CATÓLICA
ESCOLA SUPERIOR DE BIOTECNOLOGIA
PORTO

THE VALORISATION OF BROCCOLI BY-PRODUCTS TO DESIGN A NEW FUNCTIONAL PASTA

by
Mariana Sampaio Nogueira

September 2021

CATÓLICA
ESCOLA SUPERIOR DE BIOTECNOLOGIA
PORTO

THE VALORISATION OF BROCCOLI BY-PRODUCTS TO DESIGN A NEW FUNCTIONAL PASTA

Thesis presented to *Escola Superior de Biotecnologia* of *the Universidade Católica Portuguesa* to fulfil the requirements of Master of Science degree in Biotechnology and Innovation

by

Mariana Sampaio Nogueira

Supervisor: Doctor Natalia Drabińska (PhD)

Tutor: Master Ana Pimenta (MSc)

September 2021

This thesis is dedicated to my parents, Tita and Guilherme!

Resumo

A procura por novos produtos alimentares com benefícios adicionais para a saúde está a aumentar devido ao envelhecimento da população e aumento do número de consumidores preocupados com a saúde. Os subprodutos de frutas e vegetais têm sido descritos como boas fontes de compostos bioativos com atividades biológicas importantes tal como propriedades antioxidantes. As folhas de brócolos são normalmente consideradas um produto residual do processamento destes vegetais. Os estudos têm demonstrado que, à semelhança da parte edível, as folhas de brócolos contêm grandes quantidades de fitoquímicos, podendo ser utilizadas na formulação de novos produtos alimentares.

Neste contexto, o principal objetivo deste trabalho foi desenvolver um novo alimento funcional da categoria das massas através da incorporação de pó de folhas de brócolos (BLP) em concentrações até 5%. Os efeitos da adição de BLP sobre os parâmetros tecnológicos, propriedades sensoriais, composição química, perfil em compostos voláteis e capacidade antioxidante da massa foram avaliados em comparação com uma amostra controlo.

O BLP demonstrou ser uma boa fonte de proteínas e minerais e de compostos bioativos, como glucosinolatos (GLS) e compostos fenólicos. A adição de BLP resultou numa melhoria geral das propriedades nutricionais da massa, visível pelo aumento significativo do teor mineral. A incorporação de BLP influenciou o conteúdo de GLS na massa. Este foi superior ao previsto, o que pode ser explicado pela libertação de GLS durante o processamento térmico. Os compostos neoglucobrassicina e glucobrassicina foram os GLS predominantes no BLP e na massa. A adição de BLP aumentou o teor de compostos fenólicos totais da massa e a sua atividade antioxidante (determinada pelos métodos ABTS e FRAP). Durante a cozedura, a perda de matéria para a água aumentou com a adição de 2,5% de BLP, no entanto, todas as amostras permaneceram no intervalo aceitável de 8g/100g. O tempo ótimo de cozedura e a absorção de água diminuíram com a adição de BLP, enquanto o “swelling index” aumentou. Relativamente à cor e textura das amostras, os valores de L*, a*, “firmness” e “total shearing force” diminuíram à medida que a quantidade de BLP na massa era superior, enquanto o valor de b* aumentou. O perfil em compostos orgânicos voláteis da massa alterou-se após a adição de BLP, tendo sido detetados compostos que contribuíram para o aparecimento do típico odor a couve. Contudo, os resultados da análise sensorial indicaram que a adição de BLP não afetou a qualidade global da massa, uma vez que todas as amostras obtiveram ótima pontuação sensorial (9 numa escala de 10 pontos). Assim, para concentrações até 5% de BLP, a massa permaneceu aceitável ao nível da textura, aparência, odor e sabor.

Os resultados obtidos indicam que o BLP pode ser utilizado com sucesso como ingrediente de massas alimentícias. Em conclusão, a adição de BLP permitiu melhorar o potencial nutracêutico da massa sem comprometer a sua qualidade tecnológica e sensorial. Assim sendo, as folhas de brócolos são uma alternativa interessante para a indústria alimentar, para fornecer aos consumidores novos produtos da categoria das massas com benefícios adicionais para a saúde.

Palavras-chave: Folhas de brócolos; subprodutos; alimentos funcionais; propriedades tecnológicas; glucosinolatos; capacidade antioxidante.

Abstract

The demand for new food products with additional health benefits is growing mostly due to an ageing population and increasing health-conscious consumers. Fruit and vegetable by-products have been described as good sources of bioactive compounds with important biological activities such as antioxidant properties. Broccoli leaves are usually perceived as a waste product of broccoli processing. Studies have reported that similarly to the edible parts, broccoli leaves contain large amounts of phytochemicals thus they can be used to design novel food products.

In this context, the main objective of this work was to develop a new functional pasta product enriched with broccoli leaf powder (BLP) up to concentrations of 5%. The effects on the technological parameters, sensory properties, chemical composition, volatile profile, and antioxidant capacity of the fortified pasta samples were evaluated in comparison with a control sample.

BLP was a good source of nutritional components, including proteins and minerals, and bioactive compounds such as glucosinolates (GLS) and phenolics. The addition of BLP to pasta resulted in an improvement of several nutritional properties, which was demonstrated by a significant increase in the ash content. The incorporation of BLP influenced the content of GLS in pasta. GLS content was higher than expected, which could be attributed to the release of partially bound GLS during thermal processing. Neoglucobrassicin and glucobrassicin were the predominant GLS in BLP and in pasta. The addition of BLP to pasta increased their total phenolic content (TPC) and antioxidant activity (determined by ABTS and FRAP assays). Cooking loss increased with the addition of 2.5% of BLP, however, all pasta samples were in the acceptable range of 8g/100g. BLP incorporation decreased optimal cooking time and water absorption but increased the swelling index. Regarding colour and texture parameters, lightness (L*), redness (a*), firmness and total shearing force decreased as the amount of BLP in pasta increased, while yellowness (b*) increased. The volatile profile of pasta changed due to the addition of BLP. Dimethyl sulfide, detected in supplemented pasta after BLP incorporation, contributed to pasta flavour with a sulphurous cabbage-like odour. However, the sensory evaluation results indicated that the addition of BLP did not affect the overall acceptance of pasta, since all samples had great sensorial score (9 on a 10-point scale). Thus, for concentrations up to 5% of BLP, pasta remained acceptable for its texture, appearance, odour, and taste.

The obtained results indicated that BLP can be successfully used as an ingredient of pasta products. In conclusion, the addition of BLP to pasta allowed an improvement in the nutraceutical potential without compromising its technological and sensory quality. Therefore, broccoli leaves are an interesting alternative for the food industry to provide new value-added pasta products to consumers.

Keywords: Broccoli leaves; by-products; functional food; technological properties; glucosinolates; antioxidant activity.

Contents

List of Tables

1. Introduction

1.1. Health-promoting compounds and the potential for by-product utilization

Broccoli, alongside with cabbage, cauliflower, and Brussels sprouts, belongs to the *Brassica* genus of plants and is one of the most popular vegetables in the temperate climate zone (1). In the last few years, broccoli has been labelled as a health-promoting food due to its high content in bioactive phytochemicals such as glucosinolates (GLS) and isothiocyanates, phenolic compounds with high antioxidant activity, vitamins (C, E, A and K) and dietary essential minerals (Ca, Mg, Na, K, Fe, Zn, etc.) (2). In fact, epidemiological studies have shown that a diet rich in cruciferous vegetables, including broccoli, can lower the risk of some types of cancer in humans, particularly lung and those of the gastrointestinal tract (1, 3, 4).

In the last decade, GLS from broccoli have been widely investigated. GLS are a large group of sulphur-containing glucosides with chemopreventive properties, which are by themselves primarily inactive (5). Upon consumption or processing, the endogenous enzyme myrosinase hydrolyses GLS into several breakdown products, including isothiocyanates, that are mainly associated with anticarcinogenic effects (1). In human gut, the conversion of intact GLS to isothiocyanates is mediated by the microbiota of the colon (3). Formation of other hydrolytic products such as nitriles and cyanides from GLS is highly probable, but the full understanding of their biological significance and actual health effects in humans is still poorly documented (6). The chemopreventive properties associated with isothiocyanates vary from the elimination of xenobiotics, modulation of oestrogen activity and metabolism, inhibition of inflammation-induced malignancy to induction of apoptosis and inhibition of angiogenesis in a growing tumour (1). Recent data suggest that GLS and their metabolic products can inhibit cytochrome P450 enzymes, which are responsible for metabolizing and activating many carcinogenic agents (3).

Unfortunately, storage conditions and culinary processes strongly influence the content of GLS in cruciferous vegetables, including broccoli. Data suggest that combining low storage temperatures, use of radiation and controlled atmosphere packaging contributes to a good conservation of the GLS present in the food. Regarding culinary processes, the most harmless culinary thermal technique for GLS seems to be steaming, while the one that most affects their content is longitudinal boiling (3).

In the case of broccoli, glucoraphanin is the main glucosinolate (3). Glucoraphanin is the precursor of 1-Isothiocyanato-4-(methanesulfinyl)butane, known as sulforaphane, which is considered as one of the most active anticancer agents among isothiocyanates (7). In fact, evidence suggests that sulforaphane from broccoli is protective against a variety of chronic and even infectious (e.g., *Helicobacter pylori*) conditions (7). Glucoraphanin occurs in all tissues of broccoli plants, but the absolute concentration is higher in floret tissues (8). However, data suggest that leaves of broccoli also contain considerable amounts of this compound (8).

A significant portion of some Brassica crops are not harvested or utilized, which is a problem in modern agricultural production systems (8). Regarding broccoli, only 10–15% of the total aerial biomass of the

plant is consumed, which means that the possible consumption of stems and leaves would reduce the agrowaste and increase productivity and sustainability of the World's broccoli crop, by increasing yield in almost 70% (8). Broccoli leaves are rarely acceptable for food consumption, although they are rich sources of GLS, polyphenols, and other essential nutrients (8, 9). Among different tissues of broccoli, leaf exhibited the highest concentration of β-carotene, violaxanthin, neoxanthin and lutein (8). β-carotene possess provitamin A activity, which is beneficial against eye diseases (10). Therefore, compared to broccoli florets, leaves are an excellent source of vitamin A. A high carotenoid intake through diet can also be related to a reduced risk of some cancers and cardiovascular diseases (10). Moreover, broccoli leaves, in contrast with broccoli florets, are an excellent source to provide vitamin E and vitamin K for the human diet (8). Vitamin E seems to be protective against cardiovascular disease, inflammation, diabetes, prostate cancer and Alzheimer's disease (11). Vitamin K has beneficial effects on blood coagulation, bone health, and its consumption is related to a reduced risk of vascular calcification and cardiovascular disease (12).

Annually, one-third of the food produced in the world for human consumption, corresponding to approximately 1.3 billion tonnes, is lost or wasted. Quantitatively this represents a cost of around 990 billion dollars, comprising food losses and food processing wastes (13). Food processing wastes are frequently defined as by-products derived from processing raw materials to food (14). It is highly consensual among the scientific community and recognized world organizations, that the fruit and vegetable processing industry is one of the greatest producers of by-products, generally in the form of peels, kernels, pomace, unripe and/or injured fruits and vegetables (15). On average, this industry generates 50% of agrowaste by-product (16). In 2014, Europe generated 100.3 and 73.7 million metric tons of vegetables and fruit waste, corresponding to 8.6% and 8.9% of global world production, respectively. In a near future, an increasing is expected due to an increasing world population and hence food production. For this reason, there has been a social, political, and environmental pressure to improve profit and value of fruit and vegetable by-products (14). In fact, in the last few years, we have witnessed an increase in the recovery, recycling and upgrading residues from plant food processing (17).

Food production is limited by resources across much of the planet and increasing the use of vegetable by-products represents an opportunity to reduce scarcity worldwide (8). Many by-products from fruit and vegetable processing may be useful as sources of nutrients and functional ingredients, giving the opportunity to obtain added value products without increasing production costs, since by-products are low-cost sources of bioactive molecules (2). Some countries already have advanced by-product utilization, including leaves of pepper, pumpkin, sweet potato, cassava, and soybean (8). Broccoli leaves have the potential to be the next edible leafy vegetable because findings suggest that leaf tissue is a good source of health-promoting compounds (8).

Utilization of by-products as new sources of anticancer and anti-inflammatory compounds is an excellent utilization of agrowaste that will benefit human health as well as provide new sources of income for farmers (8). Since broccoli by-products and broccoli florets have similar chemical composition, broccoli leaves could constitute a functional food additive. Also, the incorporation of broccoli leaves into

functional foods could facilitate the management of vegetable processing wastes. However, broccoli leaves are rarely added to food products (9). Broccoli by-products have been used traditionally as an animal feedstuff and several investigators have further explored broccoli by-products as antimicrobial agents for foodborne or soil-borne bacteria, since the hydrolysis products of GLS have antimicrobial activity (8). Recent studies have attempted to incorporate broccoli by-products as additives to improve the quality and/or nutritional value of several foods (5, 9, 18). Broccoli leaves were successfully used to develop new gluten-free mini sponge cakes (9) and gluten-free bread (19). These studies highlight the viability of broccoli by-products as a good source of bioactive compounds, such as GLS and phenolics, for the human diet.

1.2. Functional foods: definition, development, opportunities, challenges

It has been known for a long time that the food we eat clearly affects our overall health. Although the primary role of diet is to provide adequate nutrients for our body and to satisfy our metabolic needs, some groups of foods, in addition to their nutritional properties, show other beneficial properties for health. These types of foods are generally called functional foods (20).

The concept of functional foods appeared for the first time in Japan, in the early 1980´s. Originally, foods that had the ability of modulating the body function and therefore contribute to the prevention of a disease were called functional foods (21). The current concept has resulted from the identification of the mechanisms by healthy foods modulate metabolism and health (20). The European Food Safety Authority (EFSA) defines functional foods as: "A food, which beneficially affects one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. A functional food can be a natural food or a food to which a component has been added or removed by technological or biotechnological means, and it must demonstrate their effects in amounts that can normally be expected to be consumed in the diet" (22). In this context, functional foods should be resembling to traditional foods that are consumed as part of our normal diet and they must remain as foods, not a capsule, a pill or any form of dietary supplement (20). The products produced from foods but sold in medicinal forms, which have demonstrated physiological benefits, are called nutraceuticals. Overall, both nutraceuticals and functional foods provide a means to reduce the increasing burden on the health care system (23).

For the Food and Agricultural Organization of the United Nations (FAO), a functional food should be a food similar in appearance to a conventional food, consumed as part of a usual diet, that contains biologically active components with demonstrated physiological benefits, and offers the potential of reducing the risk of chronic diseases beyond basic nutritional functions (23). In the USA, the Functional Food Center currently define functional foods as "natural or processed foods that contain known or unknown biologically active compounds, which, in defined, effective, and non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers for the prevention, management, or treatment of chronic disease or its symptoms" (21).

As it can be seen, functional foods have no global accepted definition. However, from a practical point of view, they can generally be:

- a natural food including a health promoting component;
- a food in which one of the components has been enhanced by employing certain agronomical conditions;
- a food from which a component has been removed to produce less adverse effects;
- a food to which a component has been added to provide benefits;
- a food in which a component has been modified by enzymatic, chemical or technological means to provide a benefit;
- a food in which a component has been replaced by an alternative component with favourable properties;

- a food in which the bioavailability of a component has been modified to improve the assimilation of a health promoting component (21, 24).

Other additional considerations have been proposed to define a functional food, including having a particular function when eaten such as enhancement of biological defence mechanisms; prevention of specific diseases; recovery from specific diseases; control of physical and mental disorders; and slowing of the ageing process (20).

Examples of functional foods include foods that contain specific minerals, vitamins, fatty acids or dietary fibre, foods with added biologically active substances such as phytochemicals and probiotics. However, unmodified whole foods such as fruits and vegetables, represent the simplest form of a functional food. For example, broccoli, carrots or tomatoes can be considered functional foods because they are rich in biologically active components such as sulforaphane, beta carotene and lycopene, respectively (23). Regarding broccoli, researchers have concluded that the evidence of sulforaphane health benefits is strong enough to support product development in this field (4).

The combination of increasing levels of income, rapid growth of urbanization and busy lifestyles led to a modern unbalanced diet, which generally includes large amounts of fat and sugar. Consequently, in 2016, noncommunicable diseases including heart disease, stroke and respiratory cancers caused 71% of deaths globally (25). In Europe and in the United States, cancer and cardiovascular diseases are the main causes of death. Epidemiologic studies have shown a link between the consumption of plant-derived foods such as fruits, vegetables and whole grains, and a range of health benefits. In fact, a high dietary intake of these foods is strongly related with reduced risk of developing chronic diseases and it is estimated that an appropriate diet could prevent around one-third of all cancer deaths in industrialized countries (23).

The already established important role of fruits, vegetables and wholegrain cereals in disease prevention and the latest research on dietary antioxidants has helped to provide the impetus for further developments in the functional food market in Europe (26). Yet, the principal reasons for the growth of the functional food market are health trends and current population (23).

