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Development of a gluten-free artisanal fresh pasta with thermal water and associated flours

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I dedicate this work:

TO MY DEAR PARENTS: Fathi Hasni and Noura Dhahri

No words can truly capture the depth of my respect, my undying love, my appreciation, and my gratitude for the sacrifices you’ve made for my education and well-being. I am deeply thankful for all the support and love you have shown me since childhood, and I hope to always have your blessing. This humble work is a small reflection of your hopes, the result of your countless sacrifices, though I know I can never fully repay you, May God bless you with good health, happiness, and a long life, and may I always make you proud.

IN MEMORY OF MY GRANDMOTHER: MIMA FATIMA

I wish you could have been here. May God hold your soul in His holy mercy.

TO MY DEAR SISTER AND BROTHER: GHADA AND OUSSEMA

At the memories of all the moments we've shared, I want to thank you for being in my life and for your unwavering support and love.

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Thank you for constantly pushing me to be more ambitious, for always being there for me, and for your unwavering support and endless love.

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LIST OF ABBREVIATIONS

a*: Red/green chromaticity
ANOVA: Analysis of variance
Aw: Water activity
AOAC: Association of Official AgriculturalChemists
b*: Blue/yellow chromaticity
ºC: Degrees Celsius
CeD: Celiac disease
CFU/g: Colony Forming Units per gram
C.e.I.R.S.A. (Centro interdipartimentale de Ricerca e documentazione sulla Sicurezza Alimentare, Italy)
DW: Distilled water.
DPPH: Diphenyl-1-picrylhidrazyl radical
GF: Gluten free
GAE: Gallic acid equivalents
Kcal: Kilocalorie
L*: Lightness level
Nd: Not detected
OCT: Optimal cooking time
ORAC: Oxygen radical absorbance capacity
RSM: Response surface methodology
TPC: Total phenolic compounds
TW: Thermal water

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ABSTRACT

The demand for gluten-free (GF) products is among the fastest-growing segments in the food industry, driven by consumers seeking healthier options. This trend pushes the industry to expand its offerings with innovative formulations and alternative ingredients that deliver both nutrition and health benefits. Fresh pasta is a globally popular food due to its taste and ease of preparation, but GF versions remain limited, highlighting the need for new approaches. In this context, teff, rice, and buckwheat stand out for their complementary nutritional and functional benefits. Nevertheless, they have never been explored for fresh pasta in combination. Additionally, thermal water (TW), an abundant endogenous resource in northern Portugal, is rich in minerals, representing an underutilized alternative to enrich food products with these essential nutrients. Given this, the present study aimed to develop GF fresh pasta using optimized mixtures of teff, rice, and buckwheat flours, and enrich the optimized formulation with TW. Firstly, a mixture design was applied to achieve a balance of functional qualities, namely total phenolic compounds (TPC) and antioxidant activity (DPPH radical inhibition), and technological parameters, including texture (hardness, chewiness, cohesiveness, adhesiveness), optimal cooking time, water absorption and cooking loss rates. Standard fresh wheat-based pasta was used as a reference to select the best formulations. Two optimized mixtures were chosen: F4 (27.5 g teff and 27.5 g buckwheat) and F9 (36.3g buckwheat, 9.35g rice, and 9.35g teff), which were characterized for proximate composition, mineral content, TPC, antioxidant activity (DPPH and ORAC), water activity (a_{w}), color (L*a*b*), technological properties, and microbiological quality (over seven days at 4 °C). Control samples made with distilled water (DW) were also analyzed, resulting in four formulations: F4_DW, F4_TW, F9_DW, and F9_TW. Moisture levels and a_W met fresh pasta standards (ranging from 27.19±0.89 g/100 g_{dw} to 35.26±0.57 g/100 g_{dw}, and from 0.983±0.009 to 0.987±0.004). Technological qualities (cooking loss, water absorption and cooking time) were acceptable. Regarding texture, values varied greatly between formulations for hardness and chewiness, cohesiveness and adhesiveness. The differences in the technological parameters between the samples with or without TW were low or insignificant, suggesting no effect of its incorporation on pasta quality properties. The formulations showed balanced nutritional composition, with low lipid leves and protein concentrations comparable to traditional pasta. Additionally, the formulations were rich in K, Ca, Fe, Mn and Zn. The pasta also contained TPC varying from 1.03±0.02 to 1.30±0.06 mg GAE/100 g and antioxidant activity through DPPH and ORAC assays. All formulations were free of *B. cereus* and demonstrated acceptable microbiological quality for at least three days at 4 °C, revealing that TW did not affect the microbiological quality and stability of the final product. Therefore, this study demonstrates the viability of buckwheat, teff, and rice mixtures as ingredients in gluten-free fresh pasta and confirms TW's potential as a novel ingredient in food formulations. The results provide important new information for developing high-quality gluten-free food items and a very good substitute for conventional wheat-based dishes.

Keywords: artisanal pasta, fresh pasta, gluten-free, thermal water, teff, buckwheat, rice, total phenolic compounds, antioxidant activity.

RESUMO

A procura de produtos sem glúten (GF) está entre os segmentos de crescimento mais rápido na indústria alimentar, impulsionada pelos consumidores que procuram opções mais saudáveis. Esta tendência leva a indústria a expandir as suas ofertas com fórmulas inovadoras e ingredientes alternativos que proporcionam benefícios nutricionais e para a saúde. A massa fresca é um alimento mundialmente popular devido ao seu sabor e facilidade de preparação, mas as versões GF continuam a ser limitadas, o que realça a necessidade de novas abordagens. Neste contexto, o teff, o arroz e o trigo mourisco destacam-se pelos seus benefícios nutricionais e funcionais complementares. No entanto, nunca foram explorados para massas frescas em combinação. Adicionalmente, a água termal (AT), um recurso endógeno abundante no norte de Portugal, é rica em minerais, representando uma alternativa subutilizada para enriquecer produtos alimentares com estes nutrientes essenciais. Neste contexto, o presente estudo teve como objetivo desenvolver massas frescas GF utilizando misturas optimizadas de farinhas de teff, arroz e trigo sarraceno, e enriquecer a formulação optimizada com TW. Em primeiro lugar, foi aplicado um modelo de mistura para obter um equilíbrio entre as qualidades funcionais, nomeadamente os compostos fenólicos totais (TPC) e a atividade antioxidante (inibição do radical DPPH), e os parâmetros tecnológicos, incluindo a textura (dureza, mastigabilidade, coesividade, adesividade), o tempo de cozedura ideal, a absorção de água e as taxas de perda na cozedura. Para selecionar as melhores formulações, foram utilizadas como referência massas frescas à base de trigo. Foram escolhidas duas misturas optimizadas: F4 (27,5 g de teff e 27,5 g de trigo mourisco) e F9 (36,3 g de trigo mourisco, 9,35 g de arroz e 9,35 g de teff), que foram caracterizadas quanto à composição proximal, conteúdo mineral, TPC, atividade antioxidante (DPPH e ORAC), atividade de água (aw), cor (L*a*b*), propriedades tecnológicas e qualidade microbiológica (durante sete dias a 4 °C). Foram também analisadas amostras de controlo feitas com água destilada (DW), resultando em quatro formulações: F4_DW, F4_TW, F9_DW e F9_TW. Os teores de humidade e a aw corresponderam às normas para massas frescas (variando de 27,19±0,89 g/100 gdw a 35,26±0,57 g/100 gdw, e de 0,983±0,009 a 0,987±0,004). As qualidades tecnológicas (perda de cozedura, absorção de água e tempo de cozedura) foram aceitáveis. Relativamente à textura, os valores variaram muito entre as formulações para a dureza e mastigabilidade, coesividade e adesividade. As diferenças nos parâmetros tecnológicos entre as amostras com ou sem TW foram baixas ou insignificantes, sugerindo que não há efeito da sua incorporação nas propriedades de qualidade da massa. As formulações apresentaram uma composição nutricional equilibrada, com baixos níveis de lípidos e concentrações de proteínas comparáveis às massas tradicionais. Além disso, as formulações eram ricas em K, Ca, Fe, Mn e Zn. As massas também continham TPC variando de 1,03±0,02 a 1,30±0,06 mg GAE/100 g e atividade antioxidante através de ensaios DPPH e ORAC. Todas as formulações estavam isentas de B. cereus e demonstraram uma qualidade microbiológica aceitável durante pelo menos três dias a 4 °C, revelando que a TW não afectou a qualidade microbiológica e a estabilidade do produto final. Portanto, este estudo demonstra a viabilidade de misturas de trigo sarraceno, teff e arroz como ingredientes em massas frescas sem glúten e confirma o potencial da TW como um novo ingrediente em formulações alimentares. Os resultados fornecem novas informações importantes para o desenvolvimento de alimentos sem glúten de alta qualidade e um substituto muito bom para pratos convencionais à base de trigo.

Palavras-chave: massa artesanal, massa fresca, sem glúten, água termal, teff, trigo mourisco, arroz, compostos fenólicos totais, atividade antioxidante.

1. Introduction

Due to its delectable flavor, ease of preparation, and low cost, pasta is a basic staple food item. It is often made with water, wheat flour, or semolina. The most important element that directly impacts the quality of pasta is considered to be the presence of gluten (Sozer, 2009). Nevertheless, some people with celiac disease (CeD) must avoid gluten-containing foods because they can harm the small intestine and interfere with the absorption of vital nutrients (Sapone et al., 2012). Therefore, gluten-free (GF) pasta is becoming a highly sought-after commercial good.

