 1 °C during the process.

Figure 6. Water activity analysis of the developed pastas using a water activity meter equipment.

4.2.1.3. Color

The color parameters of the developed pastas and corresponding flours were measured using a colorimeter (model CR400, Konica Minolta, New Jersey, USA) with an integrating sphere and 45º viewing angle (d/45 illumination and D illuminant). Without the need to prepare the sample, the luminosity values were determined on the surface of the samples, as illustrated in **Figure *7***. The results were expressed as L*, a* and b* values, where the luminosity parameter (L*) ranges from black (0) to white (100), the a* component from green (-60) to red (+60) and the b* component from blue (-60) to yellow (+60), with the result being the average of these three components.



A **B**

Figure 7. Colorimeter used to measure sample colors (**A**) and CIE Lab color space (**B**, Konica Minolta, 2021).

4.2.1.4. Weight loss after cooking

Determining weight loss followed the methodological approach outlined by Belorio & Gómez (2020), where mass loss was quantified by the variation between the weights before and after the cooking process, presented as a percentage (%) as described in **Equation *1*.**

Equation 1. Weight loss (%) after cooking.

$$\text{Weight loss (\%)} = \left(\frac{\text{dough before baking (g)}}{\text{dough after baking (g)}}\right) x\ 100$$

4.2.1.5. Flour viscosity profile (RVA, Rapid Viscosity Analyser)

The apparent viscosity profile of the samples was determined using a rapid visco-analyzer (RVA 4500 series, Perten Instruments, USA), following the protocol described by Curti et al. (2022). To conduct the experiment, 3.5 g of each sample (with a moisture content of 14%) were suspended in 25 g of distilled water to form an aqueous paste, which was then placed in the equipment's aluminum cans. The parameters of the paste were analyzed using Thermocline software (V 3.15, Perten Instruments, Australia). The parameters evaluated included pasting temperature (PT, temperature at which the viscosity begins to increase) in °C, peak viscosity (PV, maximum viscosity of the hot paste), final viscosity (FV, viscosity at the end of the test), decomposition (BD) and recoil (SB), expressed in units of centipoise (cp).

4.3. Nutritional composition, chemical profile and bioactive properties

The study of nutritional composition, chemical profile and bioactive properties was carried out on freeze-dried watercress, pasta functionalized with freeze-dried and fresh watercress (with different percentage substitutions), commercial pasta samples and negative control pasta.

4.3.1. Nutritional composition

4.3.1.1. Moisture

Moisture was assessed according to the protocol established by the official method of analysis No. 925.45b (AOAC, 1999). An electronic moisture balance (ADAM, PMB 163, Oxford, USA) was used to weigh approximately 2 g of each sample, and the moisture was completely removed using infrared radiation. The percentage of moisture was determined from the difference between the initial and final mass of the sample, with the results expressed in grams of moisture per 100 grams of dry weight.

4.3.1.2. Ash

To analyse the ash content, the procedure described in the official method of analysis number 935.42 (AOAC, 1999) was used. Samples of 250 mg were meticulously

weighed into previously treated porcelain crucibles, identified and weighed. The samples were then subjected to incineration in a muffle furnace (IVYMEN, N-8L, Barcelona, Spain) at 600 °C for a period of 6 hours, until white ash was obtained, indicating complete calcination. After the process, the crucibles containing the calcined samples were transferred to a desiccator and left to cool until they reached room temperature (~25°C) and then weighed until a constant weight was obtained. The percentage of ash was determined using the difference between the initial and final mass of the sample, with the results expressed in grams of ash per 100 grams of dry weight.

4.3.1.3. Total fat

The total fat content was determined according to the protocol established by the official analysis method number 989.05 (AOAC, 1999). Initially, approximately 3 g of each sample was weighed into a paper cartridge, which was then inserted into a Soxhlet-type fat extractor, using petroleum ether as the extraction solvent, at a temperature of approximately 120 °C, with a cycle duration of 6 hours. The results were expressed in grams of fat per 100 grams of dry weight and were calculated using the gravimetric difference between the initial mass of the sample and the residual mass of fat.

4.3.1.4. Crude protein

The crude protein content was determined using the macro-Kjeldahl method, as prescribed by the official method of analysis number 991.02 (AOAC, 1999), using a conversion factor of 6.25 to transform the nitrogen (N) content into total protein, as recommended by Xu et al. (2019). For the digestion process, approximately 0.25 g of sample was weighed into Kjeldahl tubes, to which two catalyst tablets (Kjeltabs) and 15 mL of concentrated sulfuric acid (H_2SO_4) were added. The tubes were then placed in a digester block at a temperature of 420 °C for 70 minutes. After the samples were completely digested and cooled, 25 mL of distilled water was added. Using a Kjeldahl analyser (Velp Scientifica UDK 152), NaOH was added to the tubes containing the digested sample by means of backward volumetry, releasing the nitrogen in the form of NH_3, which was then collected by steam distillation in a 0.1N H_2SO_4 solution.

Finally, a titration was carried out with 0.1N NaOH, using methyl red as an indicator to calculate the amount of nitrogen, applying a correction factor of N (amount of nitrogen)

x 6.25, according to **Equation *2***, and the results were expressed in grams per 100 grams of dry weight.

Equation 2. Calculation of crude protein content (g/100g).

$$Protein\ (\mathrm{g}/100\mathrm{g}) = \%\ of\ nitrogen\ (N)\ x\ Conversion\ factor$$

4.3.2. Chemical profile

The assessment of the sample chemical composition included a comprehensive characterization of profile and content of free sugars, organic acids, fatty acids, phenolic compounds, glucosinolates and minerals. These analyses were carried out using liquid and gas chromatographicy techniques.

4.3.2.1. Free sugars

The determination of free sugars followed the protocol established by Obodai et al. (2017), using a High Performance Liquid Chromatography (HPLC) system with a refractive index (RI) detector. Initially, 1 mL of internal standard (PI, melezitose, 25 mg/mL) was added to the lyophilized samples (1 g), followed by extraction with 40 mL of 80% ethanol in a thermostated bath (Julabo, SW22; Seelbach, Germany) at 80 ºC for 1 hour and 30 minutes, with stirring every 15 min. The samples were then centrifuged (K24OR refrigerated centrifuge, Centurion, West Sussex, UK) for 10 min at 3500 rpm, filtered and the supernatant transferred to a glass flask in which the ethanol was evaporated at 50 ºC under reduced pressure in a rotatory (Büchi R-210, Flawil, Switzerland). The supernatant obtained was subjected to a de-lipidization process and washed three consecutive times with 10 mL of ethyl ether, as shown in **Figure *8***. After concentration, the samples were placed in an oven at 50 ºC to remove the residual ethyl ether, after which the residue was redissolved in distilled water to a final volume of 5 mL. Finally, it was filtered through 0.2 µm nylon filters into vials and analyzed by HPLC-RI at 35 ºC using an HPLC system (Knauer, Smartline system) equipped with an IR detector (Knauer Smartline 2300) and a 100-5 NH2 Eurospher column (4.6 × 250 mm, 5 µm, Knauer). The mobile phase used was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 mL/min.

The sugars were identified using the internal standard method and by chromatographic comparison with authentic standards. Quantification was carried out by internal normalization of the chromatographic peak area using the melezitose peak (PI) as a standard. The results were expressed in g per 100 g dry weight of the sample.

Figure 8. Washing of the supernatant with diethyl ether.

4.3.2.2. Organic acids

The determination of organic acids was carried out using ultra-fast liquid chromatography (UFLC) coupled to a diode array detector (DAD; Shimadzu Corporation, Kyoto, Japan), as previously described by Barros et al. (2013). To extract the organic acids, 1 g of the sample was placed in a goblet, previously wrapped in aluminum foil, to which 25 mL of metaphosphoric acid (4.5%, v/v) was added. The mixture was magnetically stirred for 20 minutes at a room temperature of approximately 25 ºC and then filtered into a 20 mL test tube. For the chromatographic analysis, the samples were filtered through 0.2 µm nylon filters into an amber vial (1.5 mL) for subsequent analysis.

The chromatographic analysis was carried out on a Shimadzu 20A series UFLC system (Shimadzu Corporation, Kyoto, Japan). The compounds were separated on a C18 SphereClone reverse phase column (250 mm x 4.6 mm, 5 µm, Phenomenex), thermostated at 35 ºC. Detection was carried out using a diode array detector (DAD), using 215 nm and 245 nm (for ascorbic acid) as the preferred wavelengths. The mobile phase used in isocratic mode was 3.6 mM sulfuric acid (H_2SO_4), with a flow rate of 0.8 mL/min. The identification of the organic acids and their quantification was determined by comparing the retention times.

4.3.2.3. Fatty acids

From the lipid fraction previously obtained by Soxhlet extraction (section 4.3.1.3.), the fatty acids were derivatized by a transesterification process as previously described by Obodai et al. (2017). To do this, 5 mL of a methanol:sulfuric acid:toluene solution (2:1:1, *v/v/v*) was added to the lipid fraction, which was incubated in a thermostated bath (Julabo, SW22; Seelbach, Germany) for 12 hours at 50 °C and 160 rpm. After incubation, 3 mL of distilled water and 3 mL of diethyl ether were added and the mixture was vigorously stirred using a vortex (LBX V05 series, LBX Instruments, LABBOX LABWARE S.L., Barcelona, Spain). The organic phase containing the fatty acid methyl esters (FAME) was separated, dehydrated with anhydrous sodium sulphate and filtered through 0.2 µm nylon filters (Millipore) for subsequent analysis.

The fatty acid profile was determined by gas chromatography (GC 1000, DANI, Milan, Italy), equipped with a split/splitless injector at 250 °C, flame ionization detector (FID) at 250 °C and a Macherey-Nagel column (30 m × 0.32 mm ID × 0.25 µm, df, Phenomenex, Lisbon). The furnace temperature program was as follows: the initial column temperature was 100 °C for 2 minutes, then the temperature was increased at 10 31 °C/min until 140 °C, 3 °C/min until 190 °C, 30 °C/min until 260 °C for 2 minutes. Hydrogen (carrier gas) had a flow rate of 4.0 mL/min (0.61 bar), measured at 50 ºC. The split injection (1:50) was carried out at 250 ºC. For each analysis, 1 µL of the sample was injected.

The fatty acids were identified based on the relative retention times of the peaks in the standard mixture of the 37 FAMEs and the samples. The results were processed using Clarity 4.0.1.7 software (DataApex, Podohradska, Czech Republic) and expressed as a relative percentage of each fatty acid.

4.3.2.4. Mineral elements

The mineral elements were analyzed by atomic absorption spectroscopy (AAS) using a Perkin Elmer PinAAcle 900T Spectrometer (Waltham, MA, USA). Potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), zinc (Zn) and iron (Fe) were determined by flame ionization AAS, while manganese (Mn) and copper (Cu) will be analyzed by graphite furnace AAS. A sample of approximately 500 mg was placed in digestion tubes containing 10 mL of concentrated nitric acid and digested for 30 minutes at 200 °C, using a microwave power of 1200 W. After cooling, the resulting solution was

diluted to 50 mL with deionized water and submitted for analysis by AAS, after prior treatment for the specific elements. For K and Na, the sample was diluted in a solution of cerium chloride (1 g/L); for Ca and Mg, a solution of lanthanum chloride (1 g/L) was used; for Mn and Cu, a solution of magnesium nitrate (1 g/L) was used; and Fe and Zn were analyzed directly. The elements were quantified by comparing the absorbance responses with calibration curves prepared from standard solutions. The analyses were carried out in triplicate and the results expressed in mg/100 g of sample fresh weight for K, Na, Ca and Mg, and in µg/100 g of sample fresh weight for Fe, Mn, Cu and Zn.

4.3.2.5. Phenolic compounds

Phenolic compounds were analyzed according to the method of Bessada et al. (2016). The extraction of the bioactive compounds from the enriched pasta, both cooked and raw, and from the freeze-dried watercress, followed the protocol described in section 2.2. The samples were redissolved in an aqueous ethanol solution (20% v/v) to reach a final concentration of 10 mg/mL and filtered through 0.22 µm disposable filters. The analyses of the plant extracts or mass samples were carried out using a Dionex Ultimate 3000 system (UPLC and Linear Ion Trap LQT XL, Thermo Scientific, San Jose, CA, USA), equipped with a diode array detector and an electrospray ionization mass spectrometer (HPLC-DAD-ESI/MS), operating in negative mode. For the analysis, a Waters Spherisorb S3 ODS-2 reversed-phase C_{18} column (4.6 × 150 mm, 3 µm; Waters, Milford, MA, USA) was used, with an elution gradient consisting of formic acid/water (0.1%) and acetonitrile, monitored at 280, 320 and 370 nm, as previously described by Bessada et al. (2016).

Data acquisition and processing were carried out using the Xcalibur data system (Thermo Scientific, San Jose, CA, USA). To identify the compounds, the UV-Vis and mass spectra obtained were compared with data available in the literature. Where available, standards (from Extrasynthèse, Genay, France) were used to identify specific phenolic compounds. In cases where no standard was available, quantification was carried out using a calibration curve of a compound from the same phenolic group.

Quantification of the identified compounds was carried out based on 7-level calibration curves using authentic standards (apigenin-7-glucoside: y=30262x−32276; caffeic acid: y=90492x−29265; gallic acid: y=45933x−19932*; k*aempferol-3-O-glucoside: y=27328x+2683.3*;* kaempferol-3-O-rutinoside: y=20292x+2646.8*;* luteolin-

7-glucoside: y=43453x−1354.5*;* p-Coumaric acid: y=76029x+102258*;* quercetin-3-O-glucoside: y=28555x+3032.3*;* Rutin: y=23794x−46683*).* The results were expressed in mg per g of extract, obtained by re-extraction from the extracts.