In Europe, consumer interest in the relationship between diet, food intake and health has increased significantly. Nowadays, there is much greater recognition that people can reduce the risk of illness and disease and improve their state of health and wellbeing by keeping a healthy lifestyle (26). As a result, consumers desire to not only satisfy their hungry and metabolic needs but also to prevent nutrition related diseases and improve their physical and mental well-being (27). In fact, today's consumers are more prone to choose "natural" and "eco/green" label food products, which are expected to be safe and health-promoting (14). In the upcoming years, it is anticipated that there will be four consumer main trends, namely 1) health and well-being, 2) sustainability, 3) convenience, and 4) organic production and natural foods (25).

A food product can only be classified as functional after its potentially beneficial effect has been proven by well-designed and properly executed intervention studies in humans (20). Generally, seven steps are

needed to validate functional foods and bring them to market. This seven-step process addresses critical aspects in the design, development, and marketing of functional foods and consists of:

1) Establishment of the relationship between the food component and the health benefit.
2) Demonstration of efficacy and determination of the intake level necessary to achieve the desired effect.
3) Demonstration of safety at estimated use levels, including the potential for allergenicity.
4) Development of a suitable food vehicle for the bioactive component, knowing that the selection of a vehicle depends on the stability and bioavailability of the bioactive component in that food.
5) Demonstration of sufficiency of the scientific evidence for efficacy by relying on panels of independent scientists with appropriate expertise.
6) Communication of benefits to consumers.
7) In-market confirmation of efficacy and safety, including information about any adverse effects (28).

Functional foods are a good example of the advances made by industry in food technology and development. However, it is important to remember that these foods should not be an alternative to a varied and balanced diet combined with a healthy lifestyle. Other possible disadvantage of functional foods, from a health education point of view, is that they may overshadow the boundaries between food groups, because normally foods in each group should provide a specific selection of nutrients and no more. This inevitably influences the ease with which simple and practical dietary advice can be formulated (29).

Currently, small, and medium-sized enterprises are facing increasingly new challenges to achieve the successful launch of new food products according to new health trends. These challenges include making the healthy choice the easiest choice; developing value-added food products with superior quality, convenience, availability, and affordability; and achieving a sustainable food production. To overcome these challenges more quickly and be sure that the processes involved are following the existing legislation, it is necessary to establish collaborations with universities, research centres and governments (25).

Since the healthy food and wellness market segment is growing, companies must consider consumer nutrition and health as an integral part of their strategy (30). One way to reach this market is through the reformulation of food products. Efforts should be made to minimize the content of sugar, salt, and fat as well as increase fibre and vitamins. After the product is developed, companies should create a close connection with the consumer by presenting the characteristics of the product and its advantages for human health. Also, in the act of choosing, the consumer must acquire all the information he needs to choose quickly, so it is necessary to simplify the way the label and all the information is displayed on the product packaging (25).

To overcome the challenges of adding value to food products, companies will have to find processes to incorporate relevant ingredients. Also, it is essential to consider the importance of developing technologies to improve the bioactivity/bioavailability of bioactive compounds(25).

Finally, the most difficult challenge is achieving sustainability in the food production chain. However, consumer demand on this subject is growing rapidly. Thus, there are several obstacles that companies are facing, including the need to develop efficient food processing technologies to minimize waste along the chain, to recover and incorporate food by-products into the food chain, and developing new biodegradable and recyclable materials for packaging (25).

1.3. Pasta as a functional food

Nowadays, people are more aware that unhealthy lifestyles are related to several diseases, including obesity and high blood pressure. For this reason, the demand for new food products with additional health benefits is increasing (5). However, consumers are not willing to compromise taste, convenience, or good quality life by choosing functional food items. In fact, they expect those foods to have the same quality they are familiar with from the traditional ones (31).

Bakery products, for their high daily consumption, have been widely studied in the functional food development. Unlike biscuits, cookies and cakes, pasta has been recognized for its nutritional profile (31). Relatively low levels of fat and sodium and a moderate glycaemic index give a reason to consider pasta as a relatively healthy food. Among the cereal products, pasta seems a good vehicle for the incorporation of nutritional additives due to its capacity of maintaining acceptable physical and sensory properties when new ingredients are added. Moreover, pasta is a relatively low price food with a longer shelf life than other bakery products, such as bread and buns, and high worldwide consumption by people from all social groups (32). In 2018, 14.5 million tons of pasta were produced in the world and in many countries, pasta was the second most consumed cereal product, just after bread. Italy is the largest consumer of pasta in the world, with an annual pasta consumption of 23.2 kg per capita (33). In Portugal, the annual consumption was around 6.6 kg per capita in 2013 (34). Pasta is seen as a trendy food with a wide range of acceptability in many population groups, including fitness enthusiasts (32) and children (5). Due to all these qualities, the World Health Organization (WHO) and the Food and Drug Administration (FDA) consider pasta as an appropriate matrix through which functional ingredients can be delivered to the consumer (35).

Generally, the primary pasta ingredient is durum wheat semolina that, compared to other flours, confers excellent rheological properties to the dough, superior colour, good appearance and cooking quality to the final pasta (17). However, durum wheat semolina contributes with only 5% to the total world wheat production, and generally, it is sold at a higher price than the common wheat. Therefore, producing pasta with added new ingredients could be a good option to meet the demand of increasing pasta consumption (32).

During the flour processing, most of the nutrients such as essential amino acids, minerals and vitamins are removed from the wheat grains (32). As a result, different functional ingredients have been used to improve the nutritional and the functional properties of pasta (36). In recent years, many studies have been focused on the incorporation of various ingredients into pasta, namely 1) vegetables (37), 2) legumes such as peas, beans, lentils and chickpea (38, 39), 3) pseudo-cereals such as quinoa (40) and amaranth (41), and 4) animal protein sources such as fish (42, 43) and meat (44). Moreover, attempts have been taken to prepare nutrient-rich pasta fortified with different kinds of fruit and vegetable by-products, including onion skin (45), carrot leaves (46), grape marc (47), mango peel (36) and apple peel (48). However, the replacement of semolina is still a challenge for the food industry since this kind of fortification could affect pasta quality in terms of texture, colour, technological quality, and sensory properties (45).

The incorporation of vegetables in pasta products has significantly increased in the last few years. Initially, the addition of vegetables to pasta was mainly done for sensory reasons such as obtain a different colour. Nowadays, the addition of vegetables to pasta represents a way to contribute to the recommended vegetable intake per day (37). Many studies have proved that a diet rich in fruits and vegetables is protective against the onset of some chronic diseases (5). Hence, nutritionists recommend consuming at least five servings of fruit and vegetables per day to lower the risk (49). Although consumers awareness about the beneficial role of fruit and vegetables is increasing, campaigns to promote the consumption of these foods usually do not have the desired impact and fail by not being able to change consumer's behaviour (50). This may happen because "changing mind" does not necessarily mean "changing behaviour". Therefore, the enrichment of pasta, a regularly eaten product, with fruit and vegetables could be considered a strategy to increase the intake of these healthy foods without drastically changing the eating habits of the population (37). On the other hand, children tend to dislike and avoid eating vegetables, but they usually like pasta. So, incorporating vegetables into pasta might increase children's vegetable intake (5). This approach might be successful if the technology of production allows the healthy compounds to be retained during the pasta production and cooking and if the final product is acceptable for consumers in terms of sensory properties (37).

The sensory acceptability of pasta enriched with broccoli powder (derived from broccoli florets) was previously analysed and the results indicated that all pasta products remained acceptable up to 20% broccoli enrichment (5). The content of GLS in the cooked pasta products was also studied and it was identical for pasta enriched with 20 and 30% of broccoli powder. Therefore, in terms of GLS content, there is no advantage in adding more than 20% of broccoli. Regarding textural properties, the largest changes appeared between 20 and 30% of broccoli enrichment, indicating that the samples cannot cope with so high concentrations of broccoli powder (5). Even so, this was a very promising result because consuming 100 g of 16% broccoli enriched pasta may be the same as consuming a portion of broccoli of around 200 g as fresh weight (37). Nevertheless, it is important to refer that all pasta products enriched with different amounts of broccoli showed a decrease in GLS content after cooking. This reduction probably occurred because GLS are water-soluble compounds and as such, can leach from the pasta into the cooking water (5).

Although pasta making is a relatively simple and mild process, it can affect the nutritional quality of vegetable added to pasta. Drying at very high temperatures generally results in lower cooking losses and firmer structure upon cooking (51). In fact, it was observed that the reduction in glucoraphanin concentration in fresh pasta upon cooking was higher than the reduction in dry pasta upon cooking, which suggests that the lack of drying treatments increases the leaching. Additionally, it was also proposed that the boiling process can be done with less water to reduce the leaching into the cooking water (37).

In addition to what has already been mentioned, agro-industrial by-products are also being considered as nonconventional ingredients in pasta products because they are a major source of antioxidants that can be added to food products to increase their nutritional value (32). For example, apple peel and mango peel are food by-products that are good sources of bioactive compounds such as polyphenols.

In fact, it was shown that the incorporation of apple peel and mango peel powder in pasta significantly increased the total polyphenol content and the antioxidant activity (36, 48). The addition of grape marc powder to pasta also increased the polyphenolic concentration and the antioxidant activity of the cooked product (47). Onion by-products, namely onion skin powder, were also added to pasta (45). The obtained results showed an improvement in nutritional properties, which was confirmed by an increase in the content of dietary fibre, ash, total phenolic compounds, flavonoids content and antioxidant activity (45). These findings suggest that by-products can easily be an interesting alternative for the food industry to provide nutritionally enriched pasta. Moreover, using by-products represents an environmentally friendly way to manage industrial waste that can positively affect final product costs (47).

Regarding broccoli by-products, broccoli leaves were already used as a source of bioactive compounds in a new beverage (18) and were also used to fortify bakery products (9, 19). However, according to the available knowledge, broccoli leaves have never been used to enrich pasta products. In this context, the main objective of this work was to develop a new functional pasta product enriched with broccoli leaf powder (BLP) up to concentrations of 5%. The effect of BLP on the technological parameters, sensory properties, chemical composition, volatile profile, GLS content, and antioxidant capacity of pasta was evaluated.

2. Materials and Methods

2.1. Preparation of broccoli leaf powder

The leaves of broccoli (*Brassica oleracea L. var. italica* cv. Sebastian) were donated by GEMIX, a food distributor from Olsztyn (Poland).

To prepare the broccoli leaf powder (BLP), only the mature leaves without any sign of mechanical damage were selected and washed in tap water to remove soil residues. Leaves were blanched in boiling water for 1 minute to inactivate enzymes, such as myrosinase, responsible for hydrolysing biologically active compounds. The petioles and the main midribs were removed. Then, leaves were freeze-dried using the freeze dryers LABCONCO 195 with rotary vane pump BW-16 (Kansas City, MO, US) and ground into a fine powder with particle size ≤ 0.60 mm. Finally, the BLP was stored in the refrigerator in a tightly closed container for approximately 2 years until the moment of use.

2.2. Preparation of pasta

The pasta was prepared with durum semolina flour, water, olive oil and salt. All the ingredients were purchased locally. BLP was added in the following proportions: 0 % (control), 2.5 % (B2.5), and 5 % (B5) as an additional ingredient to the optimal, control pasta formulation. The percentage composition of control and fortified pasta is shown in Table 2.1.

The ingredients were placed in an electric pasta maker (Ariete Pastamatic 1581, Italy), mixed for five minutes, and extruded through a macaroni forming die. Fresh pasta after extrusion (100 g) was cooked in boiling water and then cooled at room temperature. Both, fresh and cooked pasta samples were freeze-dried, ground into a fine powder with particle size ≤ 0.60 mm and stored in the refrigerator in a tightly closed container until chemical analysis.

Table 2.1 *Percentage composition of control and fortified pasta.*

	Control	B2.5	B5
Semolina	71.8	70.5	69.3
Water	25.1	24.7	24.3
Olive oil	2.7	2.6	2.6
Salt	0.4	0.4	0.3
BLP	0.0	1.8	3.5

2.3. Cooking properties of pasta

2.3.1. Optimal cooking time

To evaluate cooking properties, 20 g of pasta were cooked in 300 mL of boiling distilled water. Optimal cooking time (OCT) was established when the starchy white core disappeared, indicating that the starch at the centre has gelatinized. All the cooking properties were evaluated after cooking at OCT.

2.3.2. Cooking loss

Cooking loss, which indicates the amount of dry matter lost in the cooking water, was evaluated according to the method described by Chillo et al. (2008) (35). The cooking water was collected in a beaker and dried in an air oven at 105°C until completely evaporated. Then, the beaker with the dry residue was weighed. Cooking loss was calculated according to Equation (2.3.1) and expressed as a percentage of the mass of the starting material.

$$Cooking\ loss = \left(\frac{mass\ of\ dry\ residue\ in\ cooking\ water}{mass\ of\ uncooked\ pasta}\right) \times 100\ \% \qquad (2.3.1)$$

2.3.3. Water absorption

Water absorption was evaluated according to the method described by Bustos et al. (2015) (31). It was determined as the percentage of weight increase in relation to uncooked pasta. Water absorption was calculated according to Equation (2.3.2).

$$Water\ absorption = \left(\frac{mass\ of cooked\ pasta - mass\ of\ uncooked\ pasta}{mass\ of\ uncooked\ pasta}\right) \times 100\ \% \qquad (2.3.2)$$

2.3.4. Swelling Index

The swelling index (SI) was evaluated according to the method described by Bustos et al. (2015) (31). It was determined by drying to constant weight the cooked pasta on a drying oven for 24 hours. The SI was calculated according to Equation (2.3.3).

$$SI = \left(\frac{mass\ of cooked\ pasta - mass\ of\ cooked\ pasta\ after\ drying}{mass\ of\ cooked\ pasta\ after\ drying}\right) \times 100\ \% \qquad (2.3.3)$$

2.4. Colour measurements

The colour of raw materials (BLP) and pasta products was determined using a HunterLab ColorFlex (Hunter Associates Laboratory, Inc., Reston, VA, USA). The measurements were performed through a 3-cm-diameter diaphragm containing an optical glass. The colour readings were taken from 10 separate points on the powder samples. The results were expressed as L* (L*= 0 (darkness) and L*= 100 (lightness)), a* (+a* = redness and –a* = greenness) and b* (+b* = yellowness and –b*=blueness). The whiteness index (WI) and the browning index (BI) were calculated according to Equations (2.4.1) and (2.4.2), described by Krupa-Kozak et al. (2020) (52).

$$WI = 100 - \sqrt{(100 - \mathrm{L}^*)^2 + a^{*2} + b^{*2}} \quad (2.4.1)$$

$$BI = \frac{100 \times (x - 0.31)}{0.17} \quad (2.4.2)$$

where:

$$x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}$$

2.5. Texture analysis

Textural properties of BLP-fortified pasta were assessed after cooking the pasta at OCT. Textural parameters of firmness and total shearing force (TSF) were determined using a TA.HD Plus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) equipped with a 5-kg load cell. The pasta samples were compressed at a constant rate of 1.0 mm/s.

2.6. Sensory analysis

The sensory evaluation of experimental pasta products (cooked at OCT) was carried out by a sensory panel consisted of 6 persons previously selected, trained and monitored according to ISO guidelines (53). One day before the analysis, a list of all descriptors of smell, appearance, texture, and taste was prepared by the panel in a round-table session using a standardised procedure (54). All attributes and scale edges were defined to equal understanding by panellists. In total, 13 attributes were evaluated (Table 2.2).

On the day of the actual analysis, the pasta samples were coded with a three-digit number and presented to the assessors all together in a random order in transparent plastic boxes. The sensory quality of products was evaluated by Quantitative Descriptive Analysis (QDA) method (54). A 10

centimetres scale with arbitrary units was used for the evaluation of each attribute. The overall quality of investigated products was estimated to summarise all evaluated attributes, their compatibility and harmonization. The assessments were carried out in a sensory laboratory room under normal lighting conditions at room temperature, fulfilling the requirements of the ISO standards (55).

A computerized sensory program (FIZZ, Biosystemes, Counternon, France) was used to evaluate and collect the results.