Growing numbers of people without celiac disease are choosing gluten-free products because of wheat allergies, irritable bowel syndrome, or non-celiac gluten sensitivity. Non-celiac gluten sensitivity causes symptoms similar to celiac disease without the autoimmune response (Sapone et al., 2012). In addition, many customers are switching to gluten-free diets because they think it will be healthier. Gluten-free diets have gained popularity beyond medical necessity, with many believing it promotes better digestion, according to Aziz et al. (2015). Due to this, there is now a greater market for gluten-free products, including GF pasta.

Owing to the unique features of wheat gluten, replacement is always a challenge. In order to achieve equivalent sensory, nutritional, and technological properties of wheat-containing products, GF pasta is usually prepared using flour mixtures able to provide complementary characteristics. Cereals (rice, corn, sorghum), minor cereals (teff, millet), pseudo cereals (buckwheat, quinoa, amaranth), and legumes are some of the most commonly utilized ingredients in GF pasta (Jnawali et al., 2016; Romano et al., 2021). Adjuvant ingredients, such as eggs and/or gums (e.g., xanthan gum, guar gum), may also be necessary (Sanguinetti et al., 2015)

Usually, the composition of the ingredients should be optimized to achieve ideal technological parameters (Marco & Rosell, 2008; De Pilli et al., 2013). In this context, mixture designs and response surface methodology (RSM) have been essential allies in developing gluten-free pasta formulations and other GF products using different ingredient combinations. Mixture designs are used to optimize formulations where the components of a mixture are the variables of interest. The great advantage of using mixture designs is the possibility of evaluating the effect of individual ingredients on the desired responses, as well as the possible interactions (synergistic or antagonistic) between them (Cornell, 2002.

Montgomery, 2005)

Teff, rice, and buckwheat flours have high phytochemical and nutritional qualities, thusit was hypothesized that by combining them, could result in a healthy nutritious GF pasta formulation. To the best of our knowledge, this grain combination has never been used to make fresh pasta before, so it is a creative way to investigate new raw materials for creating novel, health-promoting food products.

Thermal waters (TW) are natural mineral waters used at thermal sites for therapeutic purposes or health benefits (Nguyen Ba, 2020). Their varied chemical composition and richness in mineral compounds have been linked to myriad health advantages, including the reduction of inflammation-related illness symptoms (Lakhdari and Bouaicha, 2016; Ar et al., 2017; Silva et al., 2020). Portugal is one of the wealthiest nations in Europe, with a variety of properties, most of which are found in the country's northern and central areas (Viegas et al., 2019; Araujo et al., 2017). This endogenous resource has been well known in therapeutic applications. However, TW has been neglected in terms of food applications.

Due to the richness in minerals of TW (Quattrini et al., 2014; Pop et al. 2021, Oliveira et al., 2020), it was hypothesized that TW could be used as a functional ingredient to improve the mineral profile of fresh pasta. Given the scarcity of innovative products in the GF sector and the growing demand in this market, this study aimed (i) to develop an innovative formulation of GF artisanal fresh pasta (tagliatelle type) combining the nutritional and potential health benefits of buckwheat, teff, and rice; (ii) explore the potential of TW to enrich food products with mineral compounds and create novel, value-added products using endogenous resources.

1.1 Pasta formulations: main characteristics

Pasta is a dish consisting of mixing cereal flour, traditionally durum wheat semolina (Triticum turgidum, subsp. durum), and water, followed by proper physical processes like drawing, rolling, and drying to give it the desired appearance for consumers (Pagani et al., 2007). Semolina is highly used due to its intense yellow color and, remarkably, to its unique and high protein content (10.9– 13.5%), inducing high functionality and the appropriate dough structure properties (Pagani et al., 2007). It is generally consumed after being cooked in boiling water.

Moisture content is the most commonly used parameter for pasta classification. While dry pasta content a moisture percentage of less than 12% and water activity of 0.6 or less, fresh pasta moisture is around 24-35% and water activity is around 0.9 (Beltrão Martins et al., 2020; Pagani et al., 2007).

Pasta can be classified according to different parameters (Pagani et al., 2007).

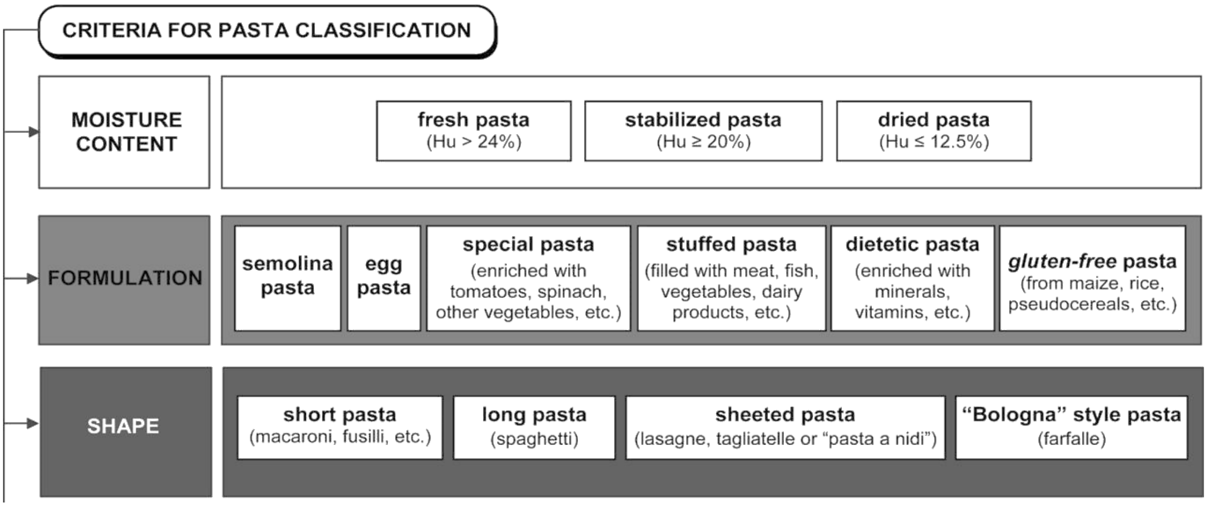


Figure 1. Classification of the different formulations of pasta. **Source**: Pagani et al., 2007.

In terms of classification based on the shaping process, fresh pasta can be categorized into "long-cut pasta" or laminated pasta, crafted from a sheet of pasta approximately 1 mm thick, which is subsequently cut into various-width "ribbons" for dishes like lasagne, tagliatelle, or fettuccine. Additionally, there are short pasta varieties with distinctive shapes (such as orecchiette, trofie, and others) and products formed through die-shaping, including fusilli, macaroni, and others on (**Figure 2**).

Fresh pasta is made using a technique called lamination or sheeting. After the ingredients (wheat and water) are kneaded slowly, without causing friction or heating of the dough, it is sent to the dough sheeter, where it is pressed into a compact sheet by a couple of smooth rolls. The next stage is the calibration stage, which comprises a sequence of steps using smooth calibrators adjusted to give the desired thickness desired (around 0.5 mm-thick) and a smooth and velvety finish (Pagani et al., 2007).

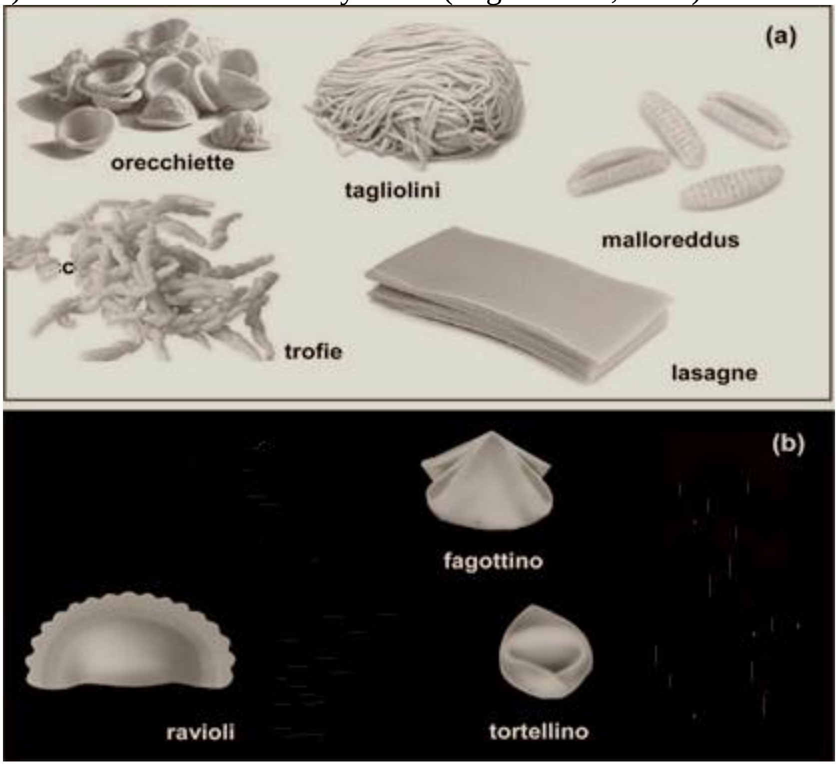


Figure 2. The various shapes of fresh pasta. (a): types of dry pasta shapes, (b): types of filled or stuffed pasta shapes Source: (Pagani et al., 2007).

Fresh pasta may have durum wheat semolina partially or entirely substituted with other cereals, plants or with raw materials from animal sources. Traditionally, added materials are eggs, and vegetables such as tomatoes, spinach, or carrots, which serve more as flavoring and coloring agents than a technological scope (Romano et al., 2021).

Currently, the use of high functionality ingredients for value addition to pasta show two main driving forces: increase the nutritional value of pasta, by partial replacement/enrichment of semolina with highly nutritious raw materials such as dietary fiber and a wide range of unconventional ingredients into dried pasta recipe; prevent allergenicity/intolerance in particular to gluten, by the complete replacement of semolina with GF raw materials (Fiorda et al., 2013; Romano et al., 2021).