4.3.2.6. Profile of glucosinolates

Freeze-dried extracts were analysed in a high-performance liquid chromatograph (HPLC-Dionex UltiMate™ 3000 series, Thermo Fisher Scientific-San Jose, CA, USA) equipped with a diode array detector (DAD) and connected in series to an Orbitrap mass spectrometer (MS, Orbitrap Exploris™ 120, Thermo Fisher Scientific-San Jose, CA, USA). Glucosinolates were separated in a Accucore™ PFP column (2.6 μm, 2.1 × 100 mm, Thermo Fisher Scientific-San Jose, CA, USA) kept at 30 °C, using 0.1% (v/v) formic acid in ultrapure water (A) and 0.1% (v/v) formic acid in acetonitrile (B) as respective mobile phases A and B. The elution gradient in A:B percentage ranged from initial 98:2 to 91:9 in 3 min, reaching 83:17 in the next 9 min, 80:20 in 1 min, 68:32 in 11 min, and 10:90 in 5 min, finally returning in 1 min to the initial condition. The initial condition was kept for further 5 min for column reconditioning, totalizing 35 min of run at a flow rate of 0.4 mL.min^{-1} (Missinou et al., 2022). The injection volume was 2 μL. UV-Vis spectra were acquired between 180 to 700 nm, and the chromatograms processed at 233, 280 and 330 nm for the different glucosinolate classes. The HPLC eluate was analysed by high-resolution, tandem mass spectrometry, and the compounds were ionized using an OptaMax NG electrospray ion source (ESI) source operating in negative mode. The spray voltage was set at 2.5 kV, the ion transfer tube temperature at 325 °C, and the vaporizer temperature at 350 °C. Nitrogen served as the sheath gas (50 arb), auxiliary gas (10 arb) and sweep gas (1 arb). Full MS and MS/MS spectra were acquired in the range from 110 to 1000 charge-to-mass ratio (*m/z*), with a resolution of 15,000 and RF lens kept at 70%. The full scan MS was followed by the top four data dependent MS^2 ($ddMS^2$) scans acquired by applying an HCD (high-energy collisional dissociation) normalized at 30% in the stepped (30, 50 and 150) mode. When needed, a dynamic exclusion strategy was employed. Data acquisition and processing were conducted with the Xcalibur™ software (Thermo Fisher Scientific, San Jose, CA, USA). For compound identification, elution order and characteristics of the UV-Vis and mass spectra (molecular ion ($[M-H]^-$), and MS/MS fragments) were interpreted and compared with literature data.

4.4. Statistical Analysis

All assays were performed in triplicate, and the values are expressed as mean ± standard deviation (SD). Significant differences between samples were analyzed using the Student's t-test with a 95% significance level, utilizing IBM SPSS Statistics for Windows, Version 23.0. (IBM Corp., Armonk, NY, USA). In cases where more than two factors were analyzed, a one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$ was used.

5. RESULTS AND DISCUSSIONS

5.1. Physical properties of flours and pastas

Color is an important factor in assessing the visual quality and market value of food products (Singh et al., 2021). Color values were measured for raw and cooked pasta samples. **Table 4** presents the color parameters (*L*, *a*, *b* values) of raw and cooked negative control (100% wheat) pasta, commercial sample of pasta, and novel functional pastas with freeze-dried and fresh watercress at different substitution levels (1.5%, 3%, and 5%) (**Table 5**).

For the raw pasta samples, the commercial sample (CS_R) exhibited a high *L* value (84.96±0.47), indicating a light color, along with a slight red hue (*a* value of 2.59±0.07) and a strong yellow hue (*b* value of 40.12±1.85). The negative control (NC_R) had a moderate *L* value (62.51±0.18), indicating it was darker than the commercial sample, with a slightly higher red hue (a value of 3.7±0.06) and a less intense yellow hue (b value of 31.84±0.35).

In the pastas with freeze-dried watercress (PFDW), increasing the substitution level led to a decrease in *L* values (39.92±1.68 at 1.5%, 33.18±0.52 at 3%, 31.47±0.25 at 5%), indicating darker colors with higher watercress content. The *a* values were negative (-4.93±0.24 to -2.27±0.07), indicating a shift towards green hues, while the *b* values also decreased (21.2±0.96 to 9.99±0.21), suggesting a reduction in the yellow hue. Similarly, pastas with fresh watercress (PFW) showed slightly higher *L* values compared to PFDW but still indicated darker colors with higher substitution levels (42.01±0.45 at 1.5%, 42.23±0.14 at 3%, 40.78±1.46 at 5%). The *a* values were more negative (-8.26±0.21 to -

10.18±0.05), showing a stronger shift towards green hues compared to PFDW, while the *b* values remained relatively stable (24.76±0.39 to 23.69±0.7), indicating a consistent yellow hue.

For the cooked pasta samples, the commercial sample (CS_C) maintained a high *L* value (84.06±0.02), indicating the color lightness was retained after cooking, with a negative a value (-4.68±0.04) showing a shift towards green hues and a slightly decreased *b* value (36.03±0.05) indicating a reduction in the yellow hue. The negative control (NC_C) exhibited an increase in *L* value (72.32±0.42), indicating a lighter color compared to its raw state, a reduced red hue (*a* value of 0.7±0.02), and a decreased *b* value (26.51±0.28), indicating a reduction in the yellow hue.

Table 4. Color parameters of raw and cooked negative control, commercial and developed novel functional pastas (Mean±SD).

	L	***a***	***b***
CS_R	84.96±0.47	2.59±0.07	40.12±1.85
NC_R	62.51±0.18	3.70±0.06	31.84±0.35
PFDW1.5_R	39.92±1.68	-4.93±0.24	21.20±0.96
PFDW3_R	33.18±0.52	-3.17±0.16	13.62±0.62
PFDW5_R	31.47±0.25	-2.27±0.07	9.99±0.21
PFW1.5_R	42.01±0.45	-8.26±0.21	24.76±0.39
PFW3_R	42.23±0.14	-8.51±0.1	24.83±0.64
PFW5_R	40.78±1.46	-10.18±0.05	23.69±0.7
CS_C	84.06±0.02	-4.68±0.04	36.03±0.05
NC_C	72.32±0.42	0.70±0.02	26.51±0.28
PFDW1.5_C	54.62±0.06	-8.75±0.1	23.10±0.01
PFDW3_C	51.35±0.21	-10.06±0.06	23.35±0.28
PFDW5_C	46.56±2.48	-7.72±0.32	19.84±0.98
PFW1.5_C	54.78±1.15	-9.57±0.01	25.63±0.02
PFW3_C	49.47±0.39	-9.60±0.16	21.49±0.13
PFW5_C	52.55±0.04	-9.41±0.01	28.85±0.18

R – Raw; C - Cooked; CS – Commercial samples; NC – negative control (100% wheat); PFDW – Pasta with freeze-dried watercress; PFW – Pasta with fresh watercress; Wheat substitutition – 1,5%, 3%, and 5%. Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same column correspond to significant differences ($p < 0.05$).

In the cooked pastas with freeze-dried watercress (PFDW), the *L* values increased upon cooking (54.62±0.06 at 1.5%, 51.35±0.21 at 3%, 46.56±2.48 at 5%), indicating lighter colors compared to their raw states. The negative *a* values became more pronounced (-8.75±0.1 to -7.72±0.32), showing a stronger shift towards green hues, and the *b* values decreased (23.1±0.01 to 19.84±0.98), indicating a reduction in the yellow hue. For the cooked pastas with fresh watercress (PFW), the *L* values remained relatively high (54.78±1.15 at 1.5%, 49.47±0.39 at 3%, 52.55±0.04 at 5%), indicating lighter colors.

The negative *a* values (-9.57±0.01 to -9.41±0.01) showed a consistent green hue, while the *b* values displayed slight variations but remained higher than PFDW (25.63±0.02 to 28.85±0.18), indicating a stronger yellow hue.

Lighter colored pastas are usually better accepted than whole wheat pasta, mainly because consumers are used to eating pasta with wheat semolina. The functional pastas showed negative *a* value, indicating a green color. On the other hand, the *b* coordinates showed a yellow color, with positive values. According to Chang and Flores (2004), a more intense yellow color is a highly desirable characteristic in pasta products because it is one of the most influential visual appeals in the acceptance of pasta.

Table 5. Visual verfication of the color parameters before and after cooking of the negative control, commercial and developed novel functional pastas.

Code name	Raw (R)	Cooked (C)
NC		
CS		
PFDW1.5		
PFDW3		

PFDW5		
PFW1.5		
PFW3		
PFW5		

The substitution of watercress in pasta formulations significantly impacts the color attributes, with higher levels of substitution resulting in darker and greener pastas. This effect is more pronounced with fresh watercress compared to freeze-dried. Cooking generally results in lighter pastas with a greater shift towards green hues and a reduction in yellow hues. These findings illustrate the influence of both ingredient substitution and cooking on the color characteristics of pasta. The cooking test provides crucial information about the behavior of the product during cooking, including the texture of the final product (Gull et al., 2018).

The results in **Table *6*** describe the physical properties of various raw pastas, including commercial samples (CS), negative control (NC, 100% wheat), and novel functional pastas with freeze-dried watercress (PFDW) and fresh watercress (PFW) at

different substitution levels (1.5%, 3%, and 5%). The properties measured are solids lost, weight gain, and water absorption (WA).

Regarding solids lost, the commercial Sample (CS_R) showed a moderate loss of solids during cooking (3.51±0.53). The negative control (NC_R) had a slightly higher loss of solids (3.84±0.26) compared to the commercial sample. For pasta with freeze-dried watercress (PFDW), at 1.5% substitution (PFDW1.5_R), solids lost were minimal (0.56±0.04), but increased at 3% substitution (PFDW3_R) to 1.99±0.16, then decreased again at 5% substitution (PFDW5_R) to 0.54±0.11. Pasta with fresh watercress (PFW) also showed variation: at 1.5% substitution (PFW1.5_R), solids lost were 1.37±0.56, at 3% substitution (PFW3_R) they were 0.72±0.01, and at 5% substitution (PFW5_R) they significantly increased to 7.93±0.04.

The solids loss values of the functional doughs were lower than those of the commercial sample, indicating a lower loss of solids. A reduced amount of residue in the cooking water is desirable for high-quality pasta, because during the cooking process, soluble starch and other soluble components, including non-starch polysaccharides, are leached into the water, making it thick. The addition of watercress resulted in a higher starch leaching content, which decreased the water absorption capacity during cooking.

Table 6. Physical properties of the raw commercial, negative control and developed novel functional pastas (Mean±SD).

	Solids lost	**Weight gain**	**WA**
CS_R	3.51±0.53	68.37±3.3	0.944±0.001
NC_R	3.84±0.26	52.79±3.11	0.931±0.004
PFDW1.5_R	0.56±0.04	44.87±1.68	0.94±0.004
PFDW3_R	1.99±0.16	40.62±1.45	0.945±0.006
PFDW5_R	0.54±0.11	58.68±1.06	0.926±0.003
PFW1.5_R	1.37±0.56	54.02±0.52	0.947±0.007
PFW3_R	0.72±0.01	69.55±1.81	0.969±0.001
PFW5_R	7.93±0.04	53.18±2.29	0.975±0.003

R – Raw; CS – Commercial samples; NC – negative control (100% wheat); PFDW – Pasta with freeze-dried watercress; PFW – Pasta with fresh watercress; Wheat substitutition – 1.5%. 3%. and 5%. Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same column correspond to significant differences ($p < 0.05$).

Regarding weight gain during cooking, the commercial sample (CS_R) exhibited substantial gain (68.37±3.3), while the negative Control (NC_R) had lower gain (52.79±3.11). For PFDW, weight gain decreased with increasing substitution: at 1.5%

(PFDW1.5_R) it was 44.87±1.68, at 3% (PFDW3_R) it decreased to 40.62±1.45, then increased at 5% (PFDW5_R) to 58.68±1.06. Similarly, PFW showed variation: at 1.5% substitution (PFW1.5_R) weight gain was 54.02±0.52, at 3% substitution (PFW3_R) it increased to 69.55±1.81, and at 5% substitution (PFW5_R) it was 53.18±2.29.

The cooked weight of the developed functional pasta increased relatively less compared to the commercial pasta. This lower increase in cooked weight can be attributed to the binding and water retention capacity of carboxymethyl cellulose and bagasse. Susanna and Prabhasankar (2013) also reported an increase in cooked weight in pasta. The addition of watercress to the flour improved the quality of the pasta by reducing the loss of solids, except for the PFW5_R sample. This result can be explained by the composition of watercress, which has a relatively high protein content compared to commercial pasta, promoting the retention of amylose during cooking. The functional doughs registered a lower weight increase than the commercial sample. The addition of watercress led to greater starch leaching, resulting in lower water absorption capacity during cooking.

Water absorption (WA) varied among samples as well. The CS_R had WA of 0.944±0.001, slightly higher than the NC_R at 0.931±0.004. For PFDW, WA at 1.5% substitution (PFDW1.5_R) was 0.94±0.004, slightly increasing at 3% (PFDW3_R) to 0.945±0.006, then decreasing at 5% (PFDW5_R) to 0.926±0.003. In contrast, PFW showed increasing WA with substitution: at 1.5% (PFW1.5_R) it was 0.947±0.007, at 3% (PFW3_R) it increased to 0.969±0.001, and at 5% (PFW5_R) it reached the highest value of 0.975±0.003.