Table 2.2 *Sensory attributes, their definitions and scale edges used in QDA of pasta products.*

Attribute	Definition	Scale edges
Odour		
flour	odour corresponding to wheat flour	none - very intensive
cabbage	odour corresponding to boiled cruciferous vegetables (e.g. broccoli, cabbage)	none - very intensive
Appearance		
creamy	colour intensity (colour intensity according to colour pattern RAL 075 90 20 - scale value 6)	light - dark
green	colour intensity (colour intensity according to colour pattern RAL 110 50 20 - scale value 8)	light - dark
Texture (manual)		
elasticity	the extent to which a piece of product returns to its original length when stretched	low - high
Texture (in the mouth)		
hardness	the force required to bite through the sample placed between the front teeth	low - high
chewiness	the multiplicity of chewing the product to prepare it to swallow	low - high
adhesiveness	degree of adhesiveness while chewing the sample 10 times	low - high
Taste		
flour	as the corresponding odour (measured in the mouth)	none - very intensive
cabbage	as the corresponding odour (measured in the mouth)	none - very intensive
sweet	basic taste illustrated by sucrose dissolved in water (3%)	
aftertaste	lingering sensation after swallowing the sample	none - very intensive
Overall quality	overall quality contains all attributes and their harmonisation	low - high

2.7. Proximate chemical composition

2.7.1. Mineral content

The ash content was determined using a standard method (56). Briefly, approximately 2 g of pasta samples or BLP were weighted into containers previously washed in aqua regia (a mixture of nitric and hydrochloric acid in 1:3 ratio (v/v)), rinsed with distilled water and heated in a muffle furnace for 1 hour at 585 ˚C. Then, samples were pre-burned until the moment when they stopped smoking. After that, samples were placed in a muffle furnace for 1 hour at 585 ˚C. After cooling in a desiccator, the samples were weighed, and the ash content was calculated according to Equation (2.7.1).

$$Ash = \frac{mass\ of\ sample\ after\ oven}{mass\ of\ sample\ taken} \times 100\ \% \quad (2.7.1)$$

2.7.2. Moisture

Moisture content was calculated using an oven drying procedure (56). First, cooked pasta (roughly 25 g) was put on a filter paper and placed on a drying oven for 24 hours. Then, dried cooked pasta was weighed and ground into a fine powder. After that, approximately 2 g were placed in glass weighing bottles and pasta samples were placed in a drying oven at 130 ˚C for 1 hour. After cooling in a desiccator, the samples were weighed, and the moisture contents were calculated according to Equation (2.7.2).

$$Moisture = X1 + X2 \quad (2.7.2)$$

where:

$$X1 = \left(1 - \frac{mass\ after\ drying\ on\ oven\ for\ 24h}{mass\ of\ sample\ taken}\right) \times 100\ \%$$

$$X2 = \left(1 - \frac{mass\ after\ drying\ 1h\ in\ oven\ at\ 130^{\circ}C}{mass\ of\ sample\ taken}\right) \times 100\ \%$$

2.7.3. Protein content

The protein content was determined with the use of the Kjeldahl method (56). This method involves a three-step approach to the quantification of protein: digestion, distillation, and titration. First,

approximately 0.5 g of pasta samples or BLP were placed in a Kjeldahl digestion tube. Then, the tube was heated together with 10 mL of concentrated sulfuric acid in the presence of a catalyst (potassium and copper sulfate tablet) in a heat block set at 400 °C for 90 minutes. In this process, the nitrogen in the sample was converted to ammonium sulfate. After digestion, the tubes were cooled at room temperature and inserted in an automatic Kjeldahl distillation system (Kjeltec™ 2200, FOSS, Hilleroed, Denmark). Ammonia was formed, distilled off and collected in a receiving flask of excess boric acid, forming ammonium borate. The boric acid was then titrated with a 0.1 N hydrochloric acid to the change of colour into pink to estimate the total nitrogen content of the sample (Equation (2.7.3)). Protein content was then calculated from the nitrogen concentration of the food using a specific conversion factor of 5.70 for wheat products (Equation (2.7.4)).

$$N\ content = \frac{Volume\ of\ HCl\ (mL) \times 0.1 \times 1{,}4007}{mass\ of\ sample\ (g)} \quad (2.7.3)$$

$$Protein\ content = N\ content \times 5.70 \quad (2.7.4)$$

2.7.4. Fat content

To determine the fat content, samples of pasta or BLP (0,5 g) were vortexed with 2 mL of *n*-hexane for 1 minute. Then, they were centrifuged for 5 minutes at 4000 rpm, and the hexane layer was collected. The process was repeated 5 times and the hexane layers were combined. In the end, the *n*-hexane was evaporated in a hitting block set at 70˚C, and the remaining residue was weighted. The percentage of fat was calculated according to Equation (2.7.5).

$$Fat\ = \left(\frac{mass\ of\ remaining\ residue}{mass\ of\ sample\ taken}\right) \times 100\ \% \quad (2.7.5)$$

2.7.5. Energy value

The total carbohydrate content was estimated by subtracting the protein, fat, ash, and moisture content from 100%. The energy value was calculated using the Equation (2.7.6). Energy values are 4.0 kcal/g for protein, 9.0 kcal/g for fat and 4.0 kcal/g for carbohydrates (57).

$$Energy\ value\ (kcal/100g) = 4 \times protein\ (g) + 9 \times fat\ (g) + 4 \times carbohydrates(g) \quad (2.7.6)$$

2.8. Determination of glucosinolate content

The GLS content of fortified pasta and BLP was determined according to the Official Journal of the European Communities (1990) (58), with slight modifications. Since the pasta samples contain a relatively high content of fat, the defatting step was required. First, 2 g of pasta samples and 5 mL of *n*-hexane were vortexed for 1 minute at a maximum speed. Then, they were centrifuged for 5 minutes at 4000 rpm and the hexane layer was removed. The process was repeated 3 times. The degreased powder was dried under a stream of nitrogen until *n*-hexane was completely removed.

Next, 0.5 g of defatted pasta samples or BLP (0.2 g) were pre-heated for 1.5 minutes in a water bath at 75˚C and then extracted with 3 mL of hot 70 % methanol (v/v) via homogenisation, using an Ultra Turrax Homogenizer (Janke & Kunkel, Germany). The homogenates were centrifuged for 5 minutes at 4000 rpm and the supernatant was collected into a 10 mL measuring flask. The extraction process was repeated 3 times. In the end, the extracts were filled up to the line with 70 % methanol.

The isolation and desulphation of GLS were carried out in ion-exchange columns. To prepare the columns, a piece of glass wool was placed at the end of a 5 mL pipette tip. Then, columns were rinsed with distilled water and 0.5 mL of a DEAE Sephadex-A25 solution was added to each column and left to settle. After rinsing 2 times with 2 mL of water, 2 mL of sample extract (or 50 µL of standard) was added and left to drain. After rinsing again with water, the columns were rinsed with 0.5 mL of a 0.02 M pyridine-acetate buffer solution. Then, 75 µL of a purified sulphatase solution was added and left to react for 16-18 hours. The desulpho-GLS obtained were eluted 3 times with 0.5 mL of HPLC grade water, which was left to drain between each elution. The eluate was collected in a 4 mL vial and stored in the freezer until the HPLC analysis.

Separation of GLS was performed in an HPLC system (LC-20) with an autosampler and the SPD-M20A DAD detector (Shimadzu, Japan). The compounds were separated in the LiChrospher® 100 RP-18 (5 µm, 250 × 4 mm) column at a flow rate of 1.2 mL/min. Desulpho-GLS were separated in a gradient of deionized water and 20% acetonitrile. Individual GLS were identified by comparing their retention times with those of standards (progoitrin, glucoraphanin, sinigrin, glucoraphenin, 4-hydroxy-glucobrassicin, glucobrassicin, 4-methoxy- glucobrassicin and neoglucobrassicin). The sample content of each GLS was quantified in response to the corresponding external standard. The results were expressed in nmol per gram of dry matter (DM) and µmol per 100 grams of fresh weight.

The predicted value (PV) of GLS in fortified pasta was calculated according to Equation (2.8.1), described by Drabińska et al. (2018) (9).

$$PV(GLS) = \left(\frac{GLS_{BLP} \times N}{100\%}\right) \quad (2.8.1)$$

Where: GLS_{BLP} is the total GLS content of BLP and N is the percentage content of BLP in the whole formulation.

2.9. Profile of volatile organic compounds

The analysis of volatile organic compounds (VOCs) was performed using a solid-phase microextraction (SPME) method followed by gas chromatography-mass spectrometry (GC-MS). The SPME was carried out using a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, Bellefonte, PA). This fibre has a complex structure that supports the extraction of volatile compounds within a wide range of polarities.

For the extraction step, 4 g of pasta cooked at OCT were weighed in a 20 mL vial, hermetically closed with an aluminium crimp cap and a silicone/PTFE septum. In the pre-incubation stage, the samples were heated for 5 minutes in the thermomixer (MultiTherm shaker, Benchmark Scientific, Edison, USA) at 60 °C at 500 rpm. Then, the fibre was exposed for 30 minutes at 60 °C at 500 rpm. Finally, the desorption of volatile compounds was performed in the injector port set at 250 °C for 10 minutes.

For the analysis, a 7890A gas chromatograph coupled with a 5975C mass selective detector (Agilent Technologies, Santa Clara, CA, USA) was used. The separation of VOCs was performed using a capillary SupelcoWAX 10 column (30 m length, 250 µm internal diameter, 0.25 µm film thickness, Supelco, Bellefonte, PA). The oven temperature was initially set at 40 °C for 2 minutes, then increased to 240 °C at 6 °C/min and held for 2 minutes. Helium was used as carrier gas at a constant flow of 1 mL/min. Samples were injected in splitless mode. The detector was operated in electron impact (EI) ionization mode at 70 eV. Scan range was from 35 to 550. The ion source temperature was at 240 °C.

The tentative identification of compounds was performed by comparison of the mass spectra with the NIST/EPA/NIH Mass Spectral Library (version 2.2, 2014, Gaithersburg, MD, USA). Only substances with a mass spectra matching score greater than 70% were maintained. For the detected compounds, the odour descriptions were assigned based on the literature and online databases.

2.10. Evaluation of antioxidant capacity

2.10.1. Preparation of extracts

Methanol extracts were obtained from pasta samples (200 mg) and BLP (100 mg) with 1 mL of 67% methanol. Ultrasonic vibration (30 seconds) and vortex (30 seconds) were repeated 3 times and the samples were centrifuged for 10 minutes at 13000 rpm at 4°C. The extraction was repeated 5 times. The supernatants were collected into a 5 mL measuring flask and filled up to the line with 67% methanol. The methanol extracts were prepared in triplicate and kept in the freezer until analysis.

2.10.2. Determination of total phenolic content

The total phenolic content (TPC) was determined in microplates with the use of the Folin-Ciocalteu reagent based on the method described by Drabińska et al. (2018) (9). Firstly, aliquots of 15 µL of

methanol extracts were placed in microplate wells. Then, 250 µL of the Folin-Ciocalteu reagent (diluted with water 1:15, v/v) was added and the mixture was incubated in dark for 10 minutes at room temperature. Next, 25 µL of 20% sodium carbonate was added to each well and the mixture was incubated for 20 minutes. The microplate was shaken automatically before readings and absorbance was measured at λ = 755 nm with the plate reader Infinite M1000 PRO (Tecan Group AG, Switzerland). Gallic acid was used for standard calibration (0.008–1.0 mg/L) and the amount of TPC was expressed in mg of gallic acid equivalents (GAE) per 1 gram of dry matter (g DM).

The PV of TPC in BLP-fortified pasta was calculated according to Equation (2.10.1), described by Drabińska et al. (2018) (9).

$$PV(TPC) = \left(TPC_c - TPCc \times \frac{N}{100\%}\right) + \left(\frac{TPC_{BLP} \times N}{100\%}\right) \quad (2.10.1)$$

Where: TPC_c is the TPC of control pasta, TPC_{BLP} is the total TPC of BLP and N is the percentage content of BLP in the whole formulation.

2.10.3. Trolox Equivalent Antioxidant Capacity assay with ABTS

The Trolox Equivalent Antioxidant Capacity (TEAC) assay was determined in microplates with the use of the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) $(ABTS)^{\bullet+}$ solution, according to the method described by Drabińska et al. (2018) (9). At first, the $ABTS^{\bullet+}$ solution was diluted with 67% methanol to reach the absorbance level of 0.70 ± 0.02 at λ = 734 nm. Then, 10 µL of the methanol extracts, as described in 2.10.1, and blanks were placed in microplate wells. Subsequently, 270 µL of the $ABTS^{\bullet+}$ solution was added to each well and the reaction occurred for 6 minutes in dark at room temperature. The microplate was shaken automatically before readings and absorbance was measured at λ = 734 nm with the plate reader mentioned above. Trolox was used for standard calibration (0.02–0.5 mg/L) and the results were expressed in mg of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent per 1 gram of dry matter (g DM).

The PV of the ABTS assay in BLP-fortified pasta was calculated according to Equation (2.10.2), described by Drabińska et al. (2018) (9).

$$PV(ABTS) = \left(ABTS_c - ABTSc \times \frac{N}{100\%}\right) + \left(\frac{ABTS_{BLP} \times N}{100\%}\right) \quad (2.10.2)$$

Where: $ABTS_c$ is the antioxidant capacity of control pasta measured in the ABTS assay, $ABTS_{BLP}$ is the antioxidant capacity of BLP measured in the ABTS assay and N is the percentage content of BLP in the whole formulation.

2.10.4. Ferric Reducing Antioxidant Potential assay

The Ferric Reducing Antioxidant Potential (FRAP) assay was determined in microplates with the use of the FRAP reagent based on the method described by Drabińska et al. (2018) (9). At first, the FRAP reagent was freshly prepared with 5 mL of 10 mM TPTZ in 40 mM hydrochloric acid, 5 mL of 20 mM ferric (III) chloride solution and 50 mL of 0.3 mM acetate buffer with pH 3.6. Then, 50 µL of the methanol extracts prepared in the TPC assay were placed in microplate wells. Next, 275 µL of the FRAP reagent was added to each well and the reaction was conducted for 5 minutes in dark at room temperature. The microplate was shaken automatically before readings and absorbance was measured at λ = 593 nm in the plate reader mentioned above. Trolox was used for standard calibration (0.008–0.5 µmol/L) and the results were expressed in µmol of Trolox equivalent per 1 gram of dry matter (g DM).

The PV of FRAP assay in BLP-fortified pasta was calculated according to Equation (2.10.3), described by Drabińska et al. (2018) (9).

$$PV(FRAP) = \left(FRAPc - FRAPc \times \frac{N}{100\%}\right) + \left(\frac{FRAPBLP \times N}{100\%}\right) \quad (2.10.3)$$

Where: $FRAP_c$ is the antioxidant capacity of control pasta measured in the FRAP assay, $FRAP_{BLP}$ is the antioxidant capacity of BLP measured in the FRAP assay and N is the percentage content of BLP.

2.11. Statistical analysis

All chemical and technological analyses were performed in triplicate. The results were subjected to one-way analysis of variance (ANOVA), and the significance of differences between the samples was determined by Fisher's LSD test at $p < 0.05$. Statistical analysis was performed using the IBM® SPSS™ Statistics 27 software.

The statistical analysis of the sensory evaluation was performed by a computerized sensory program (FIZZ, Biosystemes, Counternon, France).

3. Results

3.1. Cooking properties of pasta

The OCT, cooking loss, water absorption and swelling index of investigated pasta products are shown in Table 3.1. The OCT was the shortest for the B2.5 pasta, while control and B5 pasta had similar OCT. Control and B5 pasta also had similar cooking losses, while the highest value was observed in B2.5 pasta. Regarding water absorption, a slight decrease was observed when the BLP was added to the formulation, but the differences were not statistically significant. Lastly, control pasta presented a lower swelling index than fortified pasta.

Table 3.1 *Cooking properties of cooked pasta fortified with BLP.*

	Control	B2.5	B5
OCT (min)	3.56 ± 0.08[a]	3.03 ± 0.04[b]	3.44 ± 0.10[a]
Cooking loss (%)	5.96 ± 0.38[b]	7.47 ± 0.19[a]	5.95 ± 0.05[b]
Water absorption (%)	62.26 ± 1.36[a]	55.55 ± 5.65[a]	60.45 ± 3.88[a]
Swelling index (%)	0.82	1.08	1.07

* Values represent means ± standard deviation (SD) of 3 values.

** Different letters in the same line indicate a significant difference ($p < 0.05$).

3.2. Colour

Table 3.2 shows that the fortification of pasta with BLP significantly decreased lightness (L* value) and increased yellowness (b* value). Also, enriched pasta showed a lower a* value than control pasta, which indicates that fortified pasta is greener than control pasta. However, no statistically significant differences were observed between B2.5 and B5 samples in terms of greenness (a^* value). The WI significantly decreased with the addition of BLP, while the BI significantly increased. Among pasta, B5 presented the lowest WI and the highest BI.

Table 3.2 *Colour characteristics of BLP and cooked pasta fortified with BLP.*

	BLP	Control	B2.5	B5
L*	46.18 ± 0.03	87.51 ± 0.01[a]	76.05 ± 0.03[b]	67.61 ± 0.02[c]
a*	-9.33 ± 0.03	-0.12 ± 0.01[a]	-6.25 ± 0.02[b]	-6.26 ± 0.02[b]
b*	27.39 ± 0.06	14.05 ± 0.02[a]	28.14 ± 0.03[b]	31.48 ± 0.02[c]
WI	38.89 ± 0.05	81.20 ± 0.02[a]	62.52 ± 0.03[b]	54.41 ± 0.02[c]
BI	66.67 ± 0.29	16.99 ± 0.02[a]	38.20 ± 0.07[b]	52.57 ± 0.06[c]

* Values represent means ± SD of 3 values.