1.2 Gluten-free pasta

In pasta formulations, proteins, particularly gluten, play a crucial role as formatation components, enhancing the pasta's water absorption capacity, cohesiveness, viscosity, and elasticity, thereby improving its overall structure, texture, and sensory attributes (Jnawali et al., 2016; Romano et al., 2021). Consequently, the absence of gluten in pasta products often leads to reduced mechanical strength, necessitating the optimization of formulations by combining matrices with complementary technological characteristics and incorporating other ingredients, such as hydrocolloids, that can mimic gluten properties (Carpentieri et al., 2022; Larrosa et al., 2013; Romano et al., 2021).

Starches (potato, cassava, corn), gums (guar gum, xanthan gum), and emulsifiers are employed to enhance the viscosity, firmness, acceptability, body, and mouthfeel of the final product (Larrosa et al., 2013). Legumes, including beans, lentils, and peas, are incorporated into gluten-free pasta formulations due to their high protein content (Carpentieri et al., 2022; Jnawali et al., 2016; Khairuddin and Lasekan, 2021). Additionally, non-vegetable protein sources like eggs and whey powder have been cautiously utilized to improve the technological characteristics of gluten-free pasta (Phongthai et al., 2017; Ungureanu-Iuga et al., 2020).

Rice (*Oryza sativa* L.) and corn (*Zea mays*) flours are the most used raw materials for GF pasta. Along with wheat, they are the three primary commodities in cereals worldwide (Khairuddin and Lasekan, 2021). Rice stands out as the most significant crop in total production across the developing world, serving as a staple for many consumers (BO, 2016). The production of milled rice flour involves a wet milling process utilizing a spindle mill equipped with a sieve, all conducted at a low drying temperature (BO, 2016). The major components in rice flour are carbohydrates, predominantly starch. In comparison to other cereals, rice flour is characterized by low levels of fat (2.2 g/100g), protein (7.7 g/100g), and dietary fiber (2.2 g/100g) (BO, 2016). Despite these lower values, rice flour contains all eight essential amino acids in its composition, including lysine, threonine, methionine, tryptophan, phenylalanine, isoleucine, leucine, and valine (Yang et al., 2022). Rice flour is hypoallergenic, possesses high quality protein, exhibits excellent digestibility, and has a mild flavor and color. It has been demonstrated, when combined with other food matrices, to be an effective base for producing gluten-free (GF) pasta (Bouasla et al., 2017; Ferreira et al., 2016; Fradinho et al., 2020; Witek et al., 2020). For instance, Ferreira et al. (2016) utilized a blend of sorghum, rice, corn flour, and potato starch in the development of GF pasta. Their findings revealed that the optimal mixture consisted of sorghum flour, rice flour, and potato starch in a ratio of 40:20:40. This combination exhibited superior density, yield, weight increase, and the least loss of solids.

In addition to rice, pasta formulations have extensively explored the use of sorghum, quinoa, millet, buckwheat, and legume flours (Khairuddin and Lasekan, 2021). There has been a push for innovative raw materials as well (Fiorda et al., 2013). These novel ingredients are suggested to offer potential health benefits, providing an opportunity to address the nutritional limitations of existing commercially available gluten-free products (Jnawali et al., 2016; Khairuddin and Lasekan, 2021; Romano et al., 2021).

Among them, teff (*Eragrostis tef* (Zucc.) Trotter) (**Figure 3**) is a tropical cereal mainly cultivated in Ethiopia. It contains carbohydrates (73%), fibers (typically 3%), protein levels similar to those of wheat (11%), low-fat content, minerals, particularly iron, calcium, copper, and zinc, and a well-balanced amino acid profile, with a complete set of essential amino acids (Bultosa, 2016). Due to its superior nutritional quality compared to other cereals has been studied as a valuable ingredient to improve the quality of GF products (Campo et al., 2016).

Figure 3. Teff grain.

Teff flour has been incorporated into formulations for bread and cakes (Campo et al., 2016; Homem et al., 2021; Marti et al., 2017; Oliveira). Combined with other gluten-free matrices such as rice, buckwheat, and wheat, teff flour has demonstrated promising outcomes as a novel ingredient in bakery products without compromising technological quality (Homem et al., 2021). A separate bread-focused study found that adding 25% fermented teff flour enhanced the texture properties of gluten-free bread. Additionally, teff flour proved successful in a cake formulation, exhibiting high protein content, ash levels, satisfactory overall acceptability, and favorable technological parameters (Bultosa, 2016). However, teff has never been tested in GF fresh pasta.

Buckwheat (Fagopyrum esculentum) (Figure 4) is an herbaceous crop originating from central and northeast Asia (De Arcangelis et al., 2020). Classified as a pseudocereal, buckwheat shares some characteristics with traditional cereals regarding composition, cultivation methods, and culinary uses (De Arcangelis et al., 2020). Comprising polysaccharides and proteins, buckwheat offers a well-balanced composition of amino acids, fibers, and minerals (De Arcangelis et al., 2020). Its rising popularity stems from its gluten-free nature in baking and pasta products and the presence of various bioactive compounds with significant dietary, health, and functional properties. For instance, De Arcangelis et al. (2020) combined propylene glycol alginate (0.1%), monoglycerides of fatty acids (0.5%), and the gelatinization of mixed flour (buckwheat, corn, and rice) to produce dried pasta with optimal cooking characteristics and texture. However, this approach has not been applied to fresh pasta yet. Moreover, buckwheat has never been combined with teff to produce GF pasta, highlighting an opportunity to develop new products assembled with the excellent nutritional and bioactive properties of both flours.

Figure 4. Buckwheat grain

1.3 Pasta quality parameters

A thorough analysis of the various physical, chemical, and sensory aspects of pasta quality that affect the product's acceptability is required to determine its overall quality. Determiningthe pasta's texture, nutritional value, cooking performance, and depends consumer preference on these criteria. A product's ingredients, production methods, and cooking environment all affect its quality attributes. The main characteristics of pasta quality discussed section are color, taste, cooking loss, texture (hardness and firmness), water absorption, and nutritional quality (Padalino, Conte, & Del Nobile, 2016).

1.3.1 Water absorption

Water absorption is a key component of pasta quality, which increase the texture, yield, and overall cooking outcome. Pasta's volume and weight rise as it cooks and absorbs water, contributing to the final yield. A product with higher water absorption will have a softer texture than one with lower absorption. The water absorption capacity of pasta during boiling significantly impacts the final texture and is a key quality criterion, especially in gluten-free formulations. To achieve the right texture and mouthfeel, pasta must absorb waterwell without getting too soft or losing its form (Marti & Pagani, 2013).

1.3.2 Cooking loss

The amount of solid material that leaches into the cooking water while making pasta is called "cooking loss." It is a crucial indicator of the structural integrity of pasta, and higher quality is correlated with less cooking loss. An overcooked, sticky food with little nutritional content results from excessive cooking loss. Pasta with substantial cooking loss often frequently softer, lacks cohesion, and is generally less consumers favored. Since the gluten network that usually supports the structure of pasta made with wheat is absent from gluten-free pasta formulations, reducing cooking loss is especially crucial to maintaining product integrity (Padalino, Conte, & Del Nobile, 2016).

1.3.2 Color

Another important quality factor that affects consumers perception and preference is pasta's color. Due to the high carotenoid concentration of durum wheat, pasta prepared with this grain typically has a brilliant yellow hue, considered a quality sign. Other factors affecting color include the type of flour used, how it is processed, and whether or not natural pigments are present. The color can change significantly depending on the substitute grains or pseudocereals—such as buckwheat, teff, or rice—used in gluten-free pasta. Bright, brilliant colors are desirable in pasta, as consumers connect pale or dull-colored pasta with lesser quality. Thus, a pleasing hue is crucial for creating pasta products made with alternative grains and without gluten (Petitot et al., 2009).

1.3.4 Nutritional quality

Pasta's nutritional worth is becoming more and more significant to consumers, especially as more and more health-conscious people look for foods with greater nutritional content. While traditional pasta made from wheat is poor in fiber and protein, it is high in carbohydrates. Better nutritional profiles, such as increased protein, fiber, and mineral content, can be obtained by using alternative grain pasta made from teff, buckwheat, or other pseudocereals. For example, rice-based pasta is frequently enhanced with extra vitamins and minerals to make up for its lower nutrient density, whereas buckwheat and teff are recognized for their high mineral content. To create a successful product, pasta recipes must satisfy consumers' expectations for both taste and nutrition (Saturni et al, 2010).

1.3.5 Texture

One of the most crucial aspects of pasta quality is texture, which has a direct impact on both customer happiness and the sensory experience. Other important textural characteristics are adhesiveness, chewiness, and cohesiveness. Hardness is the pasta's resistance to deformation when an applied force is applied. Cohesiveness defines how well the pasta stays together while being chewed, chewiness shows how much effort is needed to chew the pasta, and adhesiveness shows how well the pasta sticks to other ingredients or surfaces. The entire structure of the pasta is determined by these textural characteristics combined, giving it the desired "bite" or al dente sensation. "Texture, particularly hardness, is a major quality factor in pasta and is often regarded as a benchmark for product acceptability (Motta Romero et al., 2017).

1.4 Thermal waters

Thermal waters (TW) are natural mineral waters defined as deep circulating waters of the specific bacteriological profile with physicochemical characteristics that are stable at their origin within the range of natural fluctuations and possess some therapeutic properties or health-beneficial effects (Oliveira et al., 2020). They are enriched in minerals, such as sodium, magnesium, zinc, boron, manganese, selenium, and trace elements in contact with the rocks as it crosses before gushing to the surface ofthe earth (Porowski et al., 2019). Portugal is one of the richest European countries in TWsources, and most of them are located in the Northern and central regions of the country

(Araujo et al., 2017).