In foods, a water activity higher than 0.8 indicates that they are in the high humidity phase, where there is an increase in the molecular mobility of water and other food constituents, favoring an increase in the rates of undesirable chemical reactions and microbial growth. Consequently, these foods are chemically unstable and susceptible to the development of microbial load (Damodaran & Parkin, 2019).

The physical properties of raw pastas reveal several notable trends and differences. The commercial sample (CS_R) and the negative control (NC_R) both exhibit moderate levels of solids lost during cooking, but the negative control loses slightly more solids. The weight gain is substantial in the commercial sample, but significantly lower in the negative control, indicating that commercial pasta retains more water during cooking compared to the 100% wheat pasta.

For the pastas with freeze-dried watercress (PFDW), the solids lost are minimal at lower substitution levels (1.5% and 5%) but increase at the 3% level, suggesting that the watercress content influences the integrity of the pasta matrix during cooking. The weight gain in PFDW pastas generally decreases with higher substitution levels, except for the 5% substitution which shows a higher weight gain. This could be due to the varying effects of watercress concentration on the pasta's ability to absorb water.

The pastas with fresh watercress (PFW) exhibit different trends. Solids lost are relatively low at 1.5% and 3% substitution but significantly higher at 5%, indicating that higher levels of fresh watercress may compromise the pasta's structural integrity. The weight gain increases with the 3% substitution, suggesting optimal water absorption at this level, while it is lower at 1.5% and 5%. The water absorption (WA) values are highest in PFW pastas, especially at higher substitution levels, indicating a greater ability to absorb water compared to both the commercial and negative control samples.

The study of flour viscosity profile using Rapid Viscosity Analyse (RVA) is a unique tool for product development, quality and process control and quality assurance. It is a cooking and stirring viscometer with high temperature and variable shear capacity, optimized for testing the viscous properties of starch, grains, dairy products and other foods. The pasting properties of pasta flours are shown in **Table *7*** and corresponding graphics at **Table *8***.

Table 7. Flour viscosity profile of raw commercial, negative control and developed novel functional pastas (Mean±SD)

	PV (cP)	BD (cP)	FV (cP)	SB (cP)	PT (ºC)
NC_R	10184±110.3	5195±137.2	8770.5±13.4	3781.5±40.3	66.6±0.6
PFDW1.5_R	9934±62.2	5470±281.4	8102.5±466	3638.5±122.3	66.5±0.5
PFDW3_R	9560.5±586.2	5277±261.6	7862±414.4	3578.5±89.8	66.8±0.2
PFDW5_R	10305.5±115.3	6165±149.9	7604±254.6	3463.5±10.6	65.8±0.6
PFW1.5_R	10888.5±2.1	5998.5±140.7	8575.5±105.4	3685.5±37.5	66.1±0.1
PFW3_R	10286±19.8	5927.5±126.6	7682.5±65.8	3324±80.6	51.4±1.5
PFW5_R	9846.5±96.9	5409.5±54.4	7486±149.9	3049±107.5	66.2±0.1

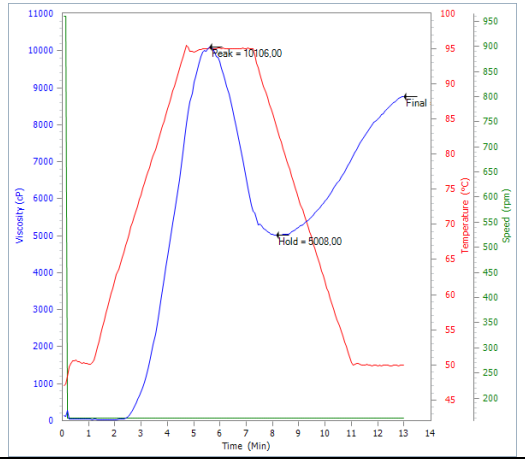
R – Raw; NC – negative control (100% wheat); PFDW – Pasta with freeze-dried watercress; PFW – Pasta with fresh watercress; Wheat substitutition – 1.5%, 3%, and 5%. PV: peak viscosity; BD: breakdown; FV: final viscosity; SB: set back; PT: Pasting Temperature. Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same column correspond to significant differences ($p < 0.05$).

The flour viscosity profile of various pasta types, including raw commercial, negative control, and novel functional pastas, reveals interesting insights into their textural characteristics. Peak viscosity (PV), which signifies the maximum viscosity attained during heating, varies notably across the samples. This is related to the extent of starch swelling before its physical breakdown during heating (Panghal et al., 2019). For instance, Pasta with fresh watercress at 1.5% wheat substitution (PFW1.5_R) demonstrates the highest peak viscosity among all variants, indicating its potential for strong gel formation during cooking. Conversely, Pasta with fresh watercress at 3% wheat substitution (PFW3_R) and Pasta with freeze-dried watercress at 3% wheat substitution (PFDW3_R) exhibit lower peak viscosity values, suggesting differences in starch gelatinization behavior. This decrease in PV can be attributed to the reduction in fiber content as the level of watercress increases in the samples. Watercress contains, on average, 1.8 g of fiber per 100 g (Pinela, 2020), while control pasta, which uses refined wheat flour, usually has between 2% and 3% dietary fiber (Sivam, 2010). This justifies the slightly higher maximum viscosity value in the control dough.

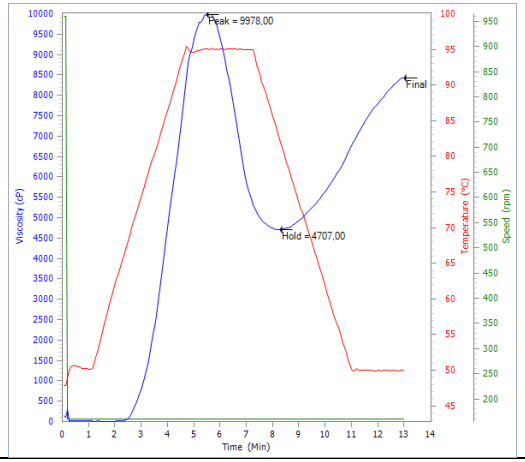
Breakdown (BD), representing the viscosity reduction during heating, also varies across the samples. PFW3_R stands out with a relatively higher breakdown compared to others, implying greater susceptibility to viscosity reduction under heating conditions. Final viscosity (FV), indicating viscosity at the end of heating, shows variation as well, with PFW1.5_R and Negative control (NC_R) displaying higher values compared to other samples, possibly due to differences in starch swelling and gel formation. The final viscosity indicates the reassociation of the starch granules during cooling and the stability of the paste throughout the heating-cooling process (Bawa et al., 2022).

Set back (SB), which measures viscosity increase after cooling and reflects starch retrogradation tendency, is notable in Pasta with freeze-dried watercress at 1.5% and 3% wheat substitution (PFDW1.5_R and PFDW3_R), suggesting potential retrogradation phenomena in these samples. Pasting temperature (PT), representing starch gelatinization onset, remains relatively consistent across most samples, indicating that the addition of watercress does not affect the temperature required to achieve total swelling of the starch granules.

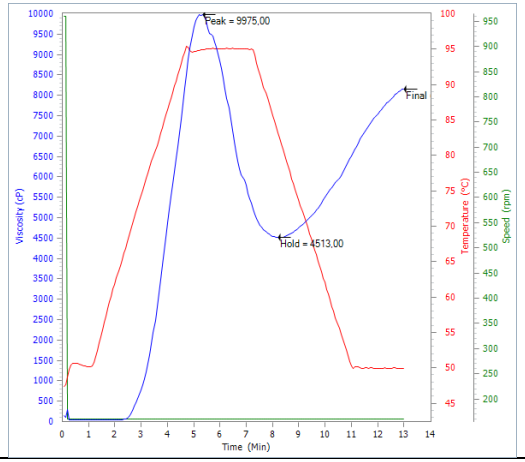
Table 8. Flour viscosity graphic profiles of the negative control (A) and developed novel functional pastas (B-G).



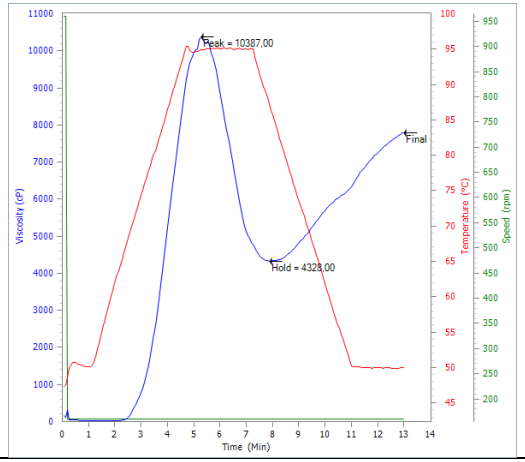
A



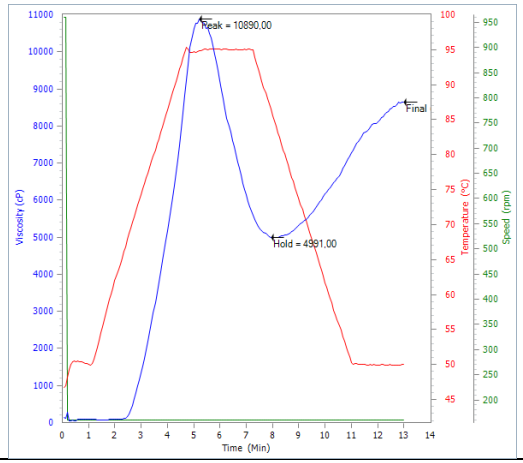
B



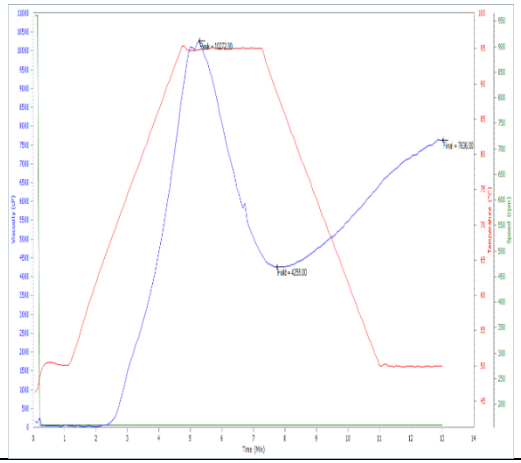
C



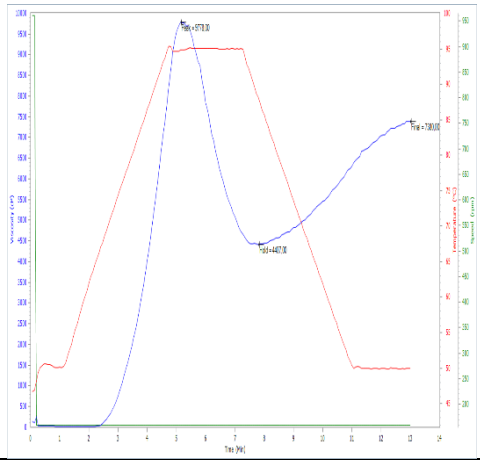
D



E



F



G

Negative control (100% wheat) (**A**); Pasta developed with freeze-dried and fresh watercress with 1.5% (**B** and **E**, respectively), 3% (**C** and **F**, respectively), and 5% (**D** and **G**, respectively) wheat substitutition.

Texture evaluation is a fundamental criterion for determining the overall quality of cooked pasta, and the textural characteristics of pasta are particularly valued by consumers. **Table *9*** presents texture evaluation of pasta samples, particularly focusing on firmness and shear force.

Table 9. Instrumental physical properties of raw and cooked negative control, commercial and developed novel functional pastas (Mean±SD).

Samples	Firmness (gf)	Cutting force (gf)
CS_R	7561.95±206.81	774.33±21.09
NC_R	3960.42±148.76	767.11±27.36
PFDW1.5_R	3026.4±24.65	750.74±31.71
PFDW3_R	3849.23±12.11	814.64±36.65
PFDW5_R	5690.82±194.54	975.58±20.16
PFW1.5_R	3026.4±24.65	750.74±31.71
PFW3_R	2635.94±55.76	475.86±15.1
PFW5_R	1512.82±69.63	253.34±7.99
CS_C	1021.25±12.17	221.46±8.81
NC_C	1079.31±16.7	236.88±3.16
PFDW1.5_C	512.81±21.88	102.95±2.6
PFDW3_C	644.3±15.31	148.36±7.19
PFDW5_C	1818.09±81.1	384.24±9.71
PFW1.5_C	1840.31±6.14	576.53±4.41
PFW3_C	1373.94±38.84	316.14±12.45
PFW5_C	1274.52±51.17	352.65±13.52

Gf- gram force, R – Raw; C - Cooked; CS – Commercial samples; NC – negative control (100% wheat); PFDW – Pasta with freeze-dried watercress; PFW – Pasta with fresh watercress; Wheat substitutition – 1,5%, 3%, and 5%. Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same column correspond to significant differences ($p < 0.05$).

The raw commercial pasta displayed the highest firmness (3960.42±148.76 gf), indicating its robust texture compared to other samples. This suggests that the commercial pasta formulation, likely optimized for texture, resulted in a product with superior firmness. However, the addition of freeze-dried watercress led to an increase in firmness, whereas the incorporation of fresh watercress resulted in a decrease. This observation underscores the importance of ingredient selection in modulating pasta texture, with watercress components such as sugars, water, and fibers influencing gluten development and water availability during pasta formation (Gull et al., 2015). Both sugars and fibers

are known to have a high affinity for water, possibly making it only partially available for the development of the gluten network (Wang et al., 2002).