**Different letters in the same line indicate a significant difference ($p < 0.05$).

3.3. Texture

Textural parameters of pasta enriched with BLP and control sample are shown in Table 3.3. There was a significant decrease in firmness and TSF when the BLP was added to the pasta formulation. However, no statistically significant differences were observed between B2.5 and B5 samples for both parameters.

Table 3.3 *Textural properties of cooked pasta fortified with BLP.*

	Control	B2.5	B5
Firmness (kg)	13.37 ± 0.17[a]	12.09 ± 0.10[b]	11.96 ± 0.24[b]
TSF[1] (kg.sec)	87.30 ± 4.11[a]	76.09 ± 1.52[b]	73.90 ± 5.53[b]

[1]Total shearing force.

* Values represent means ± SD of 3 values.

** Different letters in the same line indicate a significant difference ($p < 0.05$).

3.4. Sensory properties

Table 3.4 shows the results of the sensory evaluation of cooked pasta samples. In sum, the incorporation of BLP did not affect the overall acceptance ($p > 0.05$) of experimental pasta products, since all pasta were described as very good quality (9 score on a 10-point scale). Regarding texture attributes, no statistically significant differences were observed between the samples for hardness and chewiness. However, the degree of adhesiveness significantly increased with the increasing amount of BLP. In contrast, elasticity decreased, reaching values below 5. As expected, the cabbage odour and taste increased when the amount of BLP was increased. However, only in the pasta with the highest percentage of BLP (B5) the cabbage odour was determined above the central point of the scale. No statistically significant differences were found in the aftertaste.

Table 3.4 *Sensory properties of cooked pasta fortified with BLP.*

	Control	B2.5	B5
Flour odour	7.1[a]	1.7[b]	0.9[b]
Cabbage odour	0.0[c]	2.6[b]	5.4[a]
Creamy colour	5.6[a]	0.0[b]	0.0[b]
Green colour	0.0[c]	5.8[b]	7.9[a]
Elasticity	7.2[a]	5.5[b]	4.5[c]
Hardness	3.9[a]	3.0[a]	3.0[a]
Chewiness	3.4[a]	3.8[a]	4.0[a]
Adhesiveness	4.0[c]	5.0[b]	5.6[a]
Flour taste	5.8[a]	4.4[b]	4.1[b]
Cabbage taste	0.0[c]	0.7[b]	2.3[a]
Sweet taste	2.2[a]	1.3[b]	1.0[b]
Aftertaste	3.6[a]	3.8[a]	4.0[a]
Overall quality	**9.1[a]**	**9.1[a]**	**9.0[a]**

* Different letters in the same line indicate a significant difference ($p < 0.05$).

3.5. Proximate chemical composition

The proximate chemical composition and energy value of BLP and examined pasta products are shown in Table 3.5.

BLP was characterised by high protein content (approximately 26%), high mineral content (approximately 11%) and moderate fat content (approximately 4%). As expected, the mineral content of pasta increased ($p < 0.05$) with the addition of BLP and it was the highest in B5 pasta. The protein content also increased with the increasing amount of BLP, but the differences were not statistically significant. Regarding fat content, control pasta presented the lowest value and B2.5 pasta presented the highest. Compared to control and B5 pasta, B2.5 pasta presented the lowest carbohydrate content and the highest moisture content. Finally, the energy value significantly decreased in B2.5 pasta and increased in B5 pasta in comparison with control.

Table 3.5 *Proximate chemical composition of BLP and cooked pasta fortified with BLP.*

	BLP	Control	B2.5	B5
Moisture (%)	-	61.04 ± 0.26[a]	63.64 ± 0.01[b]	60.75 ± 0.15[a]
Ash (%)	10.94 ± 0.04	0.88 ± 0.01[a]	1.00 ± 0.03[b]	1.19 ± 0.01[c]
Protein (%)	25.66 ± 0.27	13.06 ± 0.21[a]	13.17 ± 0.21[a]	13,26 ± 0,07[a]
Fat (%)	3.94 ± 0.21	3.94 ± 0.15[a]	4.79 ± 0.04[c]	4.45 ± 0.10[b]
Carbohydrate (%)	59.46 ± 0.07	21.08 ± 0.5[a]	17.41 ± 0.18[b]	20.23 ± 0,16[a]
Energy [kcal/100g]	375.93 ± 0.89	172.01 ± 1.28[b]	165.37 ± 0.23[a]	174.43 ± 0,02[c]

* Values represent means ± SD of 3 values.

** Different letters in the same line indicate a significant difference ($p < 0.05$).

3.6. Glucosinolate content

The content of GLS in BLP is presented in Table 3.6. In total, 6 GLS were found in BLP, including 2 aliphatic (progoitrin and glucoraphanin) and 4 indole (glucobrassicin, 4-methoxy-glucobrassicin, neoglucobrassicin and 4-hydroxy-glucobrassicin) GLS. The predominant GLS were neoglucobrassicin and glucobrassicin, which accounted for 32% and 27% of total GLS, respectively. The total GLS content was estimated at 3.3 µmol/g DM.

Table 3.7 shows how the addition of BLP influenced the content of GLS in pasta products. In general, the content of GLS increased almost proportionally to an increase in BLP content. As a matter of fact, the total GLS content of experimental pasta products was 28 and 12% higher than the predicted values for B2.5 and B5, respectively.

The GLS profile of enriched pasta was identical to the GLS profile of BLP. All GLS detected in BLP were also detected in pasta, except 4-hydroxy-glucobrassicin. Neoglucobrassicin and glucobrassicin, the major compounds in BLP, were also the predominant GLS in fortified pasta, accounting for 33% and 29% of total GLS in B5, respectively. The concentrations of the analysed GLS increased significantly with an increase in BLP content, excluding progoitrin that presented a lower value in B5 pasta. Moreover, the predicted values were lower than the experimental values for all GLS, except for progoitrin in B5.

Table 3.6 *Content of GLS [µmol/g DM] in BLP.*

	BLP	
	Av	SD
Progoitrin	0.18	0.04
Glucoraphanin	0.51	0.02
Glucobrassicin	0.90	0.03
4-Methoxy-Glucobrassicin	0.62	0.03
Neoglucobrassicin	1.06	0.06
4-Hidroxy-Glucobrassicin	0.07	0.01
Total	3.34	

* Values represent means ± SD of 3 values.

Table 3.7 *Content of GLS [nmol/g DM] in cooked pasta fortified with BLP.*

	B2.5			B5		
	Av	SD	PV	Av	SD	PV
Progoitrin	5.46[a]	0.4	3.16	3.92[b]	0.8	6.20
Glucoraphanin	12.15[b]	0.2	8.96	21.58[a]	1.1	17.60
Glucobrassicin	20.01[b]	1.2	15.83	36.12[a]	0.6	31.10
4-Methoxy-Glucobrassicin	12.92[b]	1.1	10.98	22.93[a]	0.4	21.58
Neoglucobrassicin	23.42[b]	1.1	18.70	41.84[a]	1.1	36.75
Total	73.97	-	57.61	126.38	-	113.23

* Values represent means ± SD of 3 values.

** Different letters in the same line indicate a significant difference ($p < 0.05$).

Figure 3.1 shows the content of analysed GLS expressed as µmol per 100 g of cooked pasta. In general, the concentration of each GLS doubled when the percentage of BLP also doubled. Progoitrin was the only GLS with a lower concentration in B5 pasta, but the difference between samples was not statistically significant. The other GLS presented a significantly higher concentration in B5 pasta. These results indicate that consuming 100 g of fortified pasta B5 will provide 5.0 µmol of glucosinolates in total.

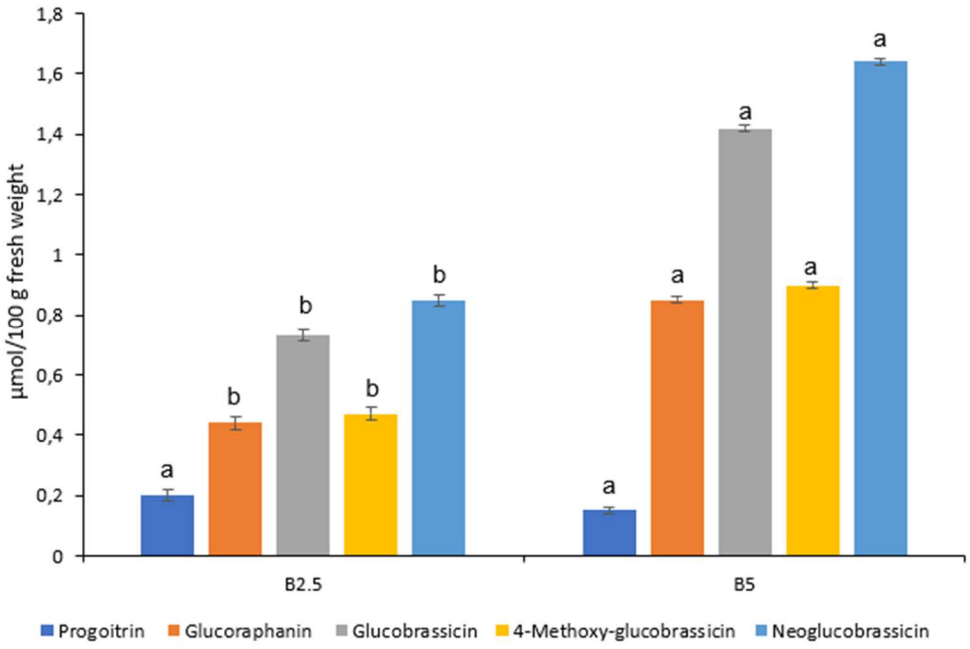

Figure 3.1 *Content of GLS [µmol/100 g fresh weight] in cooked pasta fortified with BLP. Bars are the mean ± SD of 3 values. Different letters indicate a significant difference ($p < 0.05$).*

3.7. Volatile organic compounds

In total, 97 volatile compounds were tentatively identified in BLP by GC-MS. The siloxane derivatives were not reported since they originate from the fibre and column bleeding. Regarding chemical groups, 26% of the compounds were alcohols, 23% were ketones and 22% were aldehydes. Odour descriptions were found for 55% of the compounds, which are indicated in Table 7.1 (Appendix II). In total, 31 types of odours could be assigned to the identified compounds. Fruity was the most frequently assigned odour in BLP (assigned to 23% of the compounds), followed by green (assigned to 19% of the compounds). The most representative compounds in terms of % of the total GC area were (E,E)-3,5-octadien-2-one and 1-penten-3-ol. Dimethyl sulfide detected in BLP was responsible for the sulphurous cabbage-like odour.

In the control pasta, a total of 36 volatile compounds were identified. In terms of chemical groups, 39% of the compounds were aldehydes and 31% were alcohols. Odour descriptors could be found for 29 compounds, which are shown in Table 7.1 (Appendix II). In total, 12 different types of odours were distinguished. The most frequently associated odours were citrus and fatty (assigned to 28% of the compounds). The most representative compounds in terms of % of the total GC area were hexanal, 1-hexanol, limonene and 2-pentyl-furan. A total of 13 compounds (hexanal, limonene, (E)-2-hexenal, 2-pentylfuran, 1-pentanol, octanal, 6-methyl-5-heptene-2-one, nonanal, (E)-2-octenal, 1-octen-3-ol, 2-ethyl-1-hexanol, benzaldehyde and acetophenone) were detected both in control pasta and in BLP. In fortified pasta B2.5 and B5, a total number of 83 and 87 volatile compounds has been determined,

respectively. In B2.5, the most representative compounds in terms of % of the total GC area were hexanal and 1-hexanol, while in B5 were hexanal and 1-octen-3-ol. Odour descriptors were found for 70 and 64% of the compounds in B2.5 and B5, respectively (Table 7.1, Appendix II). In both pasta, 26 types of odours could be attributed to the identified compounds. The most frequent odours descriptors were fruity and green, similarly to BLP.

A total of 28 compounds were detected in BLP and in fortified pasta, but not in control. Consequently, the odour descriptions of cabbage, pungent, sulfur, rancid, malty, and spicy were assigned to BLP and enriched pasta, but not to control pasta. In contrast, 14 compounds were detected in all types of pasta, but not in BLP.

3.8. Antioxidant capacity

As shown in Table 3.8, BLP was characterized by a high TPC (6.8 mg GAE/g DM) and a high antioxidant capacity determined by ABTS and FRAP assays. The ferric reducing power measured by FRAP was approximately 18 µmol Trolox/g DM and the scavenging ability measured by ABTS was approximately 7 mg Trolox/g DM.

The antioxidant capacity of analysed products significantly increased when the BLP was added to pasta (Table 3.8). The TPC increased ($p < 0.05$) 2.6 times, while the antioxidant capacity measured in the ABTS and FRAP assays increased ($p < 0.05$) 3.2 and 9.7 times, respectively.

In addition, it should be noted that the antioxidant capacity of fortified pasta increased significantly according to the percentage of BLP in each formulation in a dose-dependent manner. Therefore, the pasta with the highest BLP content (B5) was characterized by the highest antioxidant activity in all assays. Also, the predicted values were lower than the experimental values for both pasta in all assays.

Table 3.8 *Total phenolic content [mg GAE/g DM] and antioxidant capacity by ABTS [mg Trolox/g DM] and FRAP [µmol Trolox/g DM] of BLP and cooked pasta fortified with BLP.*

	BLP			Control			B2.5			B5		
	AV	SD	PV	AV	SD	PV	AV	SD	PV	AV	SD	PV
TPC	6,76	0,58	-	0,34[a]	0,08	-	0,58[b]	0,09	0,45	0,87[c]	0,10	0,56
ABTS	6,95	0,82	-	0,27[a]	0,04	-	0,58[b]	0,05	0,39	0,86[c]	0,08	0,50
FRAP	18,45	0,78	-	0,22[a]	0,16	-	1,24[b]	0,04	0,55	2,13[c]	0,06	0,86

* Values represent means ± SD of 3 values.

** Different letters in the same line indicate a significant difference ($p < 0.05$).

4. Discussion

Fruit and vegetable by-products have been described as good sources of bioactive compounds with important biological activities such as antioxidant properties (14). Broccoli leaves, usually perceived as a waste product, are characterised by a high content of nutrients and bioactive compounds and could be valuable as natural food ingredients (8). Recent studies have attempted to incorporate broccoli by-products, in particular leaves, as additives to improve the quality and/or nutritional value of several foods, including bakery products (9, 19). However, according to the available knowledge, broccoli leaves were never used to fortify a pasta product.

4.1. Effect of BLP on cooking properties

The cooking quality of pasta is an important consumer attribute and is assessed using OCT, cooking loss, water absorption and swelling index. As shown in Table 3.1, the OCT decreased with the addition of BLP to pasta samples. This result was somewhat expected because BLP is a material with a high water absorption index (59). However, the reduction in cooking time was only significant for B2.5 pasta, probably due to a higher moisture content (63.6%) and, consequently, faster rehydration (51). Similar results were observed by Michalak-Majewska et al. (2020), who reported that pasta fortified with onion skin powder had a shorter cooking time than control pasta (45). In addition, Silva et al. (2013) reported that the OCT of fresh pasta enriched with concentrations of broccoli up to 30% varied between 2.5 and 4.5 minutes (5). In this study, the OCT varied between 3 and 3.6 minutes, indicating that the addition of BLP did not affect this parameter.

Water absorption also decreased with the addition of BLP and, although the differences were not statistically significant, the lowest water absorption was observed for B2.5 pasta. This can be attributed to the fact that shorter cooking times can reduce the water absorption of pasta due to a lower increase in weight after cooking (45). The water absorption increases with an increased degree of starch gelatinisation (60). In this study, the water absorption did not change significantly, thus it can be suggested that the addition of BLP did not affect the degree of gelatinization of the starch. This is good because indicates that pasta properties did not get worse with BLP incorporation. In fact, in nutrition, products with relatively high water absorption index may be desirable because they swell in the stomach, which makes them more effective in giving the feeling of satiety (61).

Cooking loss indicates the amount of solid material lost in the cooking water and is one of the most important parameters determining the quality of pasta (62). In this study, the highest cooking loss value was observed for B2.5 pasta (7.47%), while control and B5 had significantly lower cooking losses (5.96% and 5.95%, respectively). According to industry guidelines, it should not exceed the technologically acceptable limit of 8% (62). All the obtained pasta types presented cooking losses below 8 g/100 g, which proves that adding BLP up to 5% did not affect the pasta quality to a significant extent. Similarly, Michalak-Majewska et al. (2020) reported that cooking losses remained below 8% for pasta fortified with 2.5%, 5% and 7.5% of onion by-product (45). These findings suggest that adding vegetables by-

products in these concentrations might be a good option to enrich pasta products, without compromising pasta quality. By contrast, the incorporation of 5% of mango peel powder resulted in cooking losses above 8%, which can be related to the high dietary fibre and/or sugar content of the mango peel powder (63).