TW are classified according to parameters such as temperature, osmotic pressure, and radioactivity. Regarding temperature, they are classified as hypothermal (<25°C), mesothermal (between 25-35°C; thermal (between 35-40°C); hyperthermal (> 40°C). Regarding to the chemical composition, natural mineral waters can be trace minerals, alkaline-bicarbonate, alkaline-earth, calcic alkaline earths, magnesium alkaline earths, sulfated, sulfurous, nitrated, chlorinated, ferruginous, radioactive, toriative and carbon dioxide (Cruz et al., 2022). Mineralization, characterized by the residue or dry extract in milligrams per liter (mg/L) at 180 °C, can be categorized as described in **Table 1** (Nguyen Ba, 2020). Portuguese TW is mostly described as weakly mineralized, sulfurous, bicarbonate or chlorinated, and sodium (Araujo et al., 2017).

Table 1. Classification of natural mineral waters based on fixed residue at 180°C.

Fixed residue at 180°C	**Definition**
< to 50 mg/L	very weakly mineralized
between 50 and 500 mg/L	weakly mineralized
500 and 1.000 mg/L	moderately mineralized between
1.000 and 1.500 mg/L	mineralized between
> 1.500 mg/L	highly mineralized or rich in mineral salts

Source: Nguyen Ba, 2020.

TW may also be classified according to their therapeutic properties, a practice called balneotherapy. Portuguese primarily therapeutic indications are for respiratory, rheumatic, and musculoskeletal systems (Oliveira et al., 2020); other studies have reported the antimicrobial activity against fungi and bacteria of TW from Portugal and other countries (Oliveira et al. 2019). Benefits to the digestive, endocrine–metabolic diseases and nephrourinary systems have also been described (Oliveira et al., 2020). These beneficial properties can be justified by the presence of dominant components, namely sulfur and bicarbonate (Araújo et al. 2017).

Additionally, to its therapeutic benefits, it has been suggested that natural mineral water, such as TW, could be introduced in the diet to boost the mineral intake. One potential advantage of minerals from TW is that they are free, so they could be more bioavailable than in usual food products. Despite this promising scenario, the study of the benefits of incorporating thermal water in food products has been limited. The interesting chemical and bioactive properties of TW described above raise suggest that TW could be used, for example, as a functional ingredient. Thus, studies investigating this possibility should be encouraged (Araújo et al. 2017).

1.5 Mixture design

When creating gluten-free pasta, it is important to carefully select and combine substitute ingredients to achieve the best texture and other quality characteristics. Gluten-free pasta formulations lack the viscoelastic proprieties of gluten, which provide them structure, elasticity, and cohesiveness, in contrast to standard semolina-based pasta. Mixture design is a statistical technique that food issue scientists use to optimize the amounts ofvarious ingredients in complex recipes in order to address this (Kamali Rousta et al., 2021).

Researchers can assess the effects of compositional changes on a mixture's outcome through mixture design. Developing new food products works well because different ingredients combine to influence the final product's texture, flavor, and cooking behavior. Based on the principle that the sum of all component proportions must equal a constant, this design approach is perfect for optimizing recipes such as gluten-free pasta, where the balance of various flours (such as rice, teff, and buckwheat) and other additives (such as gums and starches) is necessary to produce desired results (Padalino et al. (2016)

The advantages of using mixture designs in developing gluten-free product formulation may be summarized as:

- Optimization of multiple ingredients: To produce gluten-free product with the right texture and cooking qualities, it is essential to optimize various flours, starches, and other additives simultaneously. This is made possible by the design of the mixture (Mancebo et al. 2015)
- Effects of interaction: the technique sheds light on the interactions between substances. This is especially crucial for gluten-free formulations, since specific flour and gum combinations might work in concert to improve the end product's structure or flexibility (Mancebo et al. 2015)

2 Objectives

2.1 Main objective

Develop a new formulation of gluten-free fresh pasta (tagliatelle type) combining teff, buckwheat, and rice, and enrich it with thermal water.

2.2 Specific objectives

- Optimize the formulation of a gluten-free fresh pasta using rice, teff, and buckwheat through a mixture design.
- Determine the quality parameters of the formulated pasta.
- Determine the nutritional and mineral composition of the formulated pasta.
- Study the total phenolic compounds content and antioxidant activity of the produced pasta.
- Study the microbiological quality and stability of the formulated pasta.

3 Materials and methods

3.1 Raw materials

The flour and other essential ingredients for pasta production (xanthan, guar gums, eggs, and starch) were purchased in local markets in Bragança or accessible online platforms.

Thermal water samples required for the study were provided by Termas de Chaves, located in Chaves, Portugal in sterile polyethylene bottles. The mineral composition of the utilized TW was determined previously. The TW was provided by Termas de Chaves, Chaves, Portugal, with the following major compounds: K = 61 mg/L; Mg = 4.9 mg/L; Na = 614 mg/L; Ca = 23 mg/L; Cu = 0.01 mg/ L; Mn = 0.01 mg/L; Zn = 0.04 mg/L ; Fe = 0.67 mg/L (Radhouane, et al. 2024).

As a control, commercial pasta (tagliatelle type) was bought from Bragança, Portugal's local markets. The product was made in accordance with the manufacturer's instructions, which stated two minutes of boiling water for the pasta. The recipe specified that the contents included water, eggs (18%), and durum wheat semolina. According to the label's nutritional table, it has 2.9% dietary fiber, 11% proteins, 3% fats, and 50% carbohydrates. Images of the used ingredients can be found in **Figure 5**.

a. Teff flour. **b.** Buckwheat flour. **c.** Rice flour

d. Xanthan gum **e.** Guar gum

f. Commercial pasta

Figure 5. Raw materials for pasta production.

3.2 Mixture design for pasta formulation

Fresh pasta formulations using teff, buckwheat, and rice flour were developed following the methods outlined by Fradinho et al. (2020). The proportions of each flour were adjusted according to a mixture design, as detailed in **Table 2**. Initial tests indicated additional ingredients were needed to create a suitable pasta structure with minimal breakage. Therefore, various concentrations of gums, starch, and eggs were tested to achieve the desired characteristics for the base pasta formulation. For these tests, equal amounts of each flour (corresponding to formulations in experiments 10, 11, and 12 in **Table 2**) were used to evaluate the combined effect of all three flours.

After the tests, the final concentrations of the additional ingredients were set at 2.0% guar gum, 2.0% xanthan gum, 3.2% starch, and 9.0% fresh eggs. These percentages were fixed based on visual criteria of the resulting pasta that allowed to create a uniform dough that with the help of a laminator, could be rolled and cut into tagliatelle shapes. Water was added to maintain a moisture content of 28.8%, in line with fresh pasta standards (Lacivita et al., 2022).

Only distilled water was used during the optimization phase. In preparing the pasta, the flour accounted for 55 g in every 100 g of the formulation (after accounting for coadjuvants and water). The flour proportions (ranging from 0-100%) were distributed based on this final amount (as shown in **Table 2**). The flour, water, and additional ingredients were combined to form a uniform dough, then rolled and cut into tagliatelle using a laminator.

Table 2. Mixture design for the pasta formulation.

Formulation code	**% Flour (g)**		
	Teff flour	**Rice flour**	**Buckwheat flour**
F1	0	0	100 (55)
F2	0	100 (55)	0
F3	100 (55)	0	0
F4	50 (27.5)	0	50 (27.4)
F5	50	50 (27.5)	0
F6	0	50 (27.5)	50 (27.5)
F7	66 (36.3)	17 (9.35)	17 (9.35)
F8	17 (9.35)	66 (36.3)	17 (9.35)
F9	17 (9.35)	17 (9.35)	66 (36.3)
F10	33 (18.15)	33 (18.15)	33 (18.15)
F11	33 (18.15)	33 (18.15)	33 (18.15)
F12	33 (18.15)	33 (18.15)	33 (18.15)

The responses studied in the mixture design were cooking loss and cooking time, maximum water absorption, texture profile (hardness, cohesiveness, adhesiveness and chewiness), total phenolic compounds (TPC) and the inhibition of free radical diphenyl-1-picrylhidrazyl radical (DPPH), as described in section 8.5.

The responses were adjusted to appropriate mathematical models, which quality was evaluated through Analysis of Variance (ANOVA) for regression significance and lack of fit, at 95% of confidence level. The generated models were used to determine the best flour association based on minimum cooking loss, optimal cooking time, maximum water absorption, TPC and DPPH, and texture profile like wheat-based pasta.

3.3 Incorporation of thermal water

The optimized fresh pasta formulations were prepared with TW or distilled water (DW), resulting in the following samples: F4_DW (formulation 4 with distilled water), F4_TW (formulation 4 with thermal waters); F9_DW (formulation 9 with distilled water), F9_TW (formulation 9 with thermal water). The formulations were characterized for the following analyses: proximate composition (moisture, crude protein, fat, ash, carbohydrates), cooking loss, water absorption rate, optimum cooking time, water activity (a_w), texture profile (hardness, chewiness, adhesiveness, cohesiveness), mineral content, TPC, DPPH, and ORAC (Oxygen Radical Absorbance Capacity**)** assays, described in section 8.5.

3.4 Microbial stability

Pasta samples were placed in airtight, transparent polyethylene bags (dimensions 20.5x18x4 cm), sealed, and stored at 4 °C for 7 days. Three pouches of each formulation were collected on days 0, 3, and 7 for microbial load evaluation. The conducted microbial analyses were: total aerobic mesophiles, coliforms, yeasts, molds, and *Bacillus cereus*. The methods utilized are briefly described in section 8.5.