The reduction in firmness upon cooking is a common outcome attributed to starch gelatinization and water absorption. The cooked functional pasta samples exhibited comparable or even higher firmness than the cooked commercial pasta. This suggests that the formulation of functional pastas, despite ingredient substitution, maintained or even enhanced textural properties, indicating potential consumer acceptability.

The analysis of shear force, closely associated with cooked dough firmness (Larrosa et al., 2016), further corroborated the trends observed in firmness evaluation. The highest shear forces observed were in PFDW5_R (975.58±20.16 gf) and PFDW3_R (814.64±36.65 gf) samples, probably due to the amount of freeze-dried watercress replaced in the flour. Among the cooked samples, PFW1.5_C (576.53±4.41 gf) and PFDW5_C (384.24±9.71 gf) had higher shear forces than the cooked commercial dough (221.46±8.81 gf). In addition to these two samples, PFDW1.5_R and PFDW3_R also exhibited higher shear strengths compared to cooked commercial dough, indicating that, in general, the shear strength of functional doughs was higher than that of commercial dough.

5.2. Nutritional Profile and Chemical Composition of Watercress and developed Pastas

5.2.1. Nutritional composition

Watercress is a food with a high moisture content. with 92.59% water on a wet basis (**Table *10***). Among the pasta samples, those incorporating freeze-dried watercress (PFDW1.5_R, PFDW3_R, PFDW5_R) showed slightly lower moisture content compared to the negative control. However, as the proportion of freeze-dried watercress increased in the pasta formulations (1.5%, 3%, and 5%), there was no significant variation in moisture content. Notably, these values were substantially lower than that of pure watercress.

Table 10. Moisture content (%) of liophilized watercress and raw negative control and developed novel functional pastas (Mean±SD).

Samples	Moisture content (%)
Watercress	92.59±0.07[a]
NC_R	10.18±0.11[d]
PFDW1.5_R	10.06±0.06[d]
PFDW3_R	10.21±0.22[d]
PFDW5_R	10.1±0.08[d]
PFW1.5_R	23.73±0.33[c]
PFW3_R	29.9±0.06[b]
PFW5_R	30.64±0.07[b]

R – Raw; NC – negative control (100% wheat); PFDW – Pasta with freeze-dried watercress; PFW – Pasta with fresh watercress; Wheat substitutition – 1,5%, 3%, and 5%. Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same column correspond to significant differences ($p < 0.05$).

Conversely, pastas with fresh watercress (PFW1.5_R, PFW3_R, PFW5_R) demonstrated significantly higher moisture content compared to the negative control and the freeze-dried watercress pasta variants. As the percentage of fresh watercress increased in the pasta formulations (1.5%, 3%, and 5%), the moisture content notably increased. Although still lower than that of pure watercress, the moisture content in pasta with fresh watercress was markedly higher than in the other samples. The incorporation of watercress, whether fresh or freeze-dried, elevates the moisture content in pasta products compared to a pure wheat-based negative control. As expected, pasta with fresh watercress exhibits higher moisture levels compared to pasta with freeze-dried watercress.

Table *11* presents the nutritional composition of liophilized watercress and raw and cooked commercial, negative control and developed novel functional pastas.

The ash content showed a gradual increase as the substitution of flour with watercress increased. Functional pasta samples with added freeze-dried watercress had a higher ash content compared to samples with fresh watercress, both raw and cooked. The ash content is directly related to the degree of extraction of the flour and the yield during milling, as well as influencing the particle size and making the flour darker. This factor also has a direct impact on the protein content of the flour, since both are more concentrated in the aleurone layer of the wheat grain (Schimiele et al., 2011).

Plant proteins provide health benefits in addition to the essential nutrient's characteristic of each species. In recent years, there has been growing interest in the inclusion of plant proteins in food products due to changes in the human diet and the search for healthier options (Betoret et al., 2011). The protein content of the pasta differed statistically in all treatments ($p < 0.05$), increasing with the greater replacement of wheat

flour with watercress. This result is significant, as pasta is generally considered a product with low nutritional value, while watercress has a higher protein content.

Table 11. Nutritional value (total fat, crude protein, ash, and carbohydrates content, g/100g dw) and energetic value (Kcal/100g dw) of liophilized watercress and raw and cooked commercial, negative control and developed novel functional pastas (Mean±SD).

Samples	Total fat (g/100g dw)	Crude protein (g/100g dw)	Ash (g/100g dw)	Carbohydrates (g/100g dw)	Energy (Kcal/100g dw)
Watercress	3.754±0.1^{h}	39.458±0.159a	13.299±0.024a	43.489±0.035i	365.577±0.403o
CS_R	2.754±0.062^{k}	12.497±0.071^{h}	0.645±0.013^{j}	84.104±0.12^{a}	411.19±0.36^{1k}
NC_R	2.693±0.02^{k}	15.546±0.393^{e}	0.44±0.01^{m}	81.322±0.414^{d}	411.705±0.1^{k}
PFDW1.5_R	6.883±0.072^{a}	15.361±0.346^{ef}	0.557±0.004^{l}	77.199±0.422^{g}	432.189±0.342^{a}
PFDW3_R	4.819±0.082^{e}	15.885±0.365^{d}	0.662±0.005^{i}	78.633±0.288^{e}	421.446±0.427^{f}
PFDW5_R	5.287±0.063^{d}	15.461±0.347^{ef}	0.895±0.013^{g}	78.356±0.297^{e}	422.855±0.263^{e}
PFW1.5_R	3.534±0.065^{i}	14.245±0.322^{g}	0.287±0.001^{o}	81.934±0.387^{c}	416.522±0.32^{1i}
PFW3_R	3.985±0.108^{f}	14.42±0.233^{g}	0.574±0.014^{k}	81.021±0.355^{d}	417.629±0.484^{h}
PFW5_R	3.216±0.073^{j}	14.289±0.272^{g}	0.395±0.003^{n}	82.1±0.202^{c}	414.499±0.378^{j}
CS_C	2.754±0.044^{k}	12.761±0.025^{h}	0.778±0.019^{h}	83.708±0.001^{b}	410.657±0.294^{l}
NC_C	1.037±0.021^{m}	16.117±0.172^{cd}	1.038±0.00^{1f}	81.808±0.194^{d}	401.032±0.099^{n}
PFDW1.5_C	5.809±0.042^{c}	16.293±0.244^{bc}	1.421±0.022^{b}	76.476±0.263^{h}	423.364±0.297^{d}
PFDW3_C	4.753±0.093^{e}	16.502±0.077^{b}	1.299±0.021^{c}	77.445±0.192^{fg}	418.568±0.383^{g}
PFDW5_C	6.118±0.116^{b}	15.492±0.002^{ef}	1.226±0.027^{d}	77.164±0.092^{g}	425.689±0.689^{c}
PFW1.5_C	5.735±0.097^{c}	16.302±0.35^{bc}	0.279±0.001^{o}	77.684±0.447^{f}	427.555±0.48^{1b}
PFW3_C	3.869±0.044^{g}	15.32±0.281^{ef}	0.436±0.004^{m}	80.374±0.241^{e}	417.604±0.232^{h}
PFW5_C	2.458±0.003^{l}	15.168±0.165^{f}	1.177±0.019^{e}	81.197±0.142^{c}	407.584±0.061^{m}

R – Raw; C - Cooked; CS – Commercial samples; NC – negative control (100% wheat); PFDW – Pasta with freeze-dried watercress; PFW – Pasta with fresh watercress; Wheat substitutition – 1,5%, 3%, and 5%. Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same column correspond to significant differences ($p < 0.05$).

In terms of fat content, pasta with added watercress showed higher values compared to commercial pasta, playing a crucial role as the main source of energy and facilitator of the absorption of vitamins A, D, E and K, which are essential for the healthy functioning of the body (BVMS, 2011).

In plants, carbohydrates are essential for energy storage, cell wall integrity, growth, development and responses to environmental changes and stresses (Trouvelot et

al., 2014). In terms of carbohydrate content, the functional pasta had lower values than the commercial pasta. This difference can be attributed to the fact that watercress contains fewer carbohydrates (43.489±0.035i) compared to commercial pasta (84.104±0.12^{a} and 83.708±0.001b), resulting in a reduction in carbohydrate content due to the partial substitution of flour. In addition, it was observed that pasta made with fresh watercress had a slightly higher carbohydrate content than pasta made with freeze-dried watercress. Available carbohydrates represent the fraction that can be hydrolyzed by the endogenous secretions of the human digestive tract and absorbed by the body, excluding dietary fiber (Greenfield and Southgate, 2003).

Finally, the energy value of a food is the sum of the energy that comes mainly from carbohydrates, proteins and fats. The quantities of these constituents determine the total energy value. In this context, the functional pasta showed a slight divergence in energy value compared to the commercial pasta, reflecting the variations in carbohydrate, protein and fat content between the different formulations. The incorporation of watercress, whether freeze-dried or fresh, alters the nutritional composition of pasta, enriching it with total fat and impacting its energy value while maintaining comparable protein and carbohydrate levels.

5.2.2. Chemical characterization of watercress and developed pastas

5.2.2.1. Sugars

Sugars are molecules present in the metabolism of living beings and play a fundamental role in energy and cell signaling processes. Small variations in their concentrations can affect not only energy status, but also the accumulation and mobilization of carbon, directly affecting cellular activity (Pereira et al., 2022).

The free sugars content in liophilized watercress and raw and cooked commercial, negative control and developed novel functional pastas were quantified and the results are shown in **Table 12**.

Table 12. Free sugar content (g/100g dw) of liophilized watercress and raw and cooked commercial, negative control and developed novel functional pastas (Mean±SD).

Samples	Fructose	Glucose	Sucrose	Maltose	Threalose	Raffinose	Total
Watercress	0.5±0.021	0.3±0.021	0.1±0.007	0.44±0.021	0.5±0.042	nd	**1.82±0.014**
CS_R	0.1±0.007	0.11±0.014	0.37±0.007	1.33±0.134	nd	0.22±0.014	**2.12±0.148**
NC_R	0.07±0.007	0.15±0.007	0.29±0.035	1.95±0.106	nd	0.5±0.021	**2.94±0.049**
PFDW1.5_R	0.17±0.007	0.28±0.014	0.07±0.099	1.64±0.064	nd	0.47±0.021	**2.62±0.177**
PFDW3_R	0.31±0.007	0.35±0.021	0.09±0.007	1.47±0.276	nd	0.65±0.014	**2.85±0.311**
PFDW5_R	0.03±0.007	0.24±0.014	0.21±0.014	1.38±0.071	nd	0.48±0.028	**2.34±0.007**
PFW1.5_R	0.24±0.007	0.45±0.042	0.08±0.007	2.23±0.177	nd	0.74±0.071	**3.73±0.276**
PFW3_R	0.29±0.007	0.29±0.007	0.05±0.007	2.12±0.17	nd	0.57±0.071	**3.31±0.078**
PFW5_R	0.42±0.028	0.42±0.035	0.04±0.007	2.26±0.085	nd	0.79±0.134	**3.92±0.276**
CS_C	0.02±0.007	0.08±0.007	0.34±0.049	1.38±0.106	nd	0.44±0.049	**2.24±0.007**
NC_C	nd	0.07±0.007	0.15±0.014	1.76±0.156	nd	nd	**1.98±0.014**
PFDW1.5_C	0.04±0.007	0.11±0.007	0.06±0.007	1.33±0.028	nd	0.18±0.014	**1.71±0.078**
PFDW3_C	0.1±0.007	0.11±0.007	0.07±0.007	1.07±0.092	nd	0.25±0.014	**1.58±0.276**
PFDW5_C	nd	nd	nd	0.24±0.014	nd	nd	**0.24±0.177**
PFW1.5_C	0.04±0.007	0.09±0.007	nd	1.58±0.035	nd	0.12±0.007	**1.81±0.148**
PFW3_C	0.05±0.007	0.06±0.007	1.18±0.057	1.31±0.092	nd	0.13±0.014	**2.72±0.049**
PFW5_C	0.11±0	0.22±0.014	6.41±0.219	2.26±0.085	nd	6.71±0.276	**15.7±0.276**

nd- not detected; R – Raw; C - Cooked; CS – Commercial samples; NC – negative control (100% wheat); PFDW – Pasta with freeze-dried watercress; PFW – Pasta with fresh watercress; Wheat substitutition – 1.5%. 3%. and 5%. Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same column correspond to significant differences ($p < 0.05$).

The total amount of sugars in watercress was 1.82±0.014. a result similar to that found by Marchioni (2021) in watercress samples analyzed. Fructose, glucose, sucrose, maltose and trehalose were identified in watercress. Fructose and trehalose are the most abundant sugars in watercress. followed by maltose, glucose and sucrose. According to Pinela (2020) variations in sugar content can be attributed to the phenological stage of the plant. differences in annual climatic conditions the characteristics of the soil at the sampling sites and the type of plantation.

Regarding the functional pasta samples both the raw and cooked samples showed fructose, glucose, sucrose, maltose, and raffinose. The pasta samples did not contain detectable amounts of trehalose. According to Pereira (2022) trehalose levels are generally too low to make a significant contribution as a reserve or transport sugar functions that are already performed by sucrose.

Overall. the fresh watercress functional pasta samples (PFW1.5_R, PFW3_R, and PFW5_R) had a higher amount of total sugars compared to the freeze-dried samples, with

the PFW5_R sample having the highest amount of total sugars. with 3.92±0.276 for the raw sample and 15.7±0.276 for the cooked sample.