In this study, the addition of 2.5% of BLP increased the solid loss, but the addition of 5% did not. Several authors (42, 64) have reported that the quality and content of protein used in food manufacturing as well as protein interaction, are very important to form the optimum carbohydrates-protein network and obtain pasta of good cooking quality. It is possible that, due to a higher moisture content, B2.5 pasta presented a worse carbohydrates-protein network than B5 and control. As a consequence, the leaching during cooking was higher in B2.5. Thus, the higher cooking loss in B2.5 might be attributed to a weakening and disruption of the protein gluten network (42). It is likely that the addition of 2.5% of BLP, a non-gluten material, diluted the gluten strength and probably weakened the overall structure of the pasta (65). In agreement with our results, Silva et al. (2013) demonstrated that durum wheat semolina is a suitable raw material for the production of pasta filled with vegetable particles, since it does not show to be greatly affected by the type or concentration (up to 20% v/v) of broccoli particles added (5). These authors showed that gluten proteins form a very strong and elastic network that can prevent the broccoli particles from swelling and disrupt the matrix in excess. Additionally, Granito et al. (2003) reported that the use of a drying stage with higher temperatures in pasta manufacturing produces lower cooking losses (66). In the present study, fresh pasta was produced without a drying step. Even so, the cooking losses were lower than the results obtained for semolina dried macaroni with 5% addition of mango peel powder (63). These findings indicate that, even without undergoing a drying stage, pasta can be manufactured without loss of quality. The same conclusions were taken by Sant' Anna et al. (2014), who prepared fresh pasta with different concentrations of grape marc powder (47).

The swelling index of pasta is a good indication of the integrity of the protein matrix that restricts water penetration (31). It is dependent on the competition between the starch and protein for water absorption (32). In this study, control pasta showed a lower swelling index (0.82) than enriched pasta B2.5 (1.08) and B5 (1.07). Unlike other studies that also added a protein-rich fraction (39, 42), the addition of BLP did not inhibit the swelling of the starch granules. On the contrary, it seems that BLP contributed to the formation of a protein network that increased the supply of water for swelling and gelatinisation of starch granules. In general, swelling index is reduced with the addition of protein into the pasta formulation (32). The obtained results are not consistent with this observation probably because the incorporation of 2.5 and 5% of BLP into the pasta lead to an insignificant increase in the protein content (as shown in Table 3.5). On the other hand, some studies have shown a significant increase in the swelling index at increasing concentrations of dietary fibre (60). In a previous study (59), broccoli leaves flour was characterized by high content of fibre and, although the content of fibre was not evaluated in this work, this could explain the increased swelling index in enriched pasta.

4.2. Effect of BLP on technological properties (colour and texture)

Pasta colour is one of the most important factors responsible for consumer acceptance because it is related to product freshness and flavour expectations (27). In recent years, pasta products with unconventional colours have gained attention. The colour might result from the use of synthetic colourants or the addition of extracts from plants (37). Yet, the use of natural colourants is more accepted by consumers since they expect them to be safe and healthier than synthetic colourants (27).

In this study, the lightness (L* value) of pasta samples decreased as the amount of BLP in the recipe increased, indicating that the samples turned darker. This trend for change was due to the addition of a blackish ingredient (BLP). In other studies, where semolina was enriched with several additives (legume flours (65), grape marc powder (47), spirulina (27)), an increase in darkness of pasta was also observed as the concentration of substances added increased. Dark colouring might be a positive feature since consumers identify it with high-fibre products (45). The addition of BLP to pasta also resulted in a decrease of the a* values. This was expected, since the a* parameter is related to the greenness of the analysed sample and the BLP added is a by-product characterized by high green intensity (a*= -9.33 ± 0.03). However, no statistically significant differences were observed between fortified pasta B2.5 and B5 in terms of greenness (a^* value), which suggest that after cooking the green intensity is similar for concentrations up to 5% of BLP. The results of the sensory evaluation (Table 3.4) are not consistent with this observation because the green colour of B5 pasta was classified as more intense ($p < 0.05$) than that of B2.5. Although this greenish appearance of pasta could cause some concern for consumers not used to purchase this kind of products, the current tendency towards “healthier ” foods may represent an opportunity to introduce this type of pasta (67). Changes in yellow colour intensity were described by the parameter b*. This value increased as the level of BLP in pasta increased, probably due to the carotenoid pigments present in BLP. A similar increase in b* value was observed when carrot pomace powder was used to replace durum wheat semolina (68). Finally, the WI significantly decreased with the addition of BLP, while the BI significantly increased. Taking into account the results obtained for L* and a* parameters, this was expected because the addition of BLP resulted in pasta with darker colours.

From the consumer’s point of view, the textural parameters are a critical point to ensure the acceptance of food products. To a large extent, pasta texture is responsible for pasta acceptability, in particular firmness (31). Pasta firmness is defined as the peak force attained during the first compression and is closely related to the strength and integrity of the protein matrix developed during the cooking process (42). TSF is defined as the force to cut/shear. Usually, the higher the force, the firmer the sample (69). The obtained results (Table 3.3) are consistent with this observation because the pasta with the highest TSF, namely control pasta, was the one that presented higher firmness.

In this study, the addition of BLP to the control pasta formulation significantly decreased firmness and TSF by 10.5% and 15.3%, respectively. The pasta firmness decreased from 13.4 (control) to 11.9 (B5), while TSF decreased from 87.3 to 73.9. Yet, no statistically significant differences were observed between B2.5 and B5 for both parameters, which suggest that for concentrations up to 5%, the addition of BLP to pasta had the same effect on texture. In a previous study, Silva et al. (2013) demonstrated

that the largest changes in the textural properties of pasta enriched with different concentrations of broccoli powder (from florets) appear between 20% and 30% (5). Therefore, at concentrations of BLP used in this work (2.5% and 5%), pasta should remain acceptable for its texture.

Firmness is mainly related to gluten formation (31). Therefore, the addition of a non-gluten material (BLP) probably diluted the gluten strength and weakened the overall structure of the pasta (65), which led to a decrease in pasta firmness. Besides, firmness can be reduced due to a higher swelling index (32). Accordingly, in this study, control pasta presented a lower swelling index and a higher firmness than fortified pasta. Previous studies on the addition of by-products to semolina resulted in alterations in the firmness of pasta. Ajila et al. (2010) (63) reported that the addition of mango peel powder increased the firmness of the macaroni, while Gull et al. (2015) (68) reported that the use of carrot pomace powder decreased pasta firmness, probably due to the presence of sugars and fibre, which are known to have high affinity for water. Moreover, the addition of protein-rich fractions to pasta has increased the firmness value of pasta (32). These results might be attributed to the low water absorption and low swelling index of that supplemented pasta. However, it should be noted that the comparison of the obtained results with the literature was difficult due to the different tests used to assess the textural properties of pasta.

4.3. Effect of BLP on sensory properties

Cooked pasta fortified with different BLP levels was evaluated for odour, appearance, texture, taste, and overall quality, as described in Table 2.2. The sensory evaluation results (Table 3.4) indicated that the addition of BLP did not affect the overall acceptance of pasta, since all samples had a great sensorial score (9 on a 10-point scale). Thus, for concentrations up to 5% of BLP, pasta remained acceptable for its texture, appearance, odour, and taste. Silva et al. (2013) went further and reported that pasta can be fortified up to 20% of broccoli without significantly decrease its acceptability (5). As shown in Table 3.4 and as expected, the intensity of cabbage odour and taste increased ($p < 0.05$) as the concentration of BLP in pasta increased. This is probably related to the presence of numerous aroma active compounds in BLP, such as dimethyl sulfide, which is responsible for cabbage-like odour notes (Table 7.1, Appendix II). Accordingly, Silva et al. (2013) also reported that the intensity of the vegetable flavour in the mouth increased with increasing levels of broccoli added (5). However, the obtained overall acceptance results indicate that supplemented pasta would also be accepted by a large group of consumers even if the perception of cabbage flavour is present. Moreover, the addition of BLP had no adverse effect in the aftertaste, defined as “a lingering sensation after swallowing the sample”. This probably contributed to the high score obtained in overall quality for enriched pasta products.

In this study, both instrumental and sensory methods were used for texture assessment. Therefore, texture attributes such as elasticity, hardness, chewiness, and adhesiveness were evaluated by trained panellist during sensory evaluation. The results (Table 3.4) showed that the addition of BLP to pasta had no effect on hardness and chewiness but had a negative impact on adhesiveness and elasticity. Hardness was defined by the panellist as “the force required to bite through the sample placed between

the front teeth". Since control pasta presented higher firmness ($p < 0.05$) than fortified pasta (as shown in Table 3.3), it was expected that hardness was also higher in control pasta. In practical terms, this was observed but the differences between the samples were not statistically significant ($p > 0.05$). By contrast, Silva et al. (2013) reported that the hardness decreased as the concentration of broccoli powder in pasta increased (5). However, in that study higher concentrations of broccoli (up to 30%) were used, which could explain the different results.

The chewiness was defined as "the multiplicity of chewing the product to prepare it to swallow". It is related to the elastic strength of the protein matrix and decreases with cooking time due to the softening of the structure (31). In this study, the scores for chewiness were similar for all samples ($p > 0.05$), although OCT was longer for control and B5.

Adhesiveness was defined as the "degree of adhesiveness while chewing the sample 10 times". This attribute is associated with starch gelatinization, and the quantity of amylose leached to the cooking water (31). Therefore, cooking losses are intimately related to adhesiveness since a high degree of amylose leached during cooking means a high degree of amylopectin remaining in the pasta surface, which is indicative of high adhesiveness (31). In this study, adhesiveness increased ($p < 0.05$) as the amount of BLP added to pasta increased. This result makes sense for B2.5, due to the higher cooking loss, but not for B5, which solid loss was similar to control. However, the higher swelling index of enriched pasta may be associated with an increment in starch gelatinisation and, consequently, adhesiveness (60). Similarly, Michalak-Majewska et al. (2020) demonstrated that the incorporation of onion by-products (up to 7.5%) into pasta resulted in lower scores for adhesiveness (45). In contrast, Silva et al. (2013) reported that stickiness was not affected by the incorporation of broccoli powder (5).

Lastly, elasticity was defined as "the extent to which a piece of product returns to its original length when stretched". Its scores decreased ($p < 0.05$) with the addition of BLP, probably due to the less energy required to cut the fortified pasta, which is associated with the lower TSF values. Previous studies have shown that dietary fibre supplementation decreased pasta elasticity (32). For instance, the addition of brewer's spent grains (the main by-product in the brewing industry) to pasta has significantly lowered the elasticity of the dough (70). Although the content of fibre was not evaluated in this study, it is likely that BLP has a higher fibre content than semolina, which could explain the decreased in pasta elasticity.

4.4. Volatile organic compounds

Aroma has a great influence on consumer acceptability (71). Volatiles contribute to the flavour and aroma of cereal foods and are key factors for determining perceived quality. Therefore, the profiling of secondary metabolites such as VOCs by analytical techniques is widely used in food quality assessment (72). In this study, volatile composition was investigated both in BLP and in cooked pasta using an SPME method and identified by GC–MS. The SPME technology has been widely applied in the extraction of VOCs due to its simplicity, sensitivity, and solventless character compared to other methods (73).

The analysis of VOCs in plants is complex because the composition of volatile metabolites is unstable and dependent on many factors, including storage conditions, cultivars, and technological processes (73). Although some studies have already analysed the volatile profile of fresh broccoli, this was the first time that freeze-dried broccoli leaves were evaluated. The use of GC-MS allowed the tentative identification of 97 volatile compounds extracted from BLP, where the majority were alcohols. A previous study that used different extraction techniques to isolate aroma components from fresh non-stored broccoli was able to identify a larger number of VOCs (120 in total) but similarly the majority of compounds were also alcohols (73). The differences in the number of VOCs may be related to the loss of some very volatile compounds during freeze-drying. However, in that study, the second most abundant fraction was sulfur components, while in this study was ketones. Actually, only two sulfur compounds (dimethyl sulfide and dimethyl sulfoxide) were detected in BLP. The absence of high molecular sulfides such as dimethyl tri- and tetrasulfides, may be related to the fact that the SPME technique, as an absorption method, is more suitable for the extraction of low molecular weight compounds (73). Alternatively, these compounds could be lost during processing and their concentrations could be below the limit of detection. According to previous results, isothiocyanates formed an important fraction in terms of the number of VOCs in fresh raw broccoli (74). In this study, the non-detection of isothiocyanates could be related to the inactivation of myrosinase, the enzyme responsible for the hydrolytic cleavage of GLS, during blanching. On the other hand, since isothiocyanates are very volatile compounds, they could be lost during processing and storage of BLP. These compounds are usually associated with the off-flavour of cruciferous vegetables, however, in recent reports isothiocyanates did not occur as the source of any rejecting aromas (73, 74).

As described in earlier studies, broccoli is a source of numerous volatiles, and many of them display aroma activity. In this study, 31 types of odours were assigned to the identified compounds, with fruity and green being the most frequent odours in BLP. Some characteristic volatiles of broccoli reported in the literature, such as dimethyl sulfide, were also distinguished in BLP. This compound is related to putrid, cabbage-like odour notes, and it has been reported to be one the of key odorants in raw broccoli (74). By contrast, methanethiol, dimethyl disulfide and dimethyl trisulfide, well known volatile compounds of *Brassicaceae*, were not detected in BLP. Methanethiol, known for its role in creating cheddar cheese flavour (75), appeared to be the primary contributor to the unpleasant odour that broccoli develops when held in anaerobic conditions (76). Its odour has been described as an “intensely putrid, faecal-like aroma” and might be one of the reasons for the rejection of broccoli by consumers. In a recent study, methanethiol was reported as one of the most potent odorants in raw broccoli (74). The absence of this compound in BLP may be related to its high volatility or the inactivation of the enzyme responsible for its formation during blanching. Dimethyl disulfide, with a sulphurous cabbage-like odour**,** and dimethyl trisulfide, with a sulphurous alliaceous-like odour, have been detected in cruciferous vegetables, such as cabbage (77, 78) and broccoli (79). Recently, dimethyl trisulfide was recognized as a typical odour of cooked broccoli (74). Sulfur components, such as sulfides, usually have relatively low odour thresholds, which means that their odour is detectable at extremely low concentrations that are very often below the limit of detection (74). This could explain why these compounds were not detected in BLP by GC-MS. By contrast, fatty acids and lipid-derived compounds (e.g., hexanal) have been found

as quite common in vegetables and were detected in BLP. However, their impact on the aroma of broccoli has been reported as minimal (74).

Semolina and pasta are usually not considered for their aromatic properties. Nevertheless, a total of 36 volatile compounds were identified in cooked control pasta and the most frequently associated odours were citrus and fatty. Aldehydes and alcohols were the major chemical groups present, which is in agreement with Beleggia et al. (2009), who also investigated the volatile composition of cooked pasta (71). In that research, 29 volatile compounds were identified, but only 12 were common to this study, which highlights the existing differences between semolina used to produce pasta. However, in both studies, hexanal was the most abundant aldehyde in cooked pasta samples. Its presence in cereal grains has been associated with the oxidation of unsaturated fatty acids (71). Fatty acids oxidation is also the source of heptanal, octanal, nonanal, 1-hexanol, 1-octen-3-ol and 2-pentylfuran. Therefore, the presence of these compounds in pasta might be the consequence of the thermal oxidation of lipids (80). It is known that linoleic and linolenic fatty acids have much higher oxidation rates than oleic acid (81). So, the relative amounts of their oxidation products (n-hexanal, 1-hexanol, n-heptanal, 1-heptanol, 2-pentylfuran) are typically higher than those derived from oleic acid (n-octanal, n-nonanal) (80). This affirmation is in accordance with the obtained results since the percent of the total GC area is higher for linoleic and linolenic oxidation products (Table 7.1, Appendix II).

2-pentylfuran exhibits a medium-strength fruity, green, earthy beany odour with vegetable like odours (82), and it has been reported to be frequently present in samples of cooked pasta (71). This compound, as well as n-hexanal, n-nonanal and benzaldehyde, have low odour thresholds and may contribute to pasta flavour (80). Alpha-pinene and limonene also have been often found in pasta (71, 80). However, in this study, only limonene was detected by GC-MS. This compound, with a citrus odour, was detected in greater amounts in cooked pasta than in BLP. Since terpenes are known to have distinctive aromatic properties (71), limonene could also contribute to the flavour of pasta. (E)-2-nonenal and (E,E)-2,4-decadienal, both having a fatty odour, have been reported as the most potent odours in bread crumb (72). In this study, these compounds were detected in all types of cooked pasta but not in BLP, which may suggest that these aldehydes are volatiles associated with cereal products. Moreover, hexanal, nonanal and 2-nonenal have been associated with high-quality pasta and were detected in higher amounts than 2-hexenal and pentanal, which are more characteristic of lower quality pasta (82). Therefore, it may be suggested that the semolina used in this study contributed to the production of pasta of good quality.