3.5 Methods

3.5.1 Cooking loss and water absorption

The content of 25 g of pasta were cooked in a beaker with 300 mL of boiling distilled water, according to AACC method 66.50.01 (AACC International, 2000). By adding distilled water, the beaker's water level was kept at 300 mL. The sample was taken out and rinsed with 300 milliliters of distilled water after it had been cooked for the ideal amount of time. The pasta sample was weighed following a 3-minute slow draining period. The water used for cooking and washing was gathered and dried in an oven set to 105 °C until it reached a consistent weight.

The cooking loss (%) and water absorption (%) were calculated according to Eqs. (1) and (2):

$$\%\ \text{Cooking loss} = \frac{\text{weight of cooking and ricing water after drying}}{\text{weight of uncooked pasta}} * 100 \quad (1)$$

$$\%\ \text{Water absorption} = \frac{\text{Weight of cooked pasta} - \text{weight of uncooked pasta}}{\text{Weight of uncooked pasta}} * 100 \quad (2)$$

3.5.2 Optimum cooking time (OCT)

The ideal cooking duration for pasta samples was determined using AACC method 16–50 (AACC International, 1995). Twenty-five grams of pasta were boiled in a 300 mL beaker. At 30-second intervals, a pasta piece was taken out of the boiling water and compressed between glass slides to assess its cooking stage. The point at which the white center of the sample vanished was identified as the "optimal cooking time."

3.5.3 Physical parameters

3.5.3.1 Moisture

The moisture content was measured in a 2 g sample using the PMB Moisture Analyzer (Kingston, Milton Keynes, U.K.) (figure 7). The analysis was performed in triplicates for each sample.

Figure 6.PMB Moisture Analyzer (Kingston, Milton Keynes, U.K.)

3.5.3.2 Water activity

Water activity ($a_{w)}$ was measured using the AquaLab S4TE equipment (Lab-Ferrer, Cervera, Spain).

3.5.3.3 Color measurement

The color was measured using a colorimeter (model CR-400, Konica Minolta Sensing Inc, Tokyo, Japan) previously calibrated against a standard white tile. The CIE L* (lightness), a* (greenness-redness), and b* (blueness-yellowness) color space values were recorded using Spectra Magic Nx software (version CM-S100W 2.03.0006).

3.5.3.4 Texture profile

A Stable Micro Systems (Vienna Court, Godalming, UK) TA. XT Plus texture analyzer (figure 8) with a 30 kg load cell was used to analyze the texture profile (hardness, adhesiveness, cohesiveness, and chewiness). The data was processed through Exponent program.

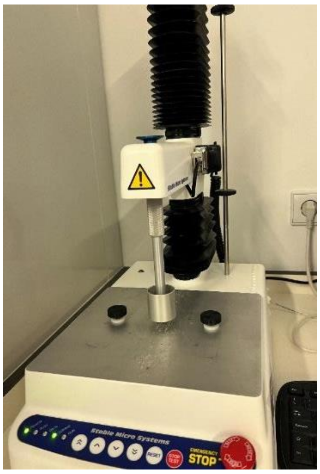


Figure 7. Texture analyser

3.6 Centesimal composition

3.6.1. Crude protein

Protein content was assessed via Kjeldahl nitrogen determination with a copper catalyst in 0.25 g of each sample, using a Kjeldahl steam distillation unit (Pro-Nitro A, Selecta, Spain) following the AOAC 920.87 Method. Nitrogen levels were converted to protein using a factor of 6.25. The analysis was conducted in triplicate for each sample. The results were expressed as g/100 g.

a. Bloc digest **b.** Kjeldahl steam distillation

Figure 8. Equipment used for protein content

3.6.2 Crude Fat

The total lipid was determined through the AOAC 989.05, 3 g of sample was treated with petroleum ether in a Soxhlet apparatus for 4 hours (**Figure 9**). The petroleum ether extract was evaporated under reduced pressure to dryness, the residues were weighed, then the lipid content was expressed as a mass percentage. The analysis was performed in triplicates for each sample. The total fat content was given as g/100 g

Figure 9. Total lipid extraction using Soxhlet apparatus.

3.6.3 Ash

The porcelain crucibles were weighed and placed in the oven. If the analysis was not carried out on the same day, the crucibles were placed in the desiccator. 250 mg of sample was weighed into the crucible, and the sample was placed in the muffle furnace (figure 11) for 5 hours at 600°C. After incineration, the weight was removed from the sample.

Figure 10. Muffle furnace.

3.6.4 Carbohydrates and Energy

Total carbohydrates were assessed by difference [100 − (g proteins + g fat + g ash)], and energetic value according to the equation: energy value [kcal/100 g dry weight (dw)] = 4 × (g protein + g carbohydrates) + 9 × (g fat).

3.7 Mineral compositions

Minerals was analyzed using atomic absorption spectrometry (iCE™ series, 3000, Thermo Scientific). Potassium (K), sodium (Na), zinc (Zn), magnesium (Mg) e lithium (Li) was determined using flame atomization, while aluminum (Al), arsenic (As), cadmium (Cd), cupper (Cu), chromium (Cr), plumb (Pb), iron (Fe), manganese (Mn), nickel (Ni) e selenium (Se) was detected through electrothermal atomization. The quantification of the mineral compounds was performed using external calibration.

3.8 Total phenolic compounds and antioxidant activity

3.8.1 Compound´s extraction

Extraction was conducted following the procedure introduced by Beta, Nam, Dexter, and Sapirstein (2005). In summary, finely ground samples (1 g) underwent two extraction cycles, involving shaking at room temperature in a 4 ml solution of methanol: water (80:20 *v/v*) for 2 hours. The resulting mixtures were then subjected to centrifugation at 1000g for 15 minutes, and the supernatants obtained after centrifugation were combined.

3.8.2 Total phenolic compounds

TPC was determined by Folin-Ciocalteu method (Singleton et al., 1999) adapted for a microplate reader. Briefly, the extract or standard solution was mixed with Folin-Cioacalteu reagent, followed by sodium carbonate. The intensity of the blue color formed after 2 h reaction was monitored at 760 nm. Results expressed as mg of gallic acid equivalents (GAE) per 100 grams of sample.

3.8.3 Radical scavenger assay through DPPH

Using a slightly modified version of the methodology outlined in the literature, the capacity to inhibit free radicals from DPPH (2,2-diphenyl-1-picrylhydrazyl) was assessed in triplicate (Ferreira et al., 2009). In short, 0.15 mL of a methanolic solution containing DPPH radicals (0.024 mM) was combined with 10 μL of methanol extract at varying doses (0.003 – 0.03 mg. mL-1). DPPH radical scavenging activity was assessed using a microplate reader (ELX800 Microplate Reader Bio-Tek Instruments, Inc.) to track the drop in absorbance at 517nm following 60 minutes in the dark at room temperature. The DPPH radical scavenging activity was calculated as a percentage using the following equation in which Abs corresponds to the absorbance of the solution with the sample extract and ABS DPPH to the initial absorbance of the DPPHsolution.

$$\% \text{ Inhibition} = [(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{blank}}] \times 100$$

3.8.4 Oxygen radical absorbance capacity (ORAC)

The tests were conducted using a microplate reader FLUOstar Omega (BMG LABTECH, Ortenberg, Germany) by the methodology outlined by Dávalos et al. (2004). The reaction media was potassium phosphate buffer (pH 7.4, 75 mM), and the fluorescent probe was fluorescein. Microplates with 20 µL of the sample or various Trolox concentrations, 120 µL of fluorescein, and 60 µL of radical AAPH (2.2′-azobis (2-methylpropionamidine) dihydrochloride) were exposed to readings every minute for a total of 80 minutes at a controlled temperature of 37°C (emission and excitation wavelengths of 485 nm and 520 nm, respectively). A regression equation involving Trolox concentrations and the net area under the fluorescein kinetic decay curve was used to calculate the ORAC values, which were then represented in µmol Trolox Equivalent per 100 g dw.

3.9 Microbiological stability

For microbial analyses, sample preparation adhered to the guidelines outlined in the International Organization for Standardization (ISO 15213-1:2023). The methodologies employed followed specific ISO standards, including ISO 4833-2:2013 for TAM, ISO 4832:2006 for coliforms, ISO 21527-2:2008 for molds and yeasts, and ISO 7932:2004 for B. cereus.

Briefly, total aerobic mesophiles were determined using the incorporation sowing approach, which involved placing 1 mL of each dilution of the material in a Petri dish and then adding 15 mL of Plate Count Agar (PCA), incubated for 72 hours at 30°C while being held inverted. Plates with 15–300 colonies were used for counting (Limit of Quantification (LOQ) = 1 log). The microbial counts were reported as Colony Forming Units per gram of sample (CFU/g).

Molds and yeasts were determined using the spread plate method, 0.2 mL of each dilution was added to Petri dishes containing 15 mL of Dichloran Rose Bengal Chloramphenicol (DRBC). The counting was done on plates with fewer than 150 colonies (LOQ = 1.7 log CFU/g), which were incubated in the upright posture at 25°C for 5 days. After 3 and 5 days of incubation, respectively, yeasts and molds were enumerated. Results were expressed as CFU/g.

Regarding coliforms, the dilutions were inoculated into VRBLA using the pour plate technique, in duplicate: 1 mL of suspension was pipetted into the plate and 15 mL of melted VRBLA (kept at 50 °C in a water bath or incubator) was poured. The mixture was homogenized and allowed to solidify. On top of the medium, a top layer of 4 mL of VRBLA was poured and allowed to solidify. The plates were then incubated at 30°C for 48 hours, in a reversed position. Colonies were counted in the plates having between 10 and 150 colonies. Typical coliform colonies appeared purple, with a diameter of 0.5 cm, and sometimes with a purple precipitate around. E. coli colonies were observed in the medium supplemented with MUG as blue fluorescent colonies when observed under long UV light (365 nm). The limit of quantification (LOQ) was 10 CFU/g.