Overall, raw watercress exhibits a higher concentration of free sugars compared to cooked watercress across all sugar types (fructose, glucose, sucrose, maltose, and threalose). This difference suggests that the cooking process might lead to the breakdown or loss of some free sugars present in raw watercress. Is even verified that the total free sugar content in raw watercress (1.82 g/100g dw) surpasses that of cooked watercress, indicating that cooking reduces the overall free sugar content in watercress.

On the other hand, freeze-dried watercress generally demonstrates lower free sugar content compared to fresh watercress. This difference suggests that the freeze-drying process might lead to the loss or alteration of some free sugars present in fresh watercress. The total free sugar content in freeze-dried watercress pasta samples (ranging from 1.58 to 3.92 g/100g dw) is notably lower than that in fresh watercress pasta samples (ranging from 3.31 to 15.7 g/100g dw). This discrepancy implies that incorporating fresh watercress into pasta formulations results in a higher concentration of free sugars compared to using freeze-dried watercress.

5.2.2.2. Organic acids

The results for organic acids content in the liophilized watercress and raw and cooked commercial, negative control and developed novel functional pasta are shown in **Table 13**. Three main organic acids were detected in all studied samples (oxalic, malic and fumaric acid).

Watercress, as an individual tested sample, exhibited the most substantial concentration of oxalic acid, with 5.182 g/100g dw detected, indicating its prominence as a source of this organic acid. Oxalic acid a significant component of many green leafy vegetables (Williams, 1978) can be lethal in high concentrations due to its antinutrient effect on the body. On the other hand, malic and fumaric acids are natural compounds that participate in the Krebs cycle which is fundamental for cellular energy production. Malic acid contributes to oral and skin health, while fumaric acid has antioxidant properties and is used in the treatment of psoriasis and multiple sclerosis. Both are considered safe in normal dietary quantities but can cause gastrointestinal discomfort if consumed in excess.

Table 13. Organic acids content (g/100g dw) of liophilized watercress and raw and cooked commercial, negative control and developed novel functional pastas (Mean±SD).

	Oxalic Acid	Malic Acid	Fumaric Acid	Total
Watercress	5.182±0.164	nd	nd	**5.182±0.164**
CS_R	tr	tr	0.023±0.002^{c}	**0.023±0.002^{ij}**
NC_R	tr	tr	tr	**tr**
PFDW1.5_R	0.117±0.01^{f}	tr	0.023±0.001^{c}	**0.14±0.009^{f}**
PFDW3_R	0.213±0.04^{c}	tr	0.027±0.001^{a}	**0.24±0.039^{d}**
PFDW5_R	0.555±0.009^{a}	0.026±0.008^{de}	0.025±0.002^{b}	**0.605±0.019^{a}**
PFW1.5_R	0.094±0.001^{g}	0.156±0.103^{a}	0.007±0.002^{h}	**0.257±0.107^{c,d}**
PFW3_R	0.13±0.001^{e}	0.135±0.023^{bc}	0.001±0.0001^{j}	**0.266±0.022^{c}**
PFW5_R	0.403±0.014^{b}	0.122±0.006^{c}	0.008±0.002^{h}	**0.533±0.023^{b}**
CS_C	tr	tr	0.023±0.002^{c}	**0.023±0.002^{ij}**
NC_C	tr	tr	0.01±0.001^{f}	**0.01±0.001^{k}**
PFDW1.5_C	0.028±0.001^{i}	tr	0.012±0.002^{ef}	**0.039±0.002^{i}**
PFDW3_C	0.093±0.019^{g}	tr	0.014±0.001^{d}	**0.107±0.02^{g}**
PFDW5_C	0.193±0.017^{d}	tr	0.011±0.001^{e}	**0.204±0.017^{e}**
PFW1.5_C	0.007±0.002^{j}	0.037±0.004^{d}	0.003±0.001^{i}	**0.047±0.001^{i}**
PFW3_C	0.036±0.009^{i}	tr	0.007±0.002^{h}	**0.043±0.011^{i}**
PFW5_C	0.072±0.009^{h}	tr	0.009±0.001^{g}	**0.081±0.01^{h}**

nd- not detected; tr- tace amounts; R – Raw; C - Cooked; CS – Commercial samples; NC – negative control (100% wheat); PFDW – Pasta with freeze-dried watercress; PFW – Pasta with fresh watercress; Wheat substitutition – 1.5%. 3%. and 5%. Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same column correspond to significant differences ($p < 0.05$).

Both raw and cooked commercial samples show trace amounts (tr) of oxalic acid, suggesting minimal presence or levels below the detection limit. However, fumaric acid is detected, with concentrations of 0.023 g/100g dw, indicating its presence in both raw and cooked commercial pasta.

Similar to the commercial samples, the negative control samples (NC_R and NC_C), also exhibit trace amounts (tr) of oxalic acid, indicating minimal presence or levels below the detection limit. Fumaric acid is detected in the cooked negative control sample (NC_C), with a concentration of 0.01 g/100g dw.

Pasta samples with freeze-dried watercress (PFDW1.5_R, PFDW3_R, PFDW5_R, PFDW1.5_C, PFDW3_C, PFDW5_C) exhibit varying levels of oxalic acid, with concentrations ranging from 0.117 to 0.605 g/100g dw, depending on the proportion of watercress incorporated. Malic acid is detected in some samples but not consistently across all formulations, suggesting variable levels based on the pasta composition and

processing. Fumaric acid is detected in all pasta samples with freeze-dried watercress, with concentrations ranging from 0.011 to 0.027 g/100g dw.

Pasta samples with fresh watercress (PFW1.5_R, PFW3_R, PFW5_R, PFW1.5_C, PFW3_C, PFW5_C) also exhibit varying levels of oxalic acid, malic acid, and fumaric acid, with concentrations dependent on the amount of watercress included in the formulation. Oxalic acid concentrations range from 0.007 to 0.403 g/100g dw, malic acid concentrations range from 0.036 to 0.156 g/100g dw, and fumaric acid concentrations range from 0.001 to 0.008 g/100g dw.

The organic acids content varies significantly among watercress and pasta samples, influenced by factors such as watercress type (freeze-dried or fresh), pasta formulation, and processing method (raw or cooked).

5.2.2.3. Fatty acids

Table *14* shows the majority composition of fatty acids. as well as the content of saturated. monounsaturated and polyunsaturated fatty acids (SFA, MUFA and PUFA, respectively) found in the freeze-dried watercress sample. A total of 19 different fatty acids were identified in the watercress sample, with a prevalence of polyunsaturated fatty acids (PUFA). followed by saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Most fatty acids found in watercress were α-linolenic acid (57.456±0.006 %). palmitic acid (17.551±0.013 %) and linoleic acid (14.859±0.013%). α-Linolenic and linoleic acids are essential for human health and should be obtained from diet (Kaur et al.. 2012). Pinela (2020) also identified these three main fatty acids in his study on watercress. Thus. although watercress is a low-fat food. it is mainly composed of n-3 PUFA (C18:3n-3), which is highly beneficial due to its positive effects on brain function and the prevention of cardiovascular, inflammatory, and autoimmune diseases (Simopoulos, 2002).

Table 14. Fatty acids content (%, relative percentage) in watercress (Mean±SD)

Relative percentagem (%)	**Watercress**
Caprylic acid (8:0)	0.155±0.004
Capric acid (10:0)	0.135±0.002
Undecanoic acid (11:0)	0.093±0.002
Lauric acid (12:0)	0.142±0.001

Myristic acid (14:0)	1.137±0.001
pentadecanoic acid (15:0)	0.127±0.001
Palmitic acid (16:0)	17.551±0.013
Palmitoleic acid (16:1)	2.481±0.015
Elaidic acid (18:1n9t)	1.066±0.006
Oleic acid (18:1n9c)	2.022±0.011
Linoleic acid (18:2n6c)	14.859±0.013
α-linolenic acid (18:3n3)	57.456±0.006
Arachidic acid (20:0)	0.359±0.012
Cis-11,14-eicosadienoic acid (20:2)	0.217±0.005
Cis-11,14,17-eicosatrienoic acid (20:3n3)	0.189±0.013
Behenic acid (22:0)	0.413±0.011
Tricosanoic acid (23:0)	0.126±0.006
Lignoceric acid (24:0)	0.887±0.01
Nervonic acid (24:1)	0.591±0.006
Saturated Fatty Acids (SFA)	**21.122±0.005**
Monosaturated Fatty Acids (MUFA)	**6.159±0.009**
Poliinsaturated Fatty acids (PUFA)	**72.721±0.015**

Table *15* and **Table *16*** show that the samples contain 27 types of fatty acids, with most of the samples containing more monounsaturated fatty acids (MUFA). This result is positive, as it is recommended to reduce the intake of saturated fatty acids due to their association with an increase in cardiovascular diseases, diabetes, cancer and other chronic diseases. On the other hand, the intake of MUFA is beneficial, since they perform relevant physiological functions, such as the prevention of cardiovascular and chronic inflammatory diseases, anti-inflammatory and antithrombotic action, influence on fetal growth and neural development, as well as participation in immunomodulatory functions (Schwingshackl et al., 2011).

Among the raw and cooked pasta, the fatty acids with the highest concentration were oleic acid (18:1n9), palmitic acid (16:0) and linoleic acid (18:2n6c). These levels are similar to those found by Prabhasankar (2009) in a study on the incorporation of Japanese knotweed into pasta. For a diet to be healthy, the food must have a balanced ratio between n-3 and n-6 fatty acids, ideally in the range of 1:2 to 1:4 (w/w) (Prabhasankar et al., 2009). Reports highlight that eating foods rich in long-chain n-3

polyunsaturated fatty acids (PUFA) can have a positive influence on blood lipid composition and the prevention of atherosclerosis (Gebauer et al., 2004).

Taking into account the variables of raw and cooked, raw pasta generally contains higher levels of SFAs compared to cooked pasta, indicating that cooking might lead to some breakdown or alteration of these fatty acids. On the other hand, cooked pasta tends to have higher MUFA levels compared to raw pasta, possibly due to the cooking process influencing the release or transformation of these fatty acids. Also, the raw pasta samples typically exhibits higher PUFA levels compared to cooked pasta, suggesting that cooking might lead to some degradation or loss of these fatty acids.

Regarding the incorporation of freeze-dried or fresh watercress in the pasta samples, freeze-dried pasta tends to have higher SFA levels compared to fresh pasta, which could be attributed to the preservation process involved in freeze-drying. However, the fresh pasta generally contains higher PUFA levels compared to freeze-dried pasta, indicating potential degradation or loss of PUFA during the freeze-drying process.

The differences clearly showed the impact of processing methods (cooking, freeze-drying) on the fatty acid composition of pasta, with variations observed in SFAs, MUFAs, and PUFAs. Additionally, the type of pasta (commercial, negative control, or developed novel functional pastas) and the substitution of ingredients also contribute to the observed differences in fatty acid content.

Table 15. Fatty acids content (%, relative percentage) in raw commercial, negative control and developed novel functional pastas (Mean±SD)

	CS_R	NC_R	PFDW1.5_R	PFDW3_R	PFDW5_R	PFW1.5_R	PFW3_R	PFW5_R
6:0	nd	nd	6.196±0.418	nd	nd	nd	0.487±0.01	nd
8:0	0.051±0.002	nd	nd	nd	nd	nd	nd	nd
10:0	0.041±0.001	nd	nd	nd	nd	nd	nd	nd
12:0	0.008±0.001	nd	nd	nd	nd	nd	nd	0.036±0.001
14:0	0.41±0.007	0.409±0.012	0.398±0.001	0.434±0.04	0.41±0.024	0.576±0.023	0.414±0.034	0.423±0.016
14:1	0.068±0.004	nd	0.07±0.001	nd	nd	nd	nd	0.1±0.001
15:0	0.127±0	0.093±0	0.094±0	0.07±0.001	0.063±0.004	nd	nd	0.091±0.001
15:1	nd	nd	nd	0.293±0.009	nd	nd	nd	
16:0	25.872±0.014	26.873±0.094	27.635±0.424	28.444±0.636	25.29±0.197	29.547±0.178	27.747±0.153	26.911±0.101
16:1	2.227±0.02	2.487±0.052	3.445±0.114	3.879±0.24	1.672±0.04	4.814±0.086	3.374±0.037	3.845±0.037
17:0	0.26±0.011	0.243±0.017	0.206±0.01	0.195±0.007	0.228±0.011	nd	nd	0.186±0.004
18:1n9	37.312±0.044	41.129±0.098	1.842±0.206	42.326±0.952	41.477±0.484	38.779±0.373	38.38±0.033	40.571±0.092
18:2n6	29.233±0.013	24.207±0.006	nd	20.44±0.134	24.671±0.468	22.371±0.096	25.138±0.035	23.375±0.208
18:3n6	0.145±0.007	0.099±0.001	nd	nd	nd	nd	nd	nd
18:3n3	2.092±0.028	1.251±0.105	1.754±0.038	1.816±0.119	2.625±0.022	1.443±0.008	2.045±0.092	1.991±0.089
20:0	0.121±0.001	0.09±0	nd	nd	0.073±0.005	nd	nd	0.079±0.002
20:1	0.403±0.008	0.276±0.01	0.291±0.014	nd	0.327±0.024	0.353±0.004	0.344±0.031	0.067±0.001
20:2	0.109±0.001	0.159±0.005	0.116±0.005	nd	0.234±0.003	nd	nd	0.114±0.006
20:3n6	0.055±0.001	0.074±0.006	0.125±0.004	nd	0.184±0.005	nd	nd	nd