Regarding fortified pasta B2.5 and B5, a total of 83 and 87 volatile compounds were identified by GC-MS, respectively. The most frequently attributed odours were, like in BLP, fruity and green, highlighting the influence of the addition of BLP in the aroma of pasta. As shown in Table 7.1 (Appendix II), the volatile profile of pasta changed due to the addition of BLP since 28 compounds that were not detected in control pasta, were then detected in enriched pasta. These compounds contributed to the appearance of odours such as cabbage, sulfur, pungent, rancid, malty, and spicy in pasta. The sensory evaluation results (Table 3.4) confirmed this hypothesis since the perception of cabbage flavour was present in fortified pasta. This also proves that dimethyl sulfide, detected in supplemented pasta after BLP

incorporation, contributed greatly to pasta flavour, probably due to its low odour threshold. Broccoli leaves have been reported as great sources of carotenoids, specially β-carotene (8). Accordingly, dihydroactinidolide and β-ionone, two volatiles that derive from the degradation of β-carotene, were identified in fortified pasta after BLP addition. These compounds could significantly influence the aroma of pasta because carotenoid degradation has been described as one of the main metabolic pathways for the production of important aromatic compounds in several foods such as tomato, watermelon (83), and wine (84). However, the profile of carotenoids was not analysed in this study and requires further confirmation.

4.5. Proximate chemical composition

The chemical composition of a by-product should be analysed before its incorporation into a food matrix. As already stated, the BLP was characterised by high protein, high mineral, and moderate fat content (Table 3.5). The moisture content was not evaluated because the broccoli leaves were freeze-dried to remove the water, as mentioned in section 2.1. The relatively high potential of BLP as a functional food ingredient is related, among others, to its high mineral content. In fact, it has been reported that florets flour show a lower amount of ash than leaves flour (59), which highlights the feasibility of utilization of broccoli by-products for the human diet.

In a previous study, the proximate chemical composition of broccoli leaf flour was also analysed (59). In comparison with the BLP used in this study, the broccoli leaf flour presented a lower protein content (12.13% VS 25.66%) but a higher content of ash (14.67% VS 10.94%), fat (6.72% VS 3.94%) and total carbohydrate (66.48% VS 59.46%). The disparity between the results might be related to the use of different broccoli species and different stages of harvest. Moreover, it has been reported that the ash content varies according to the mineral status of the soil (85). Also, differences in the preparation method could have affected the nutritional properties of the powders. While BLP resulted from leaves that were freeze-dried, broccoli leaf flour used by Campas-Baypoli et al. (2009) resulted from leaves that were dried in a convection oven at 60°C (59).

Drabińska et al. (2018) studied the chemical composition of the same BLP but fresh (9). Although the BLP used in this study was stored for a few years, the protein and ash contents obtained were very similar. These findings suggest that despite the storage, the proximate composition of BLP remained stable. A recent study by Sedlar et al. (2021) indicated that broccoli leaves were characterised with the highest content of protein in comparison with other vegetable by-products (86). Accordingly, the incorporation of BLP into the pasta resulted in an increase in the protein content (Table 3.5). However, it was a very small increase ($p > 0.05$). On the other hand, a significant ($p < 0.05$) increase in the ash content was observed due to a higher mineral content delivered by BLP. Semolina usually has a relatively high protein content and since BLP was added in such small concentrations, the increase in protein was not that evident compared to the increase in ash, which content is typically very low in semolina. Consequently, changes in ash content were more notable than changes in protein content. The addition of BLP to the pasta also resulted in a significant ($p < 0.05$) increase in the fat content. In

fact, it has been reported that broccoli leaves are a rich source of polyunsaturated fatty acids, mainly α-linolenic, linoleic, and palmitic acids (59). However, the profile of fatty acids was not analysed in this study and requires further confirmation. Krupa-Kozak et al. (2021) evaluated the nutritional value of gluten-free bread enriched with BLP and found that the addition of BLP significantly increased the protein, fat, and ash contents of bread (19). Similarly, Ranawana et al. (2016) showed that the addition of freeze-dried broccoli significantly increased the protein, fat, and total mineral contents in oil-free wheat bread (87). In this study, the addition of BLP to the pasta significantly increased the fat and ash contents, but not the protein content. The lack of significant changes in the protein content may be related to the higher proportion of fat in pasta formulation than in bread formulation.

Finally, in comparison with control and B5, enriched pasta B2.5 presented a higher moisture content and a lower carbohydrate content (Table 3.5). Consequently, the energy value of B2.5 was the lowest. This increase in the moisture content of cooked pasta supplemented with 2.5% of BLP can be attributed to a worse protein-polysaccharides interaction when compared to control and B5 (42).

4.6. Glucosinolate profile

Besides proteins and mineral compounds, the BLP analysed in this work was a good source of bioactive compounds, in particular GLS. Based on the obtained results (Table 3.6), the predominant glucosinolates in BLP were neoglucobrassicin and glucobrassicin. In contrast, Liu et al. (2018) showed that the major glucosinolates in broccoli leaf tissues were neoglucobrassicin and glucoraphanin (8), while Drabińska et al. (2018) demonstrated that glucobrassicin and glucoraphanin were the main GLS in fresh BLP (9). Although there is some similarity between the different results, in this study glucoraphanin only accounted for 15% of the total GLS, which was surprising. These differences are probably related to the storage of the BLP and suggest that glucoraphanin is not very stable during storage. Foods with high glucoraphanin content may have beneficial effects on health, mainly due to sulforaphane (88). This isothiocyanate is produced during glucoraphanin degradation and has been linked with the anticarcinogenic effects of Brassica vegetables (7). However, it has also been reported that the hydrolysis products of neoglucobrassicin and glucobrassicin, namely N-methoxyindole-3-carbinol and indole-3-carbinol, are able to inhibit the growth of human colon cancer cells (89). Thus, the anticarcinogenic effect of cruciferous vegetables, such as broccoli, is likely caused by the combined effect of various compounds, including sulforaphane, N-methoxyindole-3-carbinol and indole-3-carbinol. It is important to point out that even if myrosinase, the enzyme responsible for hydrolyzing GLS, is inactivated during blanching (as mentioned in section 2.1), the conversion of GLS to isothiocyanates is mediated by the colonic microbiota (90). For that reason, it is important to deliver the maximum amount of GLS in the intact form (not degraded thermally since thermal degradation can direct the hydrolysis into cyanides formation), as the gut microbiota will transform GLS into isothiocyanates.

In comparison with the obtained results, the total content of GLS in BLP estimated by Drabińska et al. (2018) was 45% higher (9). This highest concentration is probably related to the freshness of the BLP used in that study. Additionally, the GLS composition was slightly different. In that study, 9 GLS were

detected in BLP: 5 (glucoraphanin, glucobrassicin, neoglucobrassicin, 4-methoxy-glucobrassicin and 4-hydroxy-glucobrassicin) were common to this study; but 4 (glucoiberin, gluconasturtiin, unidentified GLS 1 and 2) were only detected in their study. In return, progoitrin was only identified in this research. These differences can be attributed to 1) lack of standards for all the GLS, which lead to misidentification of some compounds; 2) storage of the BLP for a few years, which could influence the content and composition of glucosinolates.

The stability of bioactive compounds after processing should be evaluated to assess the feasibility of functional food. In this particular case, the stability of glucosinolates in pasta products was estimated during cooking. As expected, glucosinolates were detected in pasta fortified with BLP. Surprisingly, the total GLS content of experimental pasta products was 28 and 12% higher than the predicted values for B2.5 and B5, respectively, calculated considering the content of GLS in BLP and the amount added to the pasta. This observation could be attributed to the degradation of plant tissue during thermal processing and the release of partially bound GLS from cell walls (91). Similarly, other authors reported that the total GLS content of mini sponge cakes fortified with BLP was higher than the predicted values (9). In addition, it should be noted that the increase observed in B2.5 was higher than in B5, which could be attributed to the presence of synergistic interactions between bioactive compounds and food ingredients (92).

The GLS detected in BLP were also detected in enriched pasta, except 4-hydroxy-glucobrassicin. Hanschen et al. (2012) reported that indole glucosinolates containing a hydroxyl group are more thermolabile (93). Therefore, it is likely that 4-hydroxy-glucobrassicin was lost during boiling of the pasta. In general, the predicted values of the analysed glucosinolates were lower than the experimental values. As expected, neoglucobrassicin and glucobrassicin were the predominant GLS in pasta. However, it was the glucoraphanin concentration that increased the most in relation to the predicted values. Glucoraphanin content was 36% and 23% higher than predicted for B2.5 and B5, respectively. This unexpected higher content of glucoraphanin in fortified pasta can be attributed to its stability during thermal processing. In fact, Ciska et al. (2015) demonstrated that glucoraphanin was the most thermally stable compound in boiled Brussels sprouts (91).

Although broccoli leaves are seldom utilized for food products, they were recently used to develop gluten-free mini sponge cakes (9). In that study, the total GLS content of mini sponge cakes fortified with 2.5% and 5% of BLP was higher than that of enriched pasta. This can be explained by the fact that glucosinolates are water-soluble compounds and as such, can leach from the pasta into the cooking water (5). Previous reports showed that leaching into water is the main cause of GLS losses during food preparation with water at high temperatures (94). In fact, Silva et al. (2013) demonstrated that after cooking, pasta enriched with different amounts of broccoli exhibited a reduction in GLS content. These authors also found out that there is no advantage in adding more than 20% of broccoli to pasta because after this point the retained amount of GLS did not increase further (5). Unfortunately, the content of GLS in cooking water was not analysed in this study and should be considered in future studies. Yet, to reduce the leaching into the cooking water some authors proposed that the boiling process should be done with less water (37).

4.7. Total phenolic content and antioxidant capacity

Phenolic compounds have been studied due to their several health benefits as antioxidants, including the prevention of several chronic diseases (14). Broccoli by-products have proven to be a good source of phenolic compounds (95). In fact, Liu et al. (2018) demonstrated that broccoli leaves had higher TPC (4.14 mg GAE/g DW) than florets and stems (8). In this study, BLP was characterized by greater TPC (6.8 mg GAE/g DM), suggesting that leaves can be used as a functional ingredient. This observation was supported by other authors (9, 19), who found an even higher TPC in BLP. Thus, broccoli leaves were recently used as a source of phenolics and antioxidant compounds to improve the bioactive potential of gluten-free bread and mini sponge cakes (9, 19). Although the effects of phenolic-rich additives with high levels of antioxidant activity have been previously analysed in pasta (45, 63), this was the first time that BLP was used to fortify a pasta product. Since food is a heterogeneous matrix with several chemical properties, different methods were used to determine the antioxidant capacity of the examined products, including the ABTS and FRAP assays.

Previous studies on the addition of by-products to semolina resulted in alterations in the antioxidant capacity of pasta (32). In general, the incorporation of by-products increased the total phenolic content and antioxidant activity of pasta products. For instance, the incorporation of mango peel powder as an ingredient in macaroni formulation significantly improved the TPC and the free radical scavenging activity (63). Accordingly, the addition of BLP to control pasta formulation significantly ($p < 0.05$) increased the TPC and antioxidant capacity of the analysed products (Table 3.8). This was expected since freeze-dried BLP presented high TPC and antioxidant potential. Besides, it is well-known that freeze-drying is a method that allows preserving the nutritional value of the starting material, including bioactive compounds (96). In agreement with the obtained results, an increase of antioxidant capacity after BLP incorporation was observed for supplemented gluten-free bread (19) and mini sponge cakes (9). Moreover, Lafarga et al. (2019) reported that the TPC and antioxidant capacity of wheat-based bread fortified with broccoli by-products increased after *in vitro* digestion, suggesting the health-promoting potential of products fortified with broccoli by-products can be even higher (97). However, it was not analysed in this study and requires further investigation.

The scavenging activity measured by ABTS assay is associated with the activity of hydrophilic compounds such as polyphenols (19). Therefore, the increase in the free radical scavenging may be attributed to the increase in TPC due to BLP incorporation. Although the TPC of the fortified pasta was slightly lower than the TPC of enriched (with 5% BLP) gluten-free bread, the scavenging ability measured by ABTS was higher in experimental pasta products. This result can be attributed to the presence of other reducing compounds in pasta (e.g., organic acids and/or volatile compounds) that may be related to the greater scavenging activity obtained. Also, the lowering of scavenging activity may be caused by blocking reactive groups of polyphenols by bread components. This observation was supported by Świeca et al. (2014), who investigated the antioxidant capacity of bread enriched with quinoa leaves and found that the obtained results were lower than expected (98). In comparison with mini sponge cakes fortified with broccoli leaves, pasta products presented similar TPC and scavenging ability. However, the ferric reducing power measured by FRAP was higher in pasta products. In fact, the

greatest increase (from 0.22 to 2.13 µmol Trolox/g DM) in antioxidant capacity was noted in the FRAP assay. This method treats the antioxidants contained in the samples as reductants, providing information about the ability to reduce Fe3+ to Fe2+, hence the value reflects the reducing power of antioxidants (99). The FRAP assay has been frequently used for a rapid evaluation of the total antioxidant capacity of several foods, including pasta fortified with phenolic-rich by-products, such as onion skin powder (45) and partially-deoiled chia flour (67). In such studies, the antioxidant capacity measured by FRAP increased with increasing levels of by-products added to pasta, which is in accordance with the obtained results. Altogether, these studies support the improvement of the antioxidant activity of plain wheat pasta through the use of ingredients of natural origin that are typically perceived as food waste.

For all assays, the obtained values were higher than the predicted values (Table 3.8). This observation allows for the possibility that the pasta matrix interacted with the bioactive compounds of BLP. In fact, the presence of interactions between phenolic compounds and wheat proteins have been previously described (98). The differences observed between predicted and experimental phenolic levels and antioxidant activity are also a consequence of thermal processing. During the cooking process, a series of chemical transformations is initiated, and the degradation of food ingredients gives rise to newly formed compounds that may play an important role in the biological properties of fortified food products (100). Besides, thermal processing liberates the insoluble conjugated bound phenolics, resulting in increased antioxidant activity (98). A similar trend of enhanced antioxidant properties after cooking was observed for pasta with different levels of onion by-products (45). In this study, it is more likely that the phenolic compounds released during the boiling process are components from BLP, and not from wheat, due to its high TPC. By contrast, some authors have concluded that phenolic compounds released in the boiling process are probably components from wheat (67). On the other hand, it should be noted that the Folin–Ciocalteu's reagent can reduce not only polyphenols, but also polysaccharides and proteins because the reaction is based on electron-transfer, which can lead to an overestimation of the TPC value (101). In addition, this method is a total quantification that only provides an estimate of the total polyphenols content present. In Brassica vegetables, the most widespread groups of polyphenols are flavonoids and hydroxycinnamic acids, and both can act as antioxidants by a number of potential pathways (95). Soengas et al. (2012) indicated that differences in antioxidant activity of Brassica crops were related to differences in TPC but also to differences in phenolic composition (95). Therefore, a more detailed analysis of individual phenolic compounds should be performed in the future to better understand the relationship between the antioxidant activity and the phenolic composition of broccoli by-products.

It is well known that the antioxidant capacity of food products is strongly correlated with free phenolic content (98). In this study, the TPC of experimental pasta products was 29% and 36% higher than the predicted values for B2.5 and B5, respectively. A similar trend was reported by Drabińska et al. (2018), who investigated the antioxidant capacity of mini sponge cakes fortified with 2.5 and 5% of BLP and concluded that baking did not affect TPC or antioxidant capacity in the ABTS and FRAP assays (9). Ranawana et al. (2016) indicated that freeze-dried broccoli significantly increased the vitamin E (α- and γ-tocopherols) and carotenoid contents of bakery products (87). However, the antioxidant capacity of lipophilic compounds in mini sponge cakes fortified with BLP was lower than expected, which suggest

that lipophilic compounds were thermally degraded during baking (9). In this study, the antioxidant capacity of lipophilic compounds in fortified pasta was not evaluated, but it is likely that fat-soluble vitamins and carotenoids were partly degraded during thermal processing (9, 102). Since previous reports (8) have shown that broccoli leaves are great sources of carotenoids, specially β-carotene and lutein that are characterised by a strong antioxidant activity, and vitamins E (α- and γ-tocopherols) and K, this hypothesis should be investigated in the future. Nevertheless, this study confirmed that underestimated by-products of broccoli processing could be a valuable additive to pasta by improving its functional quality.

Some studies have demonstrated that antioxidant activity peaks when leaves are harvested at the immature stage (95, 103). Therefore, the factors affecting the content of total phenolics in broccoli by-products, namely 1) plant cultivars and genetics, 2) soil composition and growing conditions, and 3) postharvest conditions, should be taken into consideration by growers or crop breeders for more sustainable and productive broccoli systems, especially in terms of by-product utilization.