For *B. cereus*, the dilutions were inoculated into MYP (Mannitol Yolk Polymyxin) using the spread plate technique, in duplicate: 0.2 mL of suspension had been pipetted onto a plate containing 15 mL of the medium and spread with the disposable spreader. The plates were then incubated at 30°C for 24-48 hours in the reversed position. Colonies were counted in the plates having between 10 and 150 colonies. Typical B. cereus colonies appeared crenated with a diameter of 0.5 cm, blue in color, and usually surrounded by a distinct opaque zone of egg yolk precipitation of the same color as the colonies.

3.10 Statistics

The analyses were conducted in triplicate, and the results were presented as the mean ± standard deviation. Comparisons between samples were made using Analysis of Variance (ANOVA) and multiple comparison tests, specifically Tukey's test. For samples analyzed over a storage period, Repeated Measures ANOVA followed by Bonferroni's multiple comparison test was applied. Differences between samples were considered significant when $p<0.05$. The data was processed using Statistica 7.0 software.

4 Results and discussion

4.1 Mixture design for gluten-free pasta formulation

Table 3 shows the results for antioxidant activity (DPPH), TPC, pasta quality parameters (cooking loss, water absorption, OCT), and texture profile of each formulation in the mixture design, and **Figure 11** summarizes these results. **Figure 12** shows the final appearance of pasta formulations in each experimental condition.

Regarding DPPH, F1 (100% buckwheat) presented the highest percentage of inhibition (85.06%), so it has the highest antioxidant capacity, and F2 (100% rice) showed the lowest antioxidant capacity (6.26% inhibition). Thus, it is possible to conclude that buckwheat contains a higher amount of antioxidant compounds compared with teff and, finally, rice.

Regarding TPC, the formulations with the highest values (91.09 mg GAE/100 g, 77.23 mg GAE/100 g, and 83.52 mg GAE/100 g where those rich in buckwheat, namely F1, F4, and F9, respectively. On the other hand, F2 had a much lower TPC value (12.98 mg GAE/g) and was composed entirely of rice, indicating that adding buckwheat raises the TPC content of gluten-free pasta. According to Yuan et al. (2020) and Zhang et al. (2019), buckwheat is an important source of phenolic compounds linked to several health advantages, including antioxidant activity.

The OCT of all formulas was not the similar, as shown in **Table 3** and **Figure 12**, it was between 10 and 16 minutes. F10, F11, F12, F2, and F8 had the lowest OCT (10 min), and F4 the higher OCT (16 min), and finally F3, that presented a value equal to 14 min. Hence, rice flour in the formulation suggests that it contributes to the shorter cooking time, while teff and buckwheat flour cause a longer cooking time.

Water absorption is important to evaluate the cooking quality of pasta. Higher water absorption capacity results in a higher yield of final products (Mercier et al., 2020; Gallo et al., 2022). As shown in **Table 3** and **Figure 12**, F3 (100% teff) had the highest value of water absorption, which is equal to 68.19%, and the formula with the lowest value is F2 (100% rice) which has 30.32 % water absorption. Water absorption of pasta made with 100% buckwheat flour (F1) was lower but near to that with 100% teff (F3). These data suggest that teff and buckwheat flour can absorb more water than rice, indicating that adding teff and buckwheat flour may contribute to higher hydration and maybe softer or more malleable pasta dough, which could impact the final texture and cooking results.

Additionally, the data suggest that adding rice flour reduces the water absorption capacity. This could be because rice flour has less protein and fiber than teff and buckwheat (Hasmadi et al., 2020).

Cooking loss is related to solid leaching during cooking and is widely used to indicate overall cooking performance (Hager et al., 2012a; Larrosa et al., 2016).. Low amounts of residue in the cooking water indicate a higher quality of cooked pasta (Giannou et al., 2020). As shown in **Table 3**, the values of cooking loss in all pasta samples ranged from 8.00% (F3, 100% teff) to 17.90 % (F8, 66% rice, 17 % teff, 17 % buckwheat).

Regarding the studied texture parameters, the resistance of pasta to deformation under force is referred to as its hardness. It is a crucial quality factor that affects the al dente texture that buyers want. Properly hardened pasta does not get mushy since it maintains its form during heating and being chewed. Harder pasta also holds up better than overcooking, which adds to its acceptance (Baldassarre et al., 2021). Based on the flour composition, there is a noticeable variation in the hardness ratings for each of the twelve pasta formulations (**Table 3** and **Figure 12**). The pasta made with a 50% teff and 50% buckwheat blend (F4) had the greatest hardness at 2556.68 g, closely followed by F5 (50% teff, 50% rice), yielding 2475.45 g. However, F1 (100% buckwheat) has the lowest hardness of any samples, measuring 1349.65 g. This suggests that buckwheat alone produces a softer texture. Combinations with a higher teff content, such F7 (66% teff, 17% rice, 17% buckwheat), produce stiffer pasta (1779.07 g). Blends, including rice and buckwheat, like F6 (50% buckwheat, 50% rice), exhibit moderate hardness (1605.58 g). In summary, teff flour seems to be the most significant factor in pasta hardness, whereas buckwheat produces a softer texture. Teff-based recipes that use rice or buckwheat typically have much greater hardness values.

Table 3. Chemical and physicochemical composition of all formulations.

Formulation[a]	**g Teff (%)**	**g Rice (%)**	**g Buckwheat (%)**	**DPPH (%inhibition)**	**TPC**[b] **(mg GAE/100 g)**	**OCT (min)**[c]	**Water abs (%)**	**Cooking loss (%)**	**Hardness (gf)**[d]	**Adhesiveness (gs)**[e]	**Cohesiveness**	**Chewiness (gf)**[d]
F1	0	0	55(100)	85.06	91.09	13	59.05	13.71	1349.65	-75.91	0.60	613.59
F2	0	55(100)	0	6.26	12.98	10	5.71	11.97	1746.79	-161.16	0.59	880.79
F3	55(100)	0	0	31.26	47.60	14	68.19	8.00	1950.50	-88.48	0.56	879.12
F4	27.5(50)	0	27.5(50)	68.64	77.23	16	42.91	16.90	2556.68	-67.92	0.64	1122.81
F5	27.5(50)	27.5(50)	0	34.42	27.90	11	46.81	13.66	2475.45	-153.22	0.57	1246.23
F6	0	27.5(50)	27.5(50)	62.98	70.16	12	35.45	15.33	1605.58	-101.26	0.60	857.68
F7	36.3(66)	9.35(17)	9.35(17)	33.27	43.64	13	43.28	17.83	1779.07	-81.21	0.58	898.74
F8	9.35(17)	36.3(66)	9.35(17)	23.28	31.55	10	30.32	17.90	2002.66	-123.96	0.57	955.83
F9	9.35(17)	9.35(17)	36.3(66)	76.37	83.52	13	47.55	14.09	1961.99	-111.88	0.57	1022.40
F10	18.15(33)	18.15(33)	18.15(33)	36.40	43.54	10	42.92	16.08	2017.00	-76.57	0.57	1037.78
F11	18.15(33)	18.15(33)	18.15(33)	32.57	40.90	10	43.76	12.59	1740.33	-100.75	0.58	858.80
F12	18.15(33)	18.15(33)	18.15(33)	50.53	57.78	10	58.50	11.08	2168.46	-84.32	0.59	1112.04
Commercial pasta (control)				-	-	2	75.54	3.03	5191.27	-28.97	0.85	3621.84

[a] 55 g was considered 100% because it represented the total amount of flour added after discounting the amounts of the other ingredients. [b] TPC: total phenolic compounds. [c] OCT: optimal cooking time. [d] gf=gram-force, [e] gs=gram-second.