21:0	nd	nd	nd	nd	nd	nd	nd	0.133±0.004
20:4n6	0.752±0.008	1.65±0.089	1.165±0.036	1.322±0.059	1.623±0.086	1.371±0.032	1.262±0.045	1.28±0.046
22:0	0.062±0.002	nd	0.141±0.004	0.271±0.013	0.137±0.009	nd	0.125±0.008	0.161±0.007
20:5n3	nd	nd	nd	nd	nd	nd	nd	0.033±0.004
23:0	0.075±0	0.024±0.001	0.055±0.001	nd	0.044±0.002	nd	nd	0.044±0.001
24:0	0.225±0.006	0.266±0.023	0.345±0.007	nd	0.202±0.013	0.239±0.022	nd	0.152±0.006
24:1	nd	nd	nd	nd	nd	nd	nd	nd
22:6n3	0.364±0.004	0.691±0.012	0.39±0.006	0.494±0.004	0.707±0.096	0.477±0.01	0.57±0.095	0.417±0.025
SFA	**27.25±0.026^{g}**	**27.997±0.043^{f}**	**35.069±0.002^{a}**	**29.413±0.67^{c}**	**26.445±0.168^{h}**	**30.362±0.179^{b}**	**28.773±0.189^{d}**	**28.214±0.07^{e}**
MUFA	**40.01±0.012^{f}**	**43.892±0.056^{c}**	**41.918±0.109^{e}**	**46.497±0.721^{a}**	**43.475±0.468^{d}**	**43.945±0.283^{c}**	**42.098±0.1^{e}**	**44.583±0.127^{b}**
PUFA	**32.748±0.018^{a}**	**28.13±0.035^{d}**	**23.299±0.294^{h}**	**24.072±0.078^{g}**	**30.043±0.508^{b}**	**25.662±0.146^{f}**	**29.014±0.076^{c}**	**27.21±0.189^{e}**

nd- not detected; R – Raw; CS – Commercial samples; NC – negative control (100% wheat); PFDW – Pasta with freeze-dried watercress; PFW – Pasta with fresh watercress; Wheat substitutition – 1.5%. 3%. and 5%. Saturated Fatty Acids (SFA); Monosaturated Fatty Acids (MUFA); Poliinsaturated Fatty acids (PUFA) Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same line correspond to significant differences ($p < 0.05$).

Caproic acid (6:0); Caprylic acid (8:0); Capric acid (10:0); Lauric acid (12:0); Myristic acid (14:0); Myristoleic acid (14:1); Pentadecanoic acid (15:0); *Cis*-10-pentadecanoic acid (15:1); Palmitic acid (16:0); Palmitoleic acid (16:1); Heptadecanoic acid (17:0); Oleic acid (18:1n9); Linoleic acid (18:2n6c); γ-linolenic acid (18:3n6); α-linolenic acid (18: 3n3); Arachidonic acid (20:0); (20:1); *cis*-11,14-eicosadienoic acid (20:2); *cis*-8,11, 14-eicosatrienoic acid (20:3n6); heneicosanoic acid (21:0); arachidonic acid (20:4n6); behenic acid (22: 0); *cis*-5,8,11,14,17- eicosapentaenoic acid (20:5n3); tricosanoic acid (23:0); lignoceric acid (24:0); nervonic acid (24:1); *cis*-4,7,10,13,16,19- docosahexaenoic acid 22:6n3).

Table 16. Fatty acid content (%, relative percentage) in cooked commercial, negative control and developed novel functional pastas (Mean±SD)

	CS_C	NC_C	PFDW1.5_C	PFDW3_C	PFDW5_C	PFW1.5_C	PFW3_C	PFW5_C
6:0	0.013±0.001	nd	nd	nd	nd	nd	nd	nd
8:0	0.021±0.001	nd	nd	nd	nd	nd	nd	nd
10:0	0.05±0.001	nd	nd	nd	nd	nd	nd	nd
12:0	0.116±0.008	nd	nd	nd	0.027±0.001	nd	nd	0.028±0.002
14:0	0.504±0.019	0.394±0.001	0.438±0.004	0.432±0.017	0.676±0.001	0.489±0.033	0.432±0.017	0.385±0.002
14:1	0.043±0.003	0.035±0.001	0.106±0.004	0.093±0.003	0.074±0.001	0.114±0.006	0.093±0.003	0.078±0.003
15:0	0.131±0.001	0.064±0.001	0.076±0	0.099±0.001	0.146±0.011	0.104±0.005	0.099±0.001	0.102±0.008
15:1	0.053±0.004	nd	nd	nd	nd	nd	nd	nd
16:0	26.937±0.123	26.899±0.127	27.374±0.294	28.941±0.186	43.405±0.152	29.09±0.432	28.941±0.186	26.158±0.095
16:1	2.16±0.001	2.477±0.041	4.154±0.075	3.67±0.024	3.177±0.136	4.197±0.057	3.67±0.024	2.821±0.207
17:0	0.251±0.03	0.165±0.007	0.163±0.006	0.212±0.003	0.4±0.006	0.222±0.013	0.212±0.004	0.19±0.011
18:1n9	36.338±0.428	1.435±0.025	44.91±0.458	39.394±0.112	0±0	1.043±0.045	1.043±0.045	1.723±0.03
18:2n6	29.965±0.808	0.838±0.016	18.801±0.065	23.014±0.054	41.491±0.298	0.554±0.028	0.554±0.028	1.212±0.025
18:3n6	nd	nd	nd	0.17±0.001	nd	nd	nd	nd
18:3n3	1.987±0.067	1.221±0.04	1.456±0.008	1.933±0.004	4.517±0.052	1.391±0.112	0.16±0.014	1.931±0.02
20:0	nd	nd	nd	nd	nd	nd	nd	0.083±0.003
20:1	0.203±0.004	0.305±0.008	0.264±0.008	0.303±0.011	0.577±0.008	0.321±0.004	1.933±0.004	0.409±0.019
20:2	0.069±0.012	0.12±0.001	0.119±0.009	0.172±0.003	0.492±0.002	0.159±0.013	0.303±0.011	0.178±0.037
20:3n6	nd	0.091±0.006	nd	0.111±0.001	0.239±0.001	nd	nd	0.081±0.002

21:0	nd	nd	nd	nd	nd	nd	nd	nd
20:4n6	0.595±0.039	1.597±0.04	1.329±0.002	nd	3.023±0.014	1.128±0.015	0.172±0.003	0.977±0.037
22:0	0.176±0.004	0.195±0.007	0.169±0.002	0.094±0.003	0.127±0.001	0.177±0.01	0.111±0.001	0.162±0.008
20:5n3	nd	nd	nd	nd	nd	nd	nd	nd
23:0	0.106±0.008	nd	nd	nd	0.041±0.001	0.029±0.001	0.094±0.003	0.039±0.011
24:0	0.141±0.001	nd	0.275±0.001	0.229±0.013	0.278±0.003	0.366±0.001	0.229±0.013	0.283±0.011
24:1	nd	nd	nd	nd	0.068±0.001	nd	nd	nd
22:6n3	0.295±0.002	0.679±0.018	0.389±0.011	0.675±0.023	1.215±0.064	0.415±0.022	0.675±0.023	0.298±0.002
SFA	**28.444±0.112^{d}**	**27.717±0.111^{e}**	**28.494±0.291^{d}**	**30.006±0.151^{c}**	**45.098±0.151^{a}**	**30.474±0.436^{b}**	**30.117±0.149^{c}**	**27.428±0.11^{f}**
MUFA	**38.797±0.426^{f}**	**44.293±0.039**	**49.434±0.396^{a}**	**43.78±0.058^{d}**	**3.895±0.127^{g}**	**45.079±0.063^{c}**	**45.411±0.073^{b}**	**41.943±0.219^{e}**
PUFA	**32.909±0.692^{b}**	**27.937±0.004^{d}**	**22.093±0.057^{g}**	**26.261±0.023^{e}**	**50.976±0.325^{a}**	**24.586±0.303^{f}**	**24.51±0.02^{f}**	**30.631±0.109^{c}**

nd- not detected; C – Cooked; CS – Commercial samples; NC – negative control (100% wheat); PFDW – Pasta with freeze-dried watercress; PFW – Pasta with fresh watercress; Wheat substitutition – 1.5%. 3%. and 5%. Saturated Fatty Acids (SFA); Monosaturated Fatty Acids (MUFA); Poliinsaturated Fatty acids (PUFA). Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same line correspond to significant differences ($p < 0.05$).

Caproic acid (6:0); Caprylic acid (8:0); Capric acid (10:0); Lauric acid (12:0); Myristic acid (14:0); Myristoleic acid (14:1); Pentadecanoic acid (15:0); *Cis*-10-pentadecanoic acid (15:1); Palmitic acid (16:0); Palmitoleic acid (16:1); Heptadecanoic acid (17:0); Oleic acid (18:1n9); Linoleic acid (18:2n6c); γ-linolenic acid (18:3n6); α-linolenic acid (18: 3n3); Arachidonic acid (20:0); (20:1); *cis*-11,14-eicosadienoic acid (20:2); *cis*-8,11, 14-eicosatrienoic acid (20:3n6); heneicosanoic acid (21:0); arachidonic acid (20:4n6); behenic acid (22: 0); *cis*-5,8,11,14,17- eicosapentaenoic acid (20:5n3); tricosanoic acid (23:0); lignoceric acid (24:0); nervonic acid (24:1); *cis*-4,7,10,13,16,19- docosahexaenoic acid 22:6n3).

5.2.2.4. Mineral elements

The findings presented in **Table *17***) ilustrate the mineral content of watercress, highlighting the concentrations of iron (Fe), zinc (Zn), and potassium (K).

Iron is essential in the formation of hemoglobin and the oxidation of carbohydrates, proteins and fats (Adeyeye and Otokiti, 1999). Zinc is crucial for various enzymes and biological processes, such as cell division, gene expression, immune function and cognitive development. Its deficiency can cause hypogonadism, oxidative damage, immunological problems, changes in taste, neuropsychological impairment and dermatitis (Mafra, 2004). Potassium helps maintain the body's normal physiological function, normal water balance in the body and in balancing the body's pH (Tazoe et al., 2007).

The variation in mineral content among different crops can stem from various factors, as noted by Kawashima (2003). Agricultural practices, soil composition, geographical location, climate conditions, and irrigation methods can all influence the mineral composition of crops like watercress. Additionally, post-harvest handling and processing techniques may impact mineral retention and bioavailability. The rich mineral profile of watercress, particularly its high concentrations of iron, zinc, and potassium, underscores its potential as a valuable dietary source for supporting overall health and well-being. Incorporating watercress into one's diet can provide essential nutrients crucial for various physiological functions and may contribute to a balanced and nutritious diet.

Table 17. Mineral elements content of liophilized watercress (Mean±SD).

Mineral elements	Watercress
[K]/(g/Kg)	52.401±0.048
[Na]/(g/Kg)	9.533±0.273
[Ca]/(g/Kg)	6.643±0.049
[Mg]/(m/Kg)	2.515±0.004
[Fe]/(mg/Kg)	93.901±1.961
[Mn]/(mg/Kg)	51.668±0.305
[Cu]/(mg/Kg)	16.929±5.196
[Zn]/(mg/Kg)	54.487±0.719

K – potassium; Na- sodium; Ca – calcium; Mg – magnesium; Fe – iron; Mn – manganes; Cu – copper; Zn – zinc. Results expressed as mean ± standard deviation (SD).

5.2.2.5. Phenolic compounds composition

The detected compounds in watercress are shown in **Table *18*** with information on their retention time, wavelength of maximum absorption, deprotonated ion, mass fragmentation, tentative identification and quantification. **Table *19*** presentes the same type of information regarding the commercial pastas and the negative control pasta formulated with 100% wheat flour. The same compounds were identified in the developed novel functional pastas and the quantification results are present in **Table *20***.

The phenolic profile of watercress has been extensively described by other authors (Martínez-Sánchez et al., 2008; Pinela et al., 2018; Kyriakou et al., 2022), as also for pastas formulated with 100% wheat flour (Dinelli et al., 2009; Arranz et al., 2010; Dinelli et al., 2011), being clear from **Table *20*** that the developed pastas presents phenolic compounds that coincide with the wheat flour and watercress. In fact, the phenolic compound content of novel functional pastas with watercress exceeds that of commercial samples and negative controls (100% wheat), indicating the added nutritional value of watercress-enriched pasta products. Significant differences ($p < 0.05$) in phenolic compound content are observed between novel functional pastas and commercial samples or negative controls, underscoring the potential health benefits of incorporating watercress into pasta formulations.

The hydroethanolic extracts of watercress reveal a diverse array of phenolic compounds, including flavonoids and phenolic acids. These compounds contribute to the antioxidant properties and potential health benefits of watercress. For instance, quercetin-3-*O*-rutinoside-7-*O*-glucoside, a flavonoid, exhibits a relatively high concentration of 0.472 mg/g extract. This compound is known for its antioxidant and anti-inflammatory properties, potentially contributing to the health benefits associated with watercress consumption (Kwon et al., 2019). Another significant compound is caffeoyl malate, quantified at 2.364 mg/g extract. Caffeoyl malate is a phenolic acid with antioxidant properties, potentially contributing to the overall antioxidant capacity of watercress.

The comparison between raw and cooked novel functional pastas reveals changes in the concentrations of various phenolic compounds. Cooking processes may influence the stability and bioavailability of these compounds. Some compounds, such as apigenin-*C*-pentosyl-*C*-hexoside and isorhamnetin-*O*-sophoroside-*O*-hexoside, are either not detected or present in trace amounts in cooked samples, suggesting susceptibility to heat-induced degradation. On the other hand, compounds like quercetin-3-*O*-rutinoside-7-*O*-glucoside maintain relatively stable concentrations between raw and cooked samples,

indicating resistance to thermal degradation or potential formation during cooking processes.