5. Conclusions

The suitability of BLP as an ingredient in the manufacture of fortified wheat pasta was investigated based on the analysis of the technological, nutritional, and functional properties of the developed product. The obtained results indicated that BLP can be successfully used as an additive in pasta products. Its incorporation into pasta allowed an evident improvement of several nutritional properties compared with non-supplemented pasta. BLP enhanced the mineral and GLS content of pasta and their TPC and antioxidant activity mainly due to its high content of nutrients and bioactive compounds with potential health benefits. Interestingly, the GLS content in pasta was higher than expected. Thus, supplementation of pasta with BLP could be a valuable strategy for delivering compounds with anticarcinogenic properties, such as GLS, to the human body.

From the technological point of view, BLP can be an additive that enriches pasta. Nevertheless, it should be added in moderate amounts since the intensity of cabbage odour and taste increased as the concentration of BLP in pasta increased. However, the sensory evaluation results indicated that the addition of BLP did not affect the overall acceptance of pasta since all samples had great sensorial scores. Thus, for concentrations up to 5% of BLP, pasta remained acceptable for its texture, appearance, odour, and taste. This means that supplemented pasta would also be accepted by a large group of consumers even if the perception of cabbage flavour is present. Therefore, the enrichment of pasta with BLP could be considered a strategy to increase the intake of Brassica vegetables without drastically changing the eating habits of the population.

In conclusion, the addition of BLP to pasta allowed an improvement in the nutraceutical potential without compromising its technological and sensory quality. Therefore, broccoli leaves are an interesting alternative for the food industry to provide new value-added pasta products to consumers. Moreover, supplementing food products available on the market with natural sources of nutrients such as BLP is a way to minimize the use of artificial additives. Besides that, the incorporation of broccoli leaves into functional foods could facilitate the management of vegetable processing wastes, which is a current problem.

6. Future work

The obtained results indicated that BLP could be successfully used as an additive in pasta products since its incorporation allowed an evident improvement of several nutritional properties. However, investigations on the stability and interactions of phytochemicals with other food ingredients during processing and storage should be conducted in the future. Moreover, in the present study, pasta was produced without a drying stage. Therefore, the effect of an additional drying step during pasta manufacturing on the technological and functional properties of pasta should be evaluated.

In this work, the TPC was evaluated using the Folin–Ciocalteu's method, which is a total quantification and only provides an estimate of total polyphenols content present. A more detailed analysis of individual phenolic compounds should be performed in the future to better understand the relationship between the antioxidant activity and the phenolic composition of broccoli by-products. Moreover, it would be interesting to analyse the TPC and antioxidant capacity of enriched pasta after *in vitro* digestion. This analysis would provide more reliable information about the health-promoting potential of products fortified with broccoli by-products.

In this study, the antioxidant capacity of lipophilic compounds in fortified pasta was not evaluated, although it has been reported that broccoli leaves are a good source of carotenoids (β-carotene and lutein) and fat-soluble vitamins (E and K). This analysis should be performed in the future to investigate if fat-soluble vitamins and carotenoids are destroyed during thermal processing, as some reports stated.

The content of fibre was also not evaluated in this work, but it would be interesting to analyse this parameter since it may affect technological properties.

Given the current food trends, today's consumers are more prone to choose "natural" food products that are sustainable and health-promoting. Therefore, organoleptically interesting food products with a clean-label and additional health benefits, such as the functional pasta fortified with BLP developed in this study, are expected to be accepted by consumers. Thus, it is worthwhile to search for new directions and methods of utilizing vegetable by-products.

.

7. Appendixes

7.1. Appendix I – Pictures of control and supplemented pasta

Figure 7.1 *Control pasta.*

Figure 7.2 *Pasta fortified with 2.5% of BLP.*

Figure 7.3 *Pasta fortified with 5% of BLP.*

7.2. Appendix II – VOCs tentatively identified by GC-MS

Table 7.1 *Volatile compounds tentatively identified in BLP and pasta fortified with BLP. Data were expressed as percentage (%) of the total GC area.*

Volatile Compound	BLP	CONTROL	B2.5	B5	Odour description*
Dimethyl sulfide	0.30 ± 0.023	ND	0.20 ± 0.014	0.18 ± 0.01	cabbage, sulfur
Propanal	0.31 ±0.029	ND	2.44 ± 0.26	2.57 ± 0.11	pungent, sweet
Acetone	0.52 ± 0.020	ND	0.14 ± 0.042	ND	pungent, sweet
Butanal	0.13 ± 0.008	ND	ND	0.17 ± 0.02	malty, sweaty
2-Butanone	0.08 ± 0.019	ND	0.23 ± 0.024	0.11 ± 0.02	ethereal, fruity
Butanal, 2-methyl-	0.35 ± 0.027	ND	0.09 ± 0.012	0.13 ± 0.01	rancid, sulfur
Butanal, 3-methyl-	0.50 ± 0.031	ND	0.20 ± 0.023	0.24 ± 0.04	rancid, sulfur
Ethanol	0.14 ± 0.008	ND	ND	ND	alcoholic
Furan, 2-ethyl-	0.15 ± 0.024	ND	0.20 ± 0.029	0.30 ± 0.09	sweet, burnt, earthy, malty
1-Penten-3-one	0.14 ± 0.007	ND	0.08 ± 0.04	0.11 ± 0.03	green, pungent, mustard
Hexanal	3.25 ± 0.063	40.4 ± 3.69	15.1 ± 0.72	12.24 ± 0.86	green grass, fatty
2-Butenal, 2-methyl-, (E)-	0.82 ± 0.073	ND	0.25 ± 0.13	0.94 ± 0.14	green, fruity, aromatic
3-Penten-2-one	0.37 ± 0.005	ND	ND	ND	fruity, pungent
2-Pentenal, (E)-	1.28 ± 0.013	ND	0.70 ± 0.054	0.98 ± 0.03	green, apple, bitter,
1-Butanol	1.22 ± 0.024	ND	0.11 ± 0.058	0.19 ± 0.02	Malty

1-Penten-3-ol	6.74 ± 0.13	ND	1.42 ± 0.086	0.34 ± 0.05	pungent, milk-like
2-Hexanone, 4-methyl-	0.22 ± 0.021	ND	0.36 ± 0.071	ND	ethereal, bitter almond-like
Dodecane	0.42 ± 0.008	ND	ND	ND	alkane
Limonene	1.59 ± 0.072	9.81 ± 0,54	4.89 ± 0.59	5.54 ± 0.18	citrus
2-Hexenal, (E)-	1.97 ± 0.034	1.04 ± 0,08	1.89 ± 0.10	2.31 ± 0.09	green
Furan, 2-pentyl-	0.13 ± 0.021	4.15 ± 0.63	0.48 ± 0.15	0.97 ± 0.14	fruity
2-Heptanone, 6-methyl-	0.40 ± 0.015	ND	0.29 ± 0.054	0.51 ± 0.03	fruity, sour
4-Heptenal, (Z)-	0.89 ± 0.004	ND	0.41 ± 0.11	0.63 ± 0.07	rancid
1-Pentanol	0.69 ± 0.002	2.54 ± 0.34	1.42 ± 0.14	0.98 ± 0.09	fermented
2-Octanone	0.06 ± 0.007	ND	0.16 ± 0.036	0.15 ± 0.02	soapy, fruity
Octanal	0.22 ± 0.007	1.30 ± 0.22	2.59 ± 0.29	2.02 ± 0.17	green, fatty, citrus
2-Penten-1-ol, (E)-	0.25 ± 0.016	ND	ND	ND	green
2-Penten-1-ol, (Z)-	4.44 ± 0.049	ND	0.55 ± 0.079	ND	musty, compost-like
5-Hepten-2-one, 6-methyl-	5.66 ± 0.035	0.64 ± 0.08	2.98 ± 0.39	4.20 ± 0.20	citrus
Tetradecane	0.76 ± 0.080	ND	ND	ND	waxy
Nonanal	0.48 ± 0.009	1.80 ± 0.25	1.89 ± 0.14	2.13 ± 0.28	green, fatty, citrus
Dihydromyrcene	0.38 ± 0.007	ND	ND	ND	floral
2-Cyclohexen-1-one, 3,5,5-trimethyl-	1.43 ± 0.014	ND	0.65 ± 0.16	0.86 ± 0.12	woody
2-Octenal, (E)-	0.18 ± 0.007	2.26 ± 0.13	3.11 ± 0.31	2.84 ± 0.34	fatty

(Z)-linalool oxide	0.48 ± 0.016	ND	0.04 ± 0.019	0.09 ± 0.01	earthy
1-Octen-3-ol	1.01 ± 0.011	2.81 ± 0.36	6.82 ± 1.3	5.75 ± 0.66	earthy
2,4-Heptadienal, (E,E)-	1.67 ± 0.030	ND	0.49 ± 0.054	0.72 ± 0.08	green, fatty
1-Hexanol, 2-ethyl-	0.17 ± 0.004	1.18 ± 0.12	0.75 ± 0.085	0.73± 0.03	citrus
3,5-Octadien-2-one, (E,E)-	12.01 ± 0.30	ND	1.05 ± 0.14	1.93 ± 0.14	fruity
Benzaldehyde	2.40 ± 0.058	1.70 ± 0.71	0.62 ± 0.046	0.85 ± 0.15	fruity
4-Acetyl-1-methylcyclohexene	0.21 ± 0.025	ND	ND	ND	seasoning
Dimethyl Sulfoxide	0.33 ± 0.066	ND	ND	ND	alliaceous
2,6-Nonadienal, (E,Z)-	0.13 ± 0.045	ND	0.12 ± 0.018	0.24 ± 0.03	green
3,5-Heptadien-2-one, 6-methyl-, (E)-	0.72 ± 0.055	ND	0.20 ± 0.034	0.32 ± 0.08	spicy
Beta-cyclocitral	3.26 ± 0.14	ND	0.77 ± 0.11	1.51 ± 0.06	tropical, fruity
Safranal	0.30 ± 0.050	ND	0.17 ± 0.029	0.23 ± 0.02	herbal
Acetophenone	0.18 ± 0.044	0.22 ± 0.04	0.10 ± 0.006	0.13 ± 0.04	fruity
Phenol, 4-(2-propenyl)-	0.22 ± 0.019	ND	0.09 ± 0.026	0.15 ± 0.03	sweet, burned
2(5H)-Furanone, 5-ethyl-	0.26 ± 0.024	ND	0.08 ± 0.021	0.07 ± 0.01	sweet, spicy
(E)-geranyl acetone	0.68 ± 0.032	ND	0.32 ± 0.033	0.54 ± 0.07	floral
β-ionone	2.23 ± 0.041	ND	0.47 ± 0.038	0.94 ± 0.07	floral
β-ionone-5,6-epoxide	1.75 ± 0.021	ND	0.35 ± 0.046	0.70 ± 0.09	fruity
Dihydroactinidolide	2.26 ± 0.10	ND	0.26 ± 0.029	0.59 ± 0.10	fruity

Pentanal	ND	1.67 ± 0.14	1.29 ± 0.07	1.30 ± 0.04	fermented
4-Pentenal	ND	0.10 ± 0.002	ND	ND	roasted
Heptanal	ND	3.98 ± 0.30	1,87 ± 0.29	2.09 ± 0.07	fatty, citrus
Styrene	ND	0.28 ± 0.02	0.30 ± 0.077	0.27 ± 0.05	balsamic
2-Hexanone, 4-methyl-	ND	0.21 ± 0.04	ND	ND	ethereal, bitter almond-like
1-Hexanol	ND	11.0 ± 1,57	7.10 ± 0.70	4.97 ± 0.02	herbal
3-Hexen-1-ol, (Z)-	ND	0.29 ± 0.02	0.45 ± 0.045	0.44 ± 0.02	green
3-Octen-2-one	ND	0.80 ± 0.09	0,82 ± 0.023	0.55 ± 0.11	earthy
Heptanol	ND	0.85 ± 0.07	0.50 ± 0.05	0.45 ± 0.01	citrus
3-Octanol	ND	0.13 ± 0.01	0.09 ± 0.010	0.07 ± 0.01	earthy
2-Nonenal, (E)-	ND	1.47 ± 0.11	1.78 ± 0.21	2,23 ± 0,21	fatty
Linalool	ND	0.21 ± 0.04	0.59 ± 0.061	0.95 ± 0.03	citrus-like, flowery
2-Octen-1-ol, (E)-	ND	0.31 ± 0.06	2.03 ± 0.48	1.82 ± 0.32	green
1-Nonanol	ND	0.36 ± 0.02	0.31 ± 0.062	0.25 ± 0.01	floral
2,4-Nonadienal, (E,E)-	ND	0.22 ± 0.02	0.05 ± 0.017	0.04 ± 0.02	fatty
2,4-Decadienal, (E,E)-	ND	0.48 ± 0.05	0.42 ± 0.034	0.36 ± 0.04	fatty

*Odour descriptions were adapted from the literature and online databases. “ND”: not detected.

8. References

1. Ciska E, Drabińska N, Narwojsz A, Honke J. Stability of glucosinolates and glucosinolate degradation products during storage of boiled white cabbage. Food chemistry. 2016;203:340-7.
2. Domínguez-Perles R, Martínez-Ballesta MC, Carvajal M, García-Viguera C, Moreno DA. Broccoli-derived by-products--a promising source of bioactive ingredients. Journal of food science. 2010;75(4):C383-92.
3. Prieto MA, López CJ, Simal-Gandara J. Chapter Six - Glucosinolates: Molecular structure, breakdown, genetic, bioavailability, properties and healthy and adverse effects. In: Ferreira ICFR, Barros L, editors. Advances in Food and Nutrition Research. 90: Academic Press; 2019. p. 305-50.
4. Moreno DA, Carvajal M, López-Berenguer C, García-Viguera C. Chemical and biological characterisation of nutraceutical compounds of broccoli. Journal of pharmaceutical and biomedical analysis. 2006;41(5):1508-22.
5. Silva E, Gerritsen L, Dekker M, van der Linden E, Scholten E. High amounts of broccoli in pasta-like products: nutritional evaluation and sensory acceptability. Food & function. 2013;4(11):1700-8.
6. Tanii H. Allyl nitrile: Toxicity and health effects. Journal of occupational health. 2017;59(2):104-11.
7. Yagishita Y, Fahey JW, Dinkova-Kostova AT, Kensler TW. Broccoli or Sulforaphane: Is It the Source or Dose That Matters? Molecules (Basel, Switzerland). 2019;24(19):3593.
8. Liu M, Zhang L, Ser S, Cumming J, Ku K-M. Comparative Phytonutrient Analysis of Broccoli By-Products: The Potentials for Broccoli By-Product Utilization. Molecules. 2018;23:900.
9. Drabińska N, Ciska E, Szmatowicz B, Krupa-Kozak U. Broccoli by-products improve the nutraceutical potential of gluten-free mini sponge cakes. Food chemistry. 2018;267:170-7.
10. Milani A, Basirnejad M, Shahbazi S, Bolhassani A. Carotenoids: biochemistry, pharmacology and treatment. British journal of pharmacology. 2017;174(11):1290-324.
11. Saldeen K, Saldeen T. Importance of tocopherols beyond α-tocopherol: evidence from animal and human studies. Nutrition Research. 2005;25(10):877-89.
12. Beulens JW, Booth SL, van den Heuvel EG, Stoecklin E, Baka A, Vermeer C. The role of menaquinones (vitamin K_2) in human health. The British journal of nutrition. 2013;110(8):1357-68.
13. Parfitt J, Barthel M, Macnaughton S. Food Waste within Food Supply Chains: Quantification and Potential for Change to 2050. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2010;365:3065-81.
14. Trigo JP, Alexandre EMC, Saraiva JA, Pintado ME. High value-added compounds from fruit and vegetable by-products - Characterization, bioactivities, and application in the development of novel food products. Critical reviews in food science and nutrition. 2020;60(8):1388-416.
15. Gómez M, Martinez MM. Fruit and vegetable by-products as novel ingredients to improve the nutritional quality of baked goods. Critical reviews in food science and nutrition. 2018;58(13):2119-35.
16. Kowalska H, Czajkowska K, Cichowska J, Lenart A. What's new in biopotential of fruit and vegetable by-products applied in the food processing industry. Trends in Food Science & Technology. 2017;67:150-9.
17. Padalino L, D'Antuono I, Durante M, Conte A, Cardinali A, Linsalata V, et al. Use of Olive Oil Industrial By-Product for Pasta Enrichment. Antioxidants (Basel). 2018;7(4):59.
18. Dominguez-Perles R, Moreno DA, Carvajal M, Garcia-Viguera C. Composition and antioxidant capacity of a novel beverage produced with green tea and minimally-processed byproducts of broccoli. Innovative Food Science & Emerging Technologies. 2011;12(3):361-8.
19. Krupa-Kozak U, Drabińska N, Bączek N, Šimková K, Starowicz M, Jeliński T. Application of Broccoli Leaf Powder in Gluten-Free Bread: An Innovative Approach to Improve Its Bioactive Potential and Technological Quality. Foods. 2021;10(4).
20. Rincón-León F. Encyclopedia of Food Sciences and Nutrition. Edition n, editor: Academic Press; 2003.