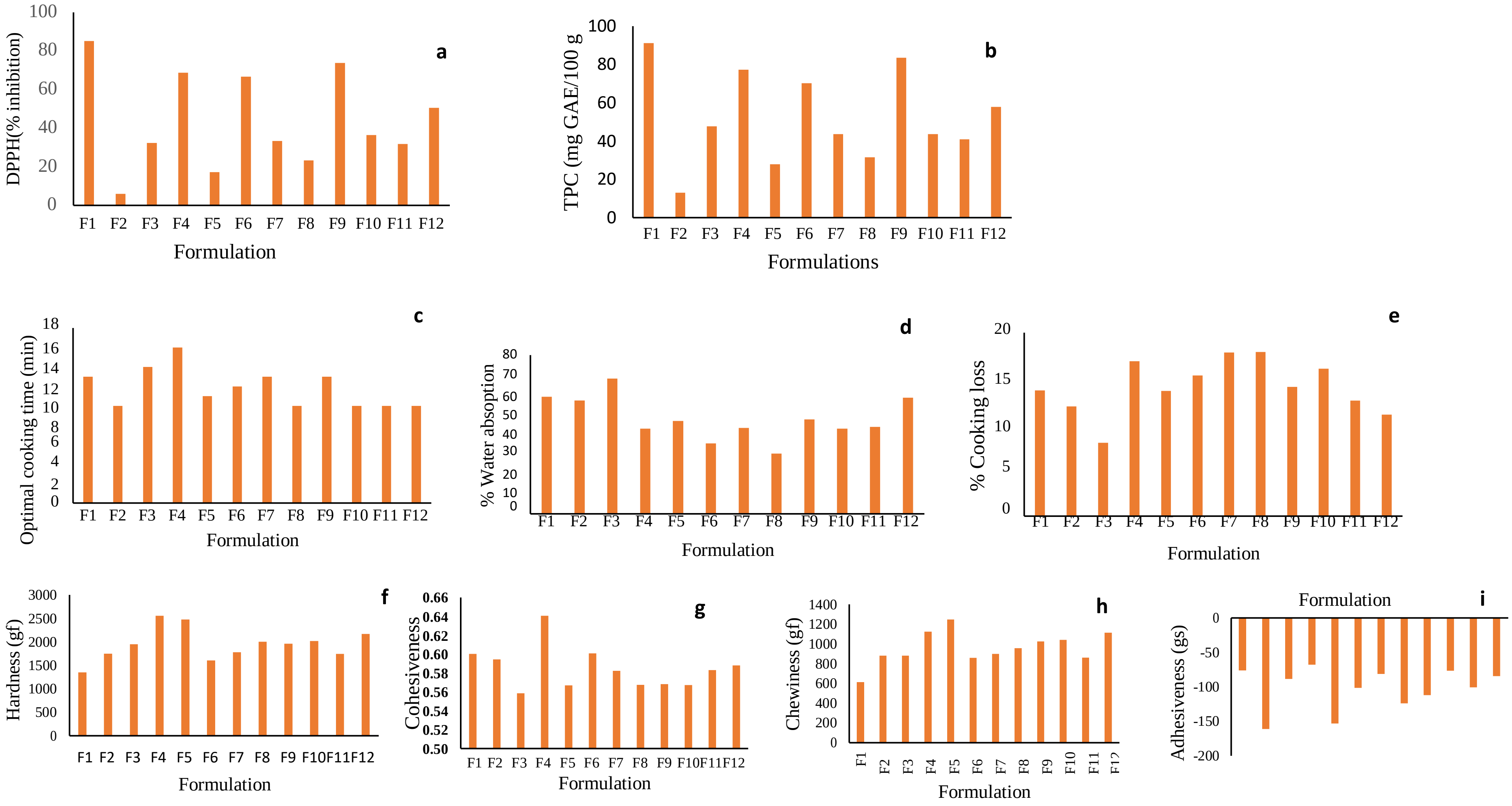
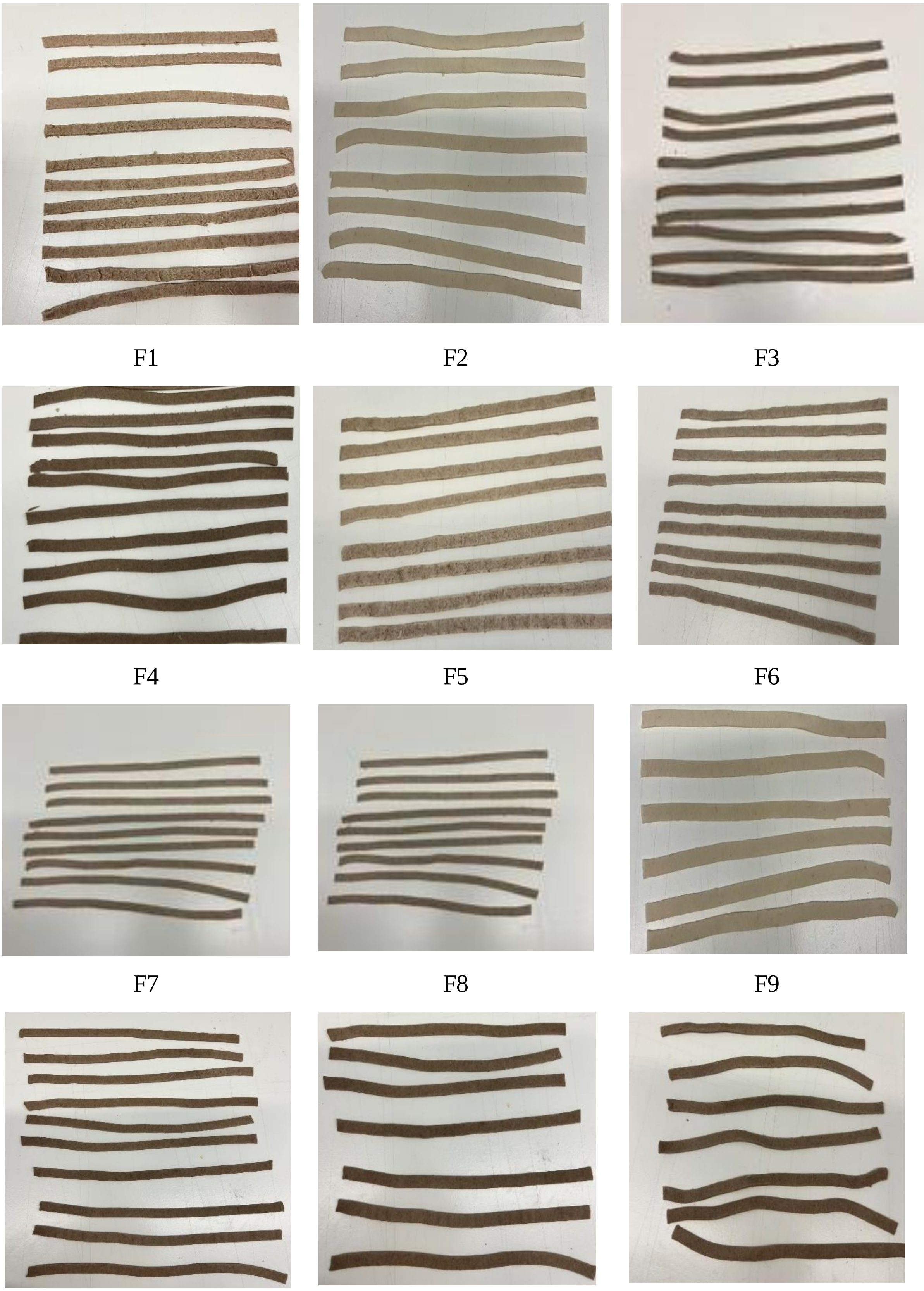


Figure 11. Summary of the results from the mixture design for TPC (a) and DPPH inhibition (%) (b), optimal cooking time (c), water absorption (d), cooking loss (e), hardness (f), cohesiveness (g), chewiness (h), adhesiveness (i).



F1 F2 F3

F4 F5 F6

F7 F8 F9

F10 F11 F12

Figure 12. Final appearances of pasta formulations. **Source:** the author.

The capacity of pasta dough to stay together while being processed and cooked is called cohesiveness. Since it indicates the pasta's capacity to remain together, a high cohesiveness value is frequently preferred because it improves the end product's firmness and texture (Hager et al., 2012a, Larrosa et al., 2016). Cohesive pasta can also hold tastes and sauces, giving customers a better sensory experience. The cohesiveness values observed in formulas F1 to F12 range between 0.56 and 0.64, showing variances in the internal bonding strength of the different pasta formulations. With equal amounts of buckwheat and teff (50% each), F4 has the most excellent cohesion value (0.64). This indicates that using these two gluten-free flours together produces pasta that cooks with good structural integrity. However, the cohesiveness values of F3 (100% teff) and F5 (50% teff, 50% rice) are lower, at 0.56 and 0.57, respectively. This suggests that the structures in these formulations may not hold as well, which could result in a softer texture. The lack of gluten in these formulations may impact their cohesion, as gluten typically aids in binding dough particles together.

The textural characteristic of pasta, known as chewiness, indicates how much force is required to chew the food. (Hager et al., 2012a, Larrosa et al., 2016). It influences the whole eating experience by combining the cohesion of the dough with the hardness of the pasta (Hager et al., 2012a, Larrosa et al., 2016). Chewing values (**Table 3** and **Figure 12**) fluctuated significantly across the various formulations. F5 (50% teff, 50% rice) has the greatest chewiness score (1246.23). Similar to F4, which has a high chewiness score of 1122.81 (50% teff, 50% buckwheat), this formulation gave denser pasta that needs more chewing effort. On the other hand, F1 (100% buckwheat) has the lowest chewiness value (613.59), suggesting a softer texture that could make the pasta less firm and more tender.

Pasta adhesiveness is correlated with the quantity of starch granules that coat the product's surface and exude from the pasta matrix into the cooking water. (Hager et al., 2012a, Larrosa et al., 2016). F2, consisting of 100% rice, exhibited the maximum negative adhesiveness value of -161.16 g/s (maximum stickiness), followed by mixtures with rice or rice predominating (F5, F6, and F8). Conversely, F4 showed the lowest negative adhesiveness value (-67.92 g/s), indicating a lower stickiness. According to these numbers, the more sticky formulas contain rice, while teff contributes to lessened stickiness.

4.2 Optimization of the fresh pasta formulation

The linear models were significant for TPC, DPPH, and water absorption, with no lack of fit observed at the 95% confidence level, according to an evaluation of the quality characteristics of the fitted mathematical models (**Table 4**). Both buckwheat and teff had a favorable impact on TPC and DPPH, according to the linear regression coefficients (**Table 4**), whereas rice flour had no discernible effect. With coefficients of 93.14 for TPC and 86.98 for DPPH, buckwheat had the greatest effect among the studied flours; teff's values were 46.53 and 35.43. This demonstrates that pasta made with teff or buckwheat has higher TPC and DPPH values than pasta made with rice flour.

Table 4. Regression coefficients and validation of the mathematical models of the mixture design.