Delving into the impact of the novel pasta formulations, the quantification of the phenolic compounds, with varying watercress concentrations, highlights the dose-dependent relationship between watercress incorporation and phenolic compound content. Meaning, pastas with higher watercress content (e.g., PFW5_R, PFW5_C, PFDW5_R, PFDW5_C) exhibit higher levels of phenolic compounds, including total phenolic compounds (TPC), total phenolic acids (TPA), and total flavonoids (TF). For instance, kaempferol-O-feruloylhexoside-O-malonylhexoside is quantified at 6.006 mg/g extract in PFW5_R. Additionally, differences in phenolic compound content are observed between freeze-dried and fresh watercress pastas, suggesting potential variations in processing methods and their effects on phenolic compound retention.

Table 18. Retention time (Rt), wavelength of maximum absorption, deprotonated ion, mass fragmentation, tentative identification, and quantification (mg/g extract) of the phenolic compounds in the hydroethanolic extracts of watercress (Mean±SD).

Peak N.	Rt (min)	λmax (nm)	[M-H]⁻ (m/z)	MS²	Tentative identification	Quantification (mg/g extract)
3	5.26	325	341	179(100),135(15)	Caffeic acid hexoside	0.122±0.001
5	7.86	349	325	163(100),145(52)	*p*-Coumaric acid hexoside	tr
6	8.76	367	431	385(34),223(89),163(5)	Roseoside	0.353±0.008
7	9.83	352	771	609(43),301(100)	Quercetin-3-*O*-rutinoside-7-*O*-glucoside	0.472±0.022
9	15.08	328	591	295(100),179(34),133(5)	Caffeoyl malate	2.364±0.063
10	17.72	352	609	301(100)	Quercetin-3-*O*-rutinoside	0.307±0.017
11	19.45	313	279	163(100),133(56)	Coumaroyl mallate	2.566±0.001
12	20.99	329	787	625(100),463(9),301(15)	Quercetin-*O*-coumaroylsophoroside	0.621±0.012
13	23.94	336	947	785(12),630(100),315(32	Isorhamnetin 3-*O*-[(2”-*O*-rhamnosyl)(6”-*O*-glucosyl)]glucosyl7-*O*-glucoside	0.589±0.009
14	24.64	333	801	639(52),463(44),315(37)	Isorhamnetin-*O*-sophoroside-*O*-hexoside	0.351±0.015
15	25.62	329	857	813(28),609(100),285(25)	Kaempferol-di-*O*-hexoside-sinapoyl acetate isomer I	1.707±0.023
16	26.37	336	917	873(49),669(100),505(12),315(11)	Isorhamnetin-*O*-hydroxyferuloylhexoside-*O*-malonylhexoside isomer I	1.368±0.016
17	27.00	334	887	843(100),639(26),505(41),315(17)	Isorhamnetin-*O*-sophoroside-*O*-malonylhexoside	1.309±0.037
18	27.98	368	917	873(52),669(100),505(14),315(10)	Isorhamnetin-*O*-hydroxyferuloylhexoside-*O*-malonylhexoside isomer II	0.158±0.002
19	28.51	343	857	813(32),609(100),285(23)	Kaempferol-di-*O*-hexoside-sinapoyl acetate isomer II	0.454±0.005
20	28.83	332	901	857(50),653(100),489(11),285(5)	Kaempferol-*O*-hydroxyferuloylglucuronide-*O*-malonylhexoside	0.595±0.143
21	29.31	332	871	827(46),623(100),489(8),285(5)	Kaempferol-*O*-feruloylhexoside-*O*-malonylhexoside	6.006±0.011
					Total Phenolic Compounds (TPC)	**19.343±0.299**
					Total Phenolic Acids (TPA)	**5.405±0.055**
					Total Flavonoids (TF)	**13.938±0.244**

tr – trace amounts; Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same line correspond to significant differences ($p < 0.05$).

Table 19. Retention time (Rt), wavelength of maximum absorption, deprotonated ion, mass fragmentation, tentative identification, and quantification (mg/g extract) of the phenolic compounds in the hydroethanolic extracts of commercial and negative control pastas (Mean±SD).

Peak N.	Rt (min)	λmax (nm)	[M-H]⁻ (m/z)	MS²	Tentative identification	Quantification (mg/g extract)			
						CS_R	NC_R	CS_C	NC_C
1	4.15	259	191	173(22),111(100)	Quinic acid	0.49±0.009	0.514±0.032	0.407±0.0002	0.34±0.003
2	5.01	334	563	545(43),503(54),473(100),443(5),413(34),383(12),353(5)	Apigenin-C-pentosyl-C-hexoside isomer I	0.151±0.008	0.134±0.008	0.148±0.001	0.127±0.001
4	5.48	280	401	-	Glycosylated pinosylvin	0.382±0.038	0.387±0.004	0.288±0.001	0.249±0.009
8	14.27	332	563	545(34),503(62),473(100),443(6),413(29),383(10),353(5)	Apigenin-C-pentosyl-C-hexoside isomer II	0.324±0.001	0.312±0.004	0.246±0.007	0.245±0.006
					Total Phenolic Compounds (TPC)	**1.347±0.036**	**1.346±0.024**	**1.088±0.007**	**0.961±0.006**
					Total Phenolic Acids (TPA)	**0.872±0.029**	**0.901±0.036**	**0.695±0.001**	**0.589±0.012**
					Total Flavonoids (TF)	**0.475±0.007**	**0.445±0.012**	**0.393±0.006**	**0.372±0.006**

R – Raw; C – Cooked; CS – Commercial samples; NC – negative control (100% wheat); Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same line correspond to significant differences ($p < 0.05$).

Table 20. Quantification (mg/g extract) of the phenolic compounds found in the hydroethanolic extracts of raw and cooked developed novel functional pastas (Mean±SD).

Peak	PFDW1.5_R	PFDW3_R	PFDW5_R	PFW1.5_R	PFW3_R	PFW5_R	PFDW1.5_C	PFDW3_C	PFDW5_C	PFW1.5_C	PFW3_C	PFW5_C
1	0.747±0.017	1.074±0.025	1.477±0.09	0.627±0.009	0.557±0.22	1.216±0.051	0.76421±0	1.743±0.066	1.444±0.07	1.064±0.016	0.959±0.025	1.86±0.006
2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4	nd	nd	nd	0.265±0.01	nd	0.375±0.006	nd	nd	nd	0.229±0.013	0.489±0.036	nd
5	tr	tr	0.027±0.002	tr	tr	tr	tr	tr	0.058±0.002	tr	tr	tr
6	nd	tr	tr	tr	tr	tr	tr	tr	tr	tr	nd	tr
7	0.073±0.002	0.0675±0.0003	0.097±0.004	0.066±0.001	0.068±0.003	0.074±0.002	0.0737±0.0003	0.079±0.003	0.099±0.002	0.073±0.001	0.0672±0.0002	0.067±0.004
8	0.243±0.004	0.24±0.001	0.229±0.02	0.221±0.009	0.222±0.018	0.284±0.002	0.28±0.018	0.256±0.004	0.274±0.016	0.243±0.008	0.24±0.006	0.23±0.004
9	0.0402±0.0001	nd	0.069±0.003	0.039±0.001	0.0519±0.0005	0.053±0.004	0.054±0.001	0.055±0.003	0.079±0.006	0.048±0.003	0.05±0.003	nd
10	0.067±0.002	0.0648±0.0002	0.071±0.003	0.0665±0.0001	0.068±0.003	0.071±0.001	0.069±0.001	0.07±0.001	0.0763±0.0001	0.068±0.001	0.0651±0.0003	0.064±0.001
11	nd	nd	tr	nd	nd	tr	nd	nd	tr	nd	nd	nd
12	0.069±0.001	0.0666±0.0004	nd	0.0679±0.0004	0.07±0.004	0.076±0.004	0.0695±0.0001	0.072±0.003	nd	0.071±0.002	0.0684±0	0.0651±0.0002
13	0.065±0	0.066±0.003	0.10621±0.00003	0.065±0.001	0.065±0.001	nd	0.067±0.001	0.0679±0.0004	0.079±0.004	0.0643±0.0003	0.066±0.001	nd
14	0.064±0.001	0.0639±0.0005	0.086±0.001	0.064±0.001	nd	0.066±0.001	0.0653±0.0002	0.066±0.001	0.069±0.003	nd	0.0651±0.0005	0.064±0.0005
15	nd	0.0026±0	0.024±0.012	0.0002±0.00001	0.0054±0.0001	0.0076±0.0005	0.00396±0.00001	0.0061±0.0001	0.039±0.003	tr	tr	tr
16	nd	0.067±0.004	0.138±0.002	0.069±0.002	0.065±0.003	0.074±0.004	0.07±0.001	0.0729±0.0002	0.091±0.002	0.066±0.001	0.0702±0.0003	0.064±0.001
17	0.064±0.001	0.066±0.003	0.099±0.001	0.069±0.001	0.065±0.003	0.077±0.005	0.072±0.001	0.075±0.001	0.0819±0.0003	0.066±0.002	0.074±0.001	0.065±0.001
18	nd	0.0649±0.0001	0.065±0.002	0.065±0.001	nd	0.064±0.001	0.064±0.0001	0.06421±0.00003	0.063±0.001	nd	0.0637±0.0004	nd
19	nd	tr	0.0102±0.0003	tr	nd	0.0093±0.0001	tr	0.00087±0.00005	tr	nd	0.00113±0	tr
20	nd	0.0017±0	0.014±0	nd	nd	0.00325±0.00003	0.000198±0.000003	tr	tr	nd	0.0006±0.0002	tr
21	tr	tr	0.013±0.001	tr	nd	0.00182±0.00001	tr	tr	0.0015±0.0001	tr	tr	tr
TPC	**0.685±0.011**	**0.771±0.012**	**1.05±0.01**	**0.792±0.004**	**0.681±0.036**	**0.861±0.019**	**0.888±0.021**	**0.884±0.016**	**1.01±0.003**	**0.698±0.019**	**0.831±0.004**	**0.619±0.012**
TPA	**0.114±0.002**	**0.0675±0.0003**	**0.193±0.003**	**0.105±0.001**	**0.12±0.003**	**0.127±0.006**	**0.128±0.001**	**0.134±0.006**	**0.235±0.006**	**0.121±0.004**	**0.117±0.003**	**0.067±0.004**
TF	**0.572±0.009**	**0.704±0.012**	**0.856±0.007**	**0.687±0.003**	**0.561±0.032**	**0.734±0.013**	**0.761±0.02**	**0.751±0.01**	**0.775±0.009**	**0.577±0.015**	**0.714±0.007**	**0.551±0.008**

nd -not detected; tr – trace amounts; R – Raw; C – Cooked; CS – Commercial samples; NC – negative control (100% wheat); PFDW – Pasta with freeze-dried watercress; PFW – Pasta with fresh watercress; Wheat substitutition – 1.5%. 3%. and 5%. Total Phenolic Compounds (TPC); Total Phenolic Acids (TPA); and Total Flavonoids (TF). Results expressed as mean ± standard deviation (SD). ANOVA - Different letters in the same line correspond to significant differences ($p < 0.05$).

5.2.2.6. Glucosinolate compounds

A total of 13 intact, naturally occurring glucosinolates (GLS) were tentatively identified in hydrophilic extracts of watercress (**Table 21, Figure 9** and **Figure 10**). This group of compounds overall share the same core structure with modifications in the molecule side chains that generate a myriad of different glucosinolates. These structurally related compounds, many of which isomers, may have no substantial differences in their physicochemical properties includind polarity, which makes both the chromatographic resolution and the assignment of peaks an analytical challenge (Andini et al., 2019). In the present study, due to chromatographic coelutions among GLS themselves and between them and polyphenols also present in the hydrophilic extract, and since some of these compounds share the same wavelengths of maximum light absorption, it was not possible to selectively detect and quantitate GLS through UV-Vis absorption (**Figure 9**). This fact is commonly reported during the chemical characterization of this group of compounds (Kyriakou et al., 2022). Thus, the glucosinolates were detected by mass spectrometry, with improved sensitivity and selectivity (**Figure 10**).

GLS were assessed in the negative ionization mode according to the presence of deprotonated molecules $[M-H]^-$ in MS spectra consistent with compounds containing sulphur and nitrogen atoms, along with characteristic fragmentation pattern in MS^2. Extracted ion chromatograms of $[M-H]^-$ of the glucosinolates tentatively identified can be seen in **Figure 10**. In the MS^2 spectra, the presence of an intense hydrogen sulphate $[HSO_4]^-$ ion at *m/z* 97 was the main characteristic observed among the compounds. The presence of diagnostic ions such as the sulphated glucosyl ion at *m/z* 259, besides those at *m/z* 195, 241 and 275, was also consistent among the identified compounds. These spectral features and the chromatographic behaviour of compounds tentatively identified herein are in line with previous reports of glucosinolate identification in cruciferous vegetables by mass spectrometry (Bianco et al., 2017; Andini et al., 2019; Missinou et al., 2022, Kyriakou et al., 2022).

Table 21. Chromatographic and spectrocopic characteristics of glucosinolates from watercress extract with their tentative identification.