21. Castillo M, Iriondo-DeHond A, Martirosyan D. Are Functional Foods Essential for Sustainable Health? 2018.
22. EUROPEIA PEECDU. REGULAMENTO (CE) N.o 1924/2006 DO PARLAMENTO EUROPEU E DO CONSELHO de 20 de Dezembro de 2006 relativo às alegações nutricionais e de saúde sobre os alimentos. Jornal Oficial da União Europeia2006.
23. Gul K, Singh AK, Jabeen R. Nutraceuticals and Functional Foods: The Foods for the Future World. Critical reviews in food science and nutrition. 2016;56(16):2617-27.
24. Howlett J. Functional Foods From Science to Health and claims. ILSI EUROPE CONCISE MONOGRAPH SERIES: ILSI EUROPE; 2008.
25. INSA, NUIG. A quadruple helix Atlantic Area healthy food Ecosystem for growth of SMEs. 2020.
26. EUFIC. What are Functional Foods and How Can They Help Us? 2006 [Available from: https://www.eufic.org/en/food-production/article/functional-foods.
27. Özyurt G, Uslu L, Yuvka I, Gökdoğan S, Atci G, Ak B, et al. Evaluation of the Cooking Quality Characteristics of Pasta Enriched with Spirulina Platensis. Journal of Food Quality. 2015;38(4):268-72.
28. Clydesdale F. Functional Foods: Opportunities & Challenges. Food Technology Magazine. 2004.
29. Foundation BN. Functional foods 2009 [Available from: https://www.nutrition.org.uk/nutritionscience/foodfacts/functional-foods.html?limitstart=0.
30. Siegrist M, Stampfli N, Kastenholz H. Consumers' willingness to buy functional foods. The influence of carrier, benefit and trust. Appetite. 2008;51(3):526-9.
31. Bustos MC, Perez GT, Leon AE. Structure and quality of pasta enriched with functional ingredients. RSC Advances. 2015;5(39):30780-92.
32. Nilusha RA, Jayasinghe J, Perera ODAN, Perera P. Development of Pasta Products with Nonconventional Ingredients and Their Effect on Selected Quality Characteristics: A Brief Overview. International Journal of Food Science. 2019;2019:1-10.
33. Organisation IP. Report on world pasta consumption & production 2018 [Available from: https://internationalpasta.org/annual-report/.
34. Real H, Barbosa M, Carvalho T. Massas alimentícias, uma abordagem técnica e científia. Colecção E-books APN: Associação Portuguesa dos Nutricionistas; 2014.
35. Chillo S, Laverse J, Falcone PM, Del Nobile MA. Quality of spaghetti in base amaranthus wholemeal flour added with quinoa, broad bean and chick pea. Journal of Food Engineering. 2008;84(1):101-7.
36. Jalgaonkar K, Jha SK, Mahawar MK. Influence of incorporating defatted soy flour, carrot powder, mango peel powder, and moringa leaves powder on quality characteristics of wheat semolina-pearl millet pasta. Journal of Food Processing and Preservation. 2018;42(4):e13575.
37. Oliviero T, Fogliano V. Food design strategies to increase vegetable intake: The case of vegetable enriched pasta. Trends in Food Science & Technology. 2016;51:58-64.
38. Wójtowicz A, Mościcki L. Influence of legume type and addition level on quality characteristics, texture and microstructure of enriched precooked pasta. LWT - Food Science and Technology. 2014;59(2, Part 1):1175-85.
39. Padalino L, Mastromatteo M, Lecce L, Spinelli S, Conte A, Del Nobile MA. Optimization and characterization of gluten-free spaghetti enriched with chickpea flour. International journal of food sciences and nutrition. 2015;66(2):148-58.
40. Lorusso A, Verni M, Montemurro M, Coda R, Gobbetti M, Rizzello CG. Use of fermented quinoa flour for pasta making and evaluation of the technological and nutritional features. LWT. 2017;78:215-21.
41. Padalino L, Conte A, Del Nobile MA. Overview on the General Approaches to Improve Gluten-Free Pasta and Bread. Foods. 2016;5(4):87.
42. Desai AS, Brennan MA, Brennan CS. Effect of Fortification with Fish (Pseudophycis bachus) Powder on Nutritional Quality of Durum Wheat Pasta. Foods. 2018;7(4):62.
43. GOES ESdR, SOUZA MLRd, MICHKA JMG, KIMURA KS, LARA JAFd, DELBEM ACB, et al. Fresh pasta enrichment with protein concentrate of tilapia: nutritional and sensory characteristics. Food Science and Technology. 2016;36:76-82.

44. Liu T, Hamid N, Kantono K, Pereira L, Farouk MM, Knowles SO. Effects of meat addition on pasta structure, nutrition and in vitro digestibility. Food chemistry. 2016;213:108-14.
45. Michalak-Majewska M, Teterycz D, Muszyński S, Radzki W, Sykut-Domańska E. Influence of onion skin powder on nutritional and quality attributes of wheat pasta. PloS one. 2020;15(1):e0227942.
46. Boroski M, de Aguiar AC, Boeing JS, Rotta EM, Wibby CL, Bonafé EG, et al. Enhancement of pasta antioxidant activity with oregano and carrot leaf. Food chemistry. 2011;125(2):696-700.
47. Sant'Anna V, Christiano F, Marczak L, Tessaro I, Thys R. The effect of the incorporation of grape marc powder in fettuccini pasta properties. LWT - Food Science and Technology. 2014;58:497–501.
48. Lončarić A, kosović I, Marko J, Žaneta U, Pilizota V. Effect of apple by-product as a supplement on antioxidant activity and quality parameters of pasta. Croatian Journal of Food Science Technology. 2014;6:97-103.
49. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer prevention: a review. Journal of the American Dietetic Association. 1996;96(10):1027-39.
50. Capacci S, Mazzocchi M, Shankar B, Brambila Macias J, Verbeke W, Pérez-Cueto FJ, et al. Policies to promote healthy eating in Europe: a structured review of policies and their effectiveness. Nutrition Reviews. 2012;70(3):188-200.
51. Piwińska M, Wyrwisz J, Kurek M, Wierzbicka A. Effect of drying methods on the physical properties of durum wheat pasta. CyTA - Journal of Food. 2016:1-6.
52. Krupa-Kozak U, Drabińska N, Rosell CM, Piłat B, Starowicz M, Jeliński T, et al. High-Quality Gluten-Free Sponge Cakes without Sucrose: Inulin-Type Fructans as Sugar Alternatives. Foods. 2020;9(12).
53. ISO. ISO 8586:2014 Sensory analysis — General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors. 2014.
54. ISO. ISO 13299:2016 Sensory analysis — Methodology — General guidance for establishing a sensory profile. 2016.
55. ISO. ISO 8589:2007 Sensory analysis — General guidance for the design of test rooms. 2010.
56. AOAC. Official Methods of Analysis of AOAC International. 18th edition ed. Gaithersburg: Association of Official Analytical Chemists; 2011.
57. NATIONS F-FAAOOTU. Food energy - methods of analysis and conversion factors. FAO FOOD AND NUTRITION PAPER 77. 2003.
58. Official Journal of the European Communities, L 170. 1990.
59. Campas-Baypoli ON, Sánchez-Machado DI, Bueno-Solano C, Núñez-Gastélum JA, Reyes-Moreno C, López-Cervantes J. Biochemical composition and physicochemical properties of broccoli flours. International journal of food sciences and nutrition. 2009;60(sup4):163-73.
60. Brennan CS, Kuri V, Tudorica CM. Inulin-enriched pasta: effects on textural properties and starch degradation. Food chemistry. 2004;86(2):189-93.
61. Sobota A, Rzedzicki Z, Zarzycki P, Kuzawińska E. Application of common wheat bran for the industrial production of high-fibre pasta. International Journal of Food Science & Technology. 2015;50(1):111-9.
62. Dick JW, Youngs VL. Durum Wheat Chemistry and Technology. Durum wheat: chemistry and technology. 1988:237-48.
63. Ajila CM, Aalami M, Leelavathi K, Rao UJSP. Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations. Innovative Food Science & Emerging Technologies. 2010;11(1):219-24.
64. Chillo S, Monro JA, Mishra S, Henry CJ. Effect of incorporating legume flour into semolina spaghetti on its cooking quality and glycaemic impact measured in vitro. International journal of food sciences and nutrition. 2010;61(2):149-60.
65. Petitot M, Boyer L, Minier C, Micard V. Fortification of pasta with split pea and faba bean flours: Pasta processing and quality evaluation. Food Research International. 2010;43(2):634-41.
66. Granito M, Torres A, Guerra M. Development and evaluation of wheat, corn, cassava and beans pasta. Interciencia. 2003;28(7):372-9+426.

67. Aranibar C, Pigni NB, Martinez M, Aguirre A, Ribotta P, Wunderlin D, et al. Utilization of a partially-deoiled chia flour to improve the nutritional and antioxidant properties of wheat pasta. LWT. 2018;89:381-7.
68. Gull A, Prasad K, Kumar P. Effect of millet flours and carrot pomace on cooking qualities, color and texture of developed pasta. LWT - Food Science and Technology. 2015;63(1):470-4.
69. Systems SM. Cutting/Shearing Tests 2019 [Available from: https://www.stablemicrosystems.com/cutting-and-shearing-testing.html.
70. Cappa C, Alamprese C. Brewer's spent grain valorization in fiber-enriched fresh egg pasta production: Modelling and optimization study. LWT - Food Science and Technology. 2017;82:464-70.
71. Beleggia R, Platani C, Spano G, Monteleone M, Cattivelli L. Metabolic profiling and analysis of volatile composition of durum wheat semolina and pasta. Journal of Cereal Science. 2009;49(2):301-9.
72. De Flaviis R, Sacchetti G, Mastrocola D. Wheat classification according to its origin by an implemented volatile organic compounds analysis. Food chemistry. 2021;341:128217.
73. Wieczorek MN, Majcher M, Jeleń H. Comparison of Three Extraction Techniques for the Determination of Volatile Flavor Components in Broccoli. Foods. 2020;9(4):398.
74. Wieczorek MN, Majcher MA, Jeleń HH. Identification of aroma compounds in raw and cooked broccoli. Flavour and Fragrance Journal. 2021;36(5):576-83.
75. Lindsay RC, Rippe JK. Enzymic Generation of Methanethiol To Assist in the Flavor Development of Cheddar Cheese and Other Foods. Biogeneration of Aromas. ACS Symposium Series. 317: American Chemical Society; 1986. p. 286-308.
76. Vermeulen C, Gijs L, Collin S. Sensorial Contribution and Formation Pathways of Thiols in Foods: A Review. Food Reviews International. 2005;21(1):69-137.
77. Wei S, Xiao X, Wei L, Li L, Li G, Liu F, et al. Development and comprehensive HS-SPME/GC–MS analysis optimization, comparison, and evaluation of different cabbage cultivars (Brassica oleracea L. var. capitata L.) volatile components. Food chemistry. 2021;340:128166.
78. CHIN H-W, LINDSAY RC. Volatile Sulfur Compounds Formed in Disrupted Tissues of Different Cabbage Cultivars. Journal of food science. 1993;58(4):835-9.
79. Hansen M, Buttery RG, Stern DJ, Cantwell MI, Ling LC. Broccoli storage under low-oxygen atmosphere: identification of higher boiling volatiles. Journal of agricultural and food chemistry. 1992;40(5):850-2.
80. Giannetti V, Boccacci Mariani M, Mannino P, Testani E. Furosine and flavour compounds in durum wheat pasta produced under different manufacturing conditions: Multivariate chemometric characterization. LWT - Food Science and Technology. 2014;56(1):15-20.
81. Rodríguez-Bernaldo De Quirós AI, López-Hernández J, González-Castro MJ, De la Cruz-García C, Simal-Lozano J. Comparison of volatile components in raw and cooked green beans by GC-MS using dynamic headspace sampling and microwave desorption. European Food Research and Technology. 2000;210(3):226-30.
82. Gaggiotti S, Shkembi B, Sacchetti G, Compagnone D. Study on volatile markers of pasta quality using GC-MS and a peptide based gas sensor array. LWT. 2019;114:108364.
83. Lewinsohn E, Sitrit Y, Bar E, Azulay Y, Ibdah M, Meir A, et al. Not just colors—carotenoid degradation as a link between pigmentation and aroma in tomato and watermelon fruit. Trends in Food Science & Technology. 2005;16(9):407-15.
84. Mendes-Pinto MM. Carotenoid breakdown products the—norisoprenoids—in wine aroma. Archives of Biochemistry and Biophysics. 2009;483(2):236-45.
85. Gidamis AB, Panga JT, Sarwatt SV, Chove BE, Shayo NB. Nutrient and Antinutrient Contents in Raw and Cooked Young Leaves and Immature Pods Of Moringa oleifera, Lam. Ecology of Food and Nutrition. 2003;42(6):399-411.
86. Sedlar T, Čakarević J, Tomić J, Popović L. Vegetable By-Products as New Sources of Functional Proteins. Plant Foods for Human Nutrition. 2021;76(1):31-6.
87. Ranawana V, Campbell F, Bestwick C, Nicol P, Milne L, Duthie G, et al. Breads Fortified with Freeze-Dried Vegetables: Quality and Nutritional Attributes. Part II: Breads Not Containing Oil as an Ingredient. Foods. 2016;5(3):62.

88. Soundararajan P, Kim JS. Anti-Carcinogenic Glucosinolates in Cruciferous Vegetables and Their Antagonistic Effects on Prevention of Cancers. Molecules. 2018;23(11):2983.
89. Neave AS, Sarup SM, Seidelin M, Duus F, Vang O. Characterization of the N-methoxyindole-3-carbinol (NI3C)–Induced Cell Cycle Arrest in Human Colon Cancer Cell Lines. Toxicological Sciences. 2005;83(1):126-35.
90. Angelino D, Jeffery E. Glucosinolate hydrolysis and bioavailability of resulting isothiocyanates: Focus on glucoraphanin. Journal of Functional Foods. 2014;7:67-76.
91. Ciska E, Drabińska N, Honke J, Narwojsz A. Boiled Brussels sprouts: A rich source of glucosinolates and the corresponding nitriles. Journal of Functional Foods. 2015;19:91-9.
92. Sęczyk Ł, Świeca M, Gawlik-Dziki U, Luty M, Czyż J. Effect of fortification with parsley (Petroselinum crispum Mill.) leaves on the nutraceutical and nutritional quality of wheat pasta. Food chemistry. 2016;190:419-28.
93. Hanschen FS, Rohn S, Mewis I, Schreiner M, Kroh LW. Influence of the chemical structure on the thermal degradation of the glucosinolates in broccoli sprouts. Food chemistry. 2012;130(1):1-8.
94. Ciska E, Kozłowska H. The effect of cooking on the glucosinolates content in white cabbage. European Food Research and Technology. 2001;212(5):582-7.
95. Soengas P, Cartea ME, Francisco M, Sotelo T, Velasco P. New insights into antioxidant activity of Brassica crops. Food chemistry. 2012;134(2):725-33.
96. Xu Y, Xiao Y, Lagnika C, Li D, Liu C, Jiang N, et al. A comparative evaluation of nutritional properties, antioxidant capacity and physical characteristics of cabbage (Brassica oleracea var. Capitate var L.) subjected to different drying methods. Food chemistry. 2020;309:124935.
97. Lafarga T, Gallagher E, Bademunt A, Viñas I, Bobo G, Villaró S, et al. Bioaccessibility, physicochemical, sensorial, and nutritional characteristics of bread containing broccoli co-products. Journal of Food Processing and Preservation. 2019;43(2):e13861.
98. Świeca M, Sęczyk Ł, Gawlik-Dziki U, Dziki D. Bread enriched with quinoa leaves – The influence of protein–phenolics interactions on the nutritional and antioxidant quality. Food chemistry. 2014;162:54-62.
99. Du B, Xu B. Oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) of β-glucans from different sources with various molecular weight. Bioactive Carbohydrates and Dietary Fibre. 2014;3(1):11-6.
100. Peng X, Ma J, Cheng K-W, Jiang Y, Chen F, Wang M. The effects of grape seed extract fortification on the antioxidant activity and quality attributes of bread. Food chemistry. 2010;119(1):49-53.
101. Vilas-Boas AA, Campos DA, Nunes C, Ribeiro S, Nunes J, Oliveira A, et al. Polyphenol Extraction by Different Techniques for Valorisation of Non-Compliant Portuguese Sweet Cherries towards a Novel Antioxidant Extract. Sustainability. 2020;12(14):5556.
102. Lešková E, Kubíková J, Kováčiková E, Košická M, Porubská J, Holčíková K. Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. Journal of Food Composition and Analysis. 2006;19(4):252-76.
103. Hwang J-H, Lim S-B. Antioxidant and anticancer activities of broccoli by-products from different cultivars and maturity stages at harvest. Prev Nutr Food Sci. 2015;20(1):8-14.