Peak [a]	RT (min) [b]	λmax (nm) [c]	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*) [d]	Tentative Identification
1	2.93	266	438	275(2),259(4),195(3),135(8),97(100),80(29)	Glucocheirolin
2	3.66	279	408	291(6),241(7),97(100)	Glucotropaeolin
3	3.90	281	388	275(3),97(100),80(45)	Progoitrin
4	4.44	286	478	275(4),195(4),97(100),80(60)	Glucosiberin
5	4.90	228	447	241(3),195(1),97(100),80(37)	Glucobracissin
6	4.99	228	520	259(6),241(5),97(100),80(33)	Glucocamelinin
7	5.00	228	422	275(3),259(4),241(1),195(1),97(100),80(26)	Gluconasturtiin
8	5.60	320	492	275(2),259(3),97(95),80(46)	Glucohirsutin
9	5.77	260, 330	402	275(2),259(3),195(2),97(100),80(34)	Methylpentyl-GLS
10	6.64	320	477	275(2),259(2),195(1),97(100),80(57)	4-Methoxyglucobracissin
12	7.38	252, 267, 334	418	275(3),259(5),195(2),97(100),80(35)	Glucoraphasatatin
12	7.81	270, 326	416	241(9),97(100)	Heptyl-GLS
13	8.61	280, 330	462	259(6),195(3),97(100),80(65)	7-(methylthio)heptyl-GLS

GLS: glucosinolate. [a] Numbered according to the MS chromatograms shown in Figure 9. [b] Retention time on PFP column. [c] Gradient of 0.1% FA in water and 0.1% formic acid in acetonitrile. [d] MS2 fragment ion (*m/z*) is accompanied by its relative abundance between parentheses.

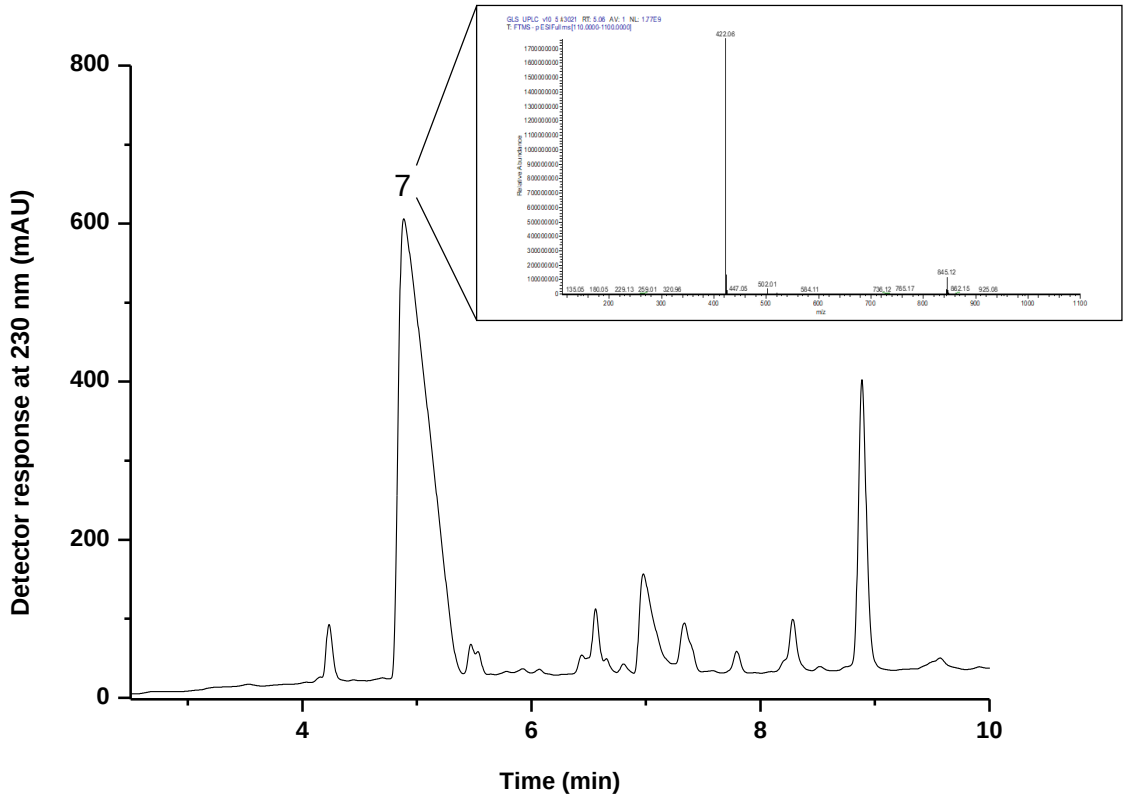


Figure 9. Representative chromatogram, obtained by HPLC-DAD, of glucosinolates in watercress extract. Only a portion of the chromatogram is shown to highlight the intensity of the chromatographic signal in the retention time of gluconasturtiin (peak 7, Table 21). Several peaks of phenolic compounds can also be observed. Inset: the MS spectra of gluconasturtiin.

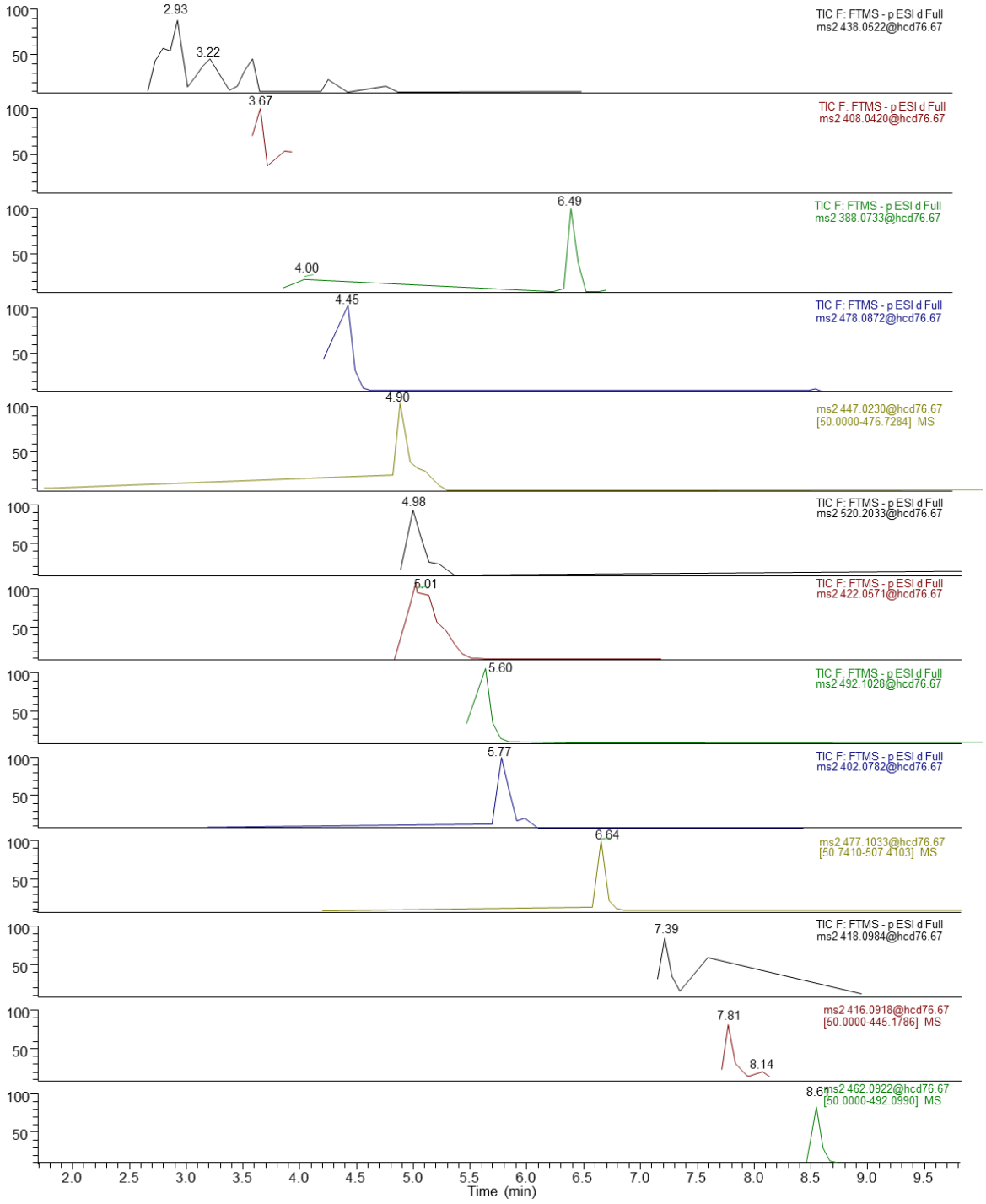


Figure 10. Extracted ion chromatograms of ions at m/z corresponding to the deprotonated molecules of glucosinolates found in watercress extract. Peaks can be noticed in the retention times of the respective compounds listed in Table 21.

The GLS found in this study are well-documented compounds in watercress or related species. In particular, gluconasturtiin (2-phenethyl glucosinolate) has been

reported as the major glucosinolate in watercress leaves, accounting not only for the largest share of compounds but also contributing for the characteristic taste of this leafy vegetable (Kykiakou et al., 2022). Despite of the peak coelution and that an absolute quantitation is not provided herein, the intensity of the chromatographic signal at 233 nm (**Figure 9**) and the considerable abundance of the deprotonated molecule of gluconasturtiin at *m/z* 422 suggest its large contribution to the chemical profile of this extract.

It is expected, therefore, that the incorporation of watercress to the pasta can contribute to the enrichment of this food product with glucosinolates, whose health-promoting activities have been largely reported.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

The comprehensive analysis presented in this study analysed the complicated relationship between ingredient composition, processing methods, and the physicochemical properties of pasta. Examining raw and cooked pasta formulations supplemented with watercress, both freeze-dried and fresh, provides valuable insights into how these additions affect various attributes such as color, texture, nutritional composition, and bioactive compounds.

The color attributes of pasta are crucial for consumer acceptance and are influenced by both raw ingredients and processing methods. The incorporation of watercress led to significant changes in color parameters, with higher substitution levels resulting in darker and greener pastas. Freeze-dried watercress generally led to darker colors compared to fresh watercress, and cooking tended to lighten the pasta while shifting towards green hues and reducing yellow hues. These findings underscore the importance of ingredient selection and processing in determining the final appearance of pasta products.

Solids loss during cooking and weight gain are essential indicators of pasta quality and texture. The addition of watercress generally reduced solids loss compared to the commercial sample, indicating improved retention of solids during cooking. However, the effect varied depending on the type and level of watercress substitution. Similarly, weight gain during cooking differed among formulations, with some showing decreased weight gain at higher substitution levels. The viscosity profile of pasta doughs provided insights into their textural characteristics and cooking behavior. Peak viscosity, breakdown, final viscosity, and setback values varied across formulations, reflecting differences in starch gelatinization, retrogradation, and overall dough stability. Watercress incorporation influenced these parameters, with variations observed based on the type and level of substitution.

On the other hand, while the commercial sample exhibited robust texture, the addition of watercress led to changes in firmness and shear strength, influenced by factors such as water retention, gluten development, and ingredient interactions. Both raw and cooked functional pasta samples showed alterations in texture compared to the commercial sample, highlighting the impact of ingredient substitution on sensory attributes.

The nutritional composition of pasta suffered significant modifications with watercress supplementation. Increases in protein and fat content were observed, reflecting the nutritive contributions of watercress, which is rich in proteins and essential fatty acids. Carbohydrate content showed variations depending on the type and level of watercress substitution, highlighting the importance of balancing nutritional profiles in functional pasta formulations. The addition of watercress enriched pasta products with essential nutrients and bioactive compounds, enhancing their nutritional value and potential health benefits. Watercress supplementation also enhanced the bioactive compound content of pasta, particularly phenolic compounds and fatty acids. These compounds contribute to antioxidant, anti-inflammatory, and other health-promoting properties, offering potential health benefits to consumers. Differences in bioactive compound profiles were observed between freeze-dried and fresh watercress formulations, as well as between raw and cooked samples, highlighting the importance of processing methods in preserving bioactive compounds.

In CONCLUSION, the integration of watercress into pasta formulations represents a promising approach to developing functional pasta products with enhanced sensory, nutritional, and health-promoting attributes. This study demonstrates the multifaceted impact of watercress supplementation on pasta properties, from color and texture to nutritional composition and bioactive compound content. Further research and optimization efforts are necessary to fully understand the potential of watercress-enriched pasta in meeting consumer preferences for healthier and more nutritious food options.

To optimize the FUTURE PERSPECTIVES of functional watercress pasta, the following approaches are suggested:

- Carry out more detailed research into the antioxidant action of pasta, taking advantage of the strong antioxidant effects of watercress;
- Evaluate the antimicrobial activity of the product, as well as its storage conditions, in order to guarantee its microbiological safety and quality over time;
- Analyze the fiber content of functional pasta, considering the potential of watercress as a source of dietary fiber, which can contribute to intestinal health and weight control;

- Assess the bioaccessibility of the bioactive compounds present in the pasta to understand whether the compounds resist to digestion conditions and are converted into their absorbable forms after the digestive process;
- Investigate the bioactive properties of the bioaccessible fraction of the pasta to assess whether its properties are maintained after human consumption and during digestion, which is crucial to ensure the nutritional and functional efficacy of the product;
- Sensory analysis of the functional pasta, especially in relation to the color and sharp flavor imparted by watercress, in order to assess the acceptability of the product for commercial purposes, taking into account consumer preferences;
- Improvement of the chromatographic and spectroscopic method of glucosinolate analysis, to be able to quantify the glucosinolates.

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