Responses	**Indicated model**	**Regression Coefficients**			**Regression significance ($p<0.05$)**	**Lack of fit ($p>0.05$)**
		Teff	Rice	Buckwheat		
Total phenolic compounds (mg GAEg/100 g)	Linear	46.53 (6.53)	12.44 (6.53)	93.14 (6.53)	<0.001	0.6625
DPPH	Linear	35.43 (6.44)	5.11 (6.44)	86.98 (6.44)	<0.001	0.7242
Water absorption	Linear	68.19 (7.13)	5.97 (7.3)	59.05 (7.13)	0.021	0.7107

Regarding water absorption, the regression coefficients indicated that teff and buckwheat positively influenced this parameter, while rice had no significant impact (**Table 4**). The slightly higher coefficient for teff (68.19 compared to 59.05 for buckwheat) suggests a stronger effect on water absorption. Indeed, teff had the highest water absorption rate (68%) among the pure flours.

While buckwheat and teff each had a considerable effect on these responses, there were no significant interactions, either positive or negative, between them or in their binary or ternary combinations with rice.

The optimized formulation could not be predicted from the other technological parameters since no trustworthy models could be developed for them. Because pasta quality depends on these characteristics, the optimal formulation was chosen from the testing findings by striking a balance between acceptable technological properties and high TPC and DPPH values. The characteristics of a commercial semolina-based tagliatelle pasta that was made in accordance with the manufacturer's directions were taken into consideration as goal values. (Larrosa et al., 2016.) (**Table 3**).

In summary, minimum cooking loss and high water absorption are characteristics of high-quality pasta (Hager et al., 2012a; Larrosa et al., 2016). Additionally, pasta should retain its structure; integrity depends on its strong cohesion and adhesiveness. Furthermore, the quality of pasta is mostly determined by its hardness and chewiness, which are related to how strong its structure is.

All formulations were shown to have lower water absorption rates and decreased cohesiveness, chewiness, adhesiveness (more negative results), and hardness than the commercial semolina-based pasta. As opposed to conventional wheat pasta, they had greater cooking loss and OCT values.

Consequently, after aligning high TPC and DPPH values with the quality properties closest to those of commercial wheat pasta (**Table 3**), formulation F9 comprising 36.3 g of buckwheat (66%), 9.35 g of rice (17%), and 9.35 g of teff (19%) was selected as the best formulation. Formulation F4, which contains 27.5 g of buckwheat (50%) and 27.5 g of teff (50%), was also chosen for further study because it exhibited technological parameters such as hardness, chewiness, and adhesiveness that were similar to those of the control pasta and ranked highly in TPC and DPPH. These formulations are shown in **Figure 13**.

a. F4 **b.** F9

Figure 13. Final Formulations.

4.3 Characterization of final formulas with and without TW

Table 5 and Table 6 show the proximate composition, texture, color and mineral content of the formulated pasta.

As shown in **Table 5**, lightness (L*) for F9 is higher than F4. When F4 moves from DW (56.18±1.24) to TW (52.65±2.78), it does not darken significantly, but F9 is constantly lighter (63.17±1.07 to 60.70±0.52). For redness (a*), values in both formulas are similar, exhibiting a modest redness, and there is little difference between DW and TW for F4 (5.04±0.68) and F9 (5.25±0.34 to 5.58±0.19). Regarding yellowness (b*), the samples did not differ statistically. In conclusion, F9 seems lighter, and this is normal due to the importantexistence of teff and buckwheat in F4. However, the information showed that TW had no discernible effect on any of the assessed properties.

The range of water activity (aw) was 0.983±0.009 to 0.987±0.004 (**Table 5**), which is within the normal range of 0.92 to 0.96 for this kind of product (Kumari et al., 2020). This feature was not considerably affected by the addition of TW.

Table 5. Chemical, physicochemical and technological properties of the developed pastaformulations.

Physicochemical and technological parameters		**F4_DW**	**F4_TW**	**F9_DW**	**F9_TW**
Color parameters	L*^{a}	56.18±1.24^{b}	52.65±2.78^{b}	63.17±1.07^{a}	60.70±0.52^{a}
	a*	5.04±0.68	5.03±0.16	5.25±0.34	5.58±0.19
	b*	18.04±0.29	16.80±0.12	16.91±0.49	17.56±0.97
Water activity	a_w	0.985±0.003	0.987±0.004	0.983±0.009	0.984±0.004
Texture parameters	Hardness (gf)	3026.89±57.88	3036.98±168.66	1809.55±265.28	1967.82±139.94
	Cohesiveness	0.64±0.05	0.63±0.05	0.58±0.007	0.59±0.01
	Chewiness (gf)	1552.67±43.33	1566.04±90.64	1154.49±230.67	1170.72±134.40
	Adhesiveness (gs)	-90.31±8.42	-88.62±9.64	-92.24±14.86	-81.813±5.33
Cooking parameters	Water absorption (%)	62.07±4.39	68.08±8.93	59.86±5.93	64.86±1.22
	Cooking loss (%)	15.18±0.33	17.26±0.18	14.51±0.63	13.18±0.25
	Optimal cooking time (min)	16	16	13	13
Proximate composition (g/100 gdw)	Moisture	31.43±0.53^{b}	27.19±0.89^{a}	35.26±0.57^{c}	27.94±0.97^{a}
	Ashes	2.34±0.07^{a}	2.12±0.06^{a}	1.73±0.08^{b}	1.83±0.12^{b}
	Proteins	12.87±0.68^{a}	12.31±0.33^{a}	13.27±0.89^{b}	13.00±0.88^{b}
	Lipids	2.33±0.11^{a}	2.37±0.12^{a}	1.32±0.04^{b}	0.85±0.01^{c}
	Carbohydrates	54.96±0.71^{ab}	51.39±1.10^{bc}	48.68±1.51^{c}	56.09±1.92^{a}
	Energetic value (Kcal/100 g)	295.03±2.58	276.19±3.75	258.71±2.39	285.17±4.11

Results are expressed as mean±standard deviation. dw: dry weight. Different lowercase letters in a row indicate significant statistical difference between the samples at 95% confidence level. ^{a}Colour values for the control pasta: L* = 82.20 _ 0.63, a* = _5.20 _ 0.22, b* = 35.70 _ 0.73. F4_DW, and F4_TW = formulation 4 (27.5 g teff, 27.5 g buckwheat flour) + distilled water (DW) or thermal waters (TW).

F9_DW and F9_TW = formulation 9 (36.3 g buckwheat, 9.35 g teff, 9.35 g rice flour) + distilled water (DW) or thermal waters (TW).

Table 6. Mineral composition of the developed pasta formulations.

Mineral Compounds	**F4_DW**	**F4_TW**	**F9_DW**	**F9_TW**
K (g/100 g_{dw})	0.31±0.02	0.26±0.02	0.25±0.02	0.29±0.08
Na (g/100 g_{dw})	0.14±0.01	0.13±0.01	0.12±0.01	0.16±0.04
Ca (mg/100 g_{dw})	29.59±3.65^{a}	30.86±3.93^{a}	16.22±1.48^{b}	18.12±1.26^{b}
Mg (mg/100 g_{dw})	0.13±0.01	0.11±0.01	0.11±0.01	0.12±0.03
Fe (mg/100 g_{dw})	7.71±0.43^{a}	7.37±0.73^{ab}	5.75±0.45^{b}	4.91±1.05^{b}
Mn (mg/100 g_{dw})	12.20±0.48	12.66±0.27	5.33±0.09	5.04±0.38
Cu (mg/100 g_{dw})	0.79±0.02	0.80±0.03	0.81±0.02	0.89±0.22
Zn (mg/100 g_{dw})	9.14±0.35^{a}	8.33±1.00^{ab}	8.01±0.26^{ab}	6.76±0.95^{b}

Results are expressed as mean±standard deviation. dw: dry weight. Different lowercase letters in a row indicate significant statistical difference between the samples at 95% confidence level.

Regarding the proximate composition (**Table 5)**, the moisture content was within the predicted minimum range of 24% (Padalino et al., 2016), ranging from 27.19 ± 0.89 to 35.26 ± 0.57 g per 100 g. The results for ashes of F4_TW (2.12%) and F4 (2.34%) are very close, and the same for F9 (1.72%) and F9_TW (1.83%) but F9_DW and F9_TW had lower ash than F4_DW and F4_TW. These results indicate that the thermal water does not affect the ash content of the pasta.

Proteins are crucial components of food, given their significant biological roles encompassing structural, enzymatic, energetic, hormonal, and defense functions (Boye et al., 2010). The protein content in the pasta is shown in **Table 5**. The protein content exhibited by both formulations (F4 and F9) exhibits no changes when substituting distilled water (DW) for thermal water (TW), as the protein content for F4_DW ranges from 12.87% to 12.31% (F4_TW), while the protein content of F9_ DW goes from 13.27% to 13.00% (F9_TW).

The lipid content of F4_TW and F4_DW do not differ, measuring 2.33% and 2.37%, respectively. For F9, the lipid content drops from 1.32% (F9_DW) to 0.85% (F9_TW). According to this, it seems that thermal water may interact with lipid molecules differently depending on the formulation, which could impact lipid extraction or retention. Similarly, **Table 5** also shows that thermal water affects the carbohydrate content differently depending on the formulation. While in F4, the carbohydrate content did not change significantly between F4_DW (54.96%) and F4_TW (51.39%), F9 ranged from 48.68% (F9_DW) to 56.09% (F9_TW). These changes imply that thermal water may have varying effects on the extraction or retention of carbohydrates in different formulations, maybe as a result of variations in the interactions between the components of each formula and the composition of the water (Xu et al., 2021).

The results of the individual mineral content is given in **Table 6**. The use of thermal water does not affect the mineral components of the pasta. This shows that teff, buckwheat, and rice flours are rich in minerals. As shown in **Table 6**, we can observe that formula F4 has higher levels of Ca, Fe, Mn, and Zn compared to F9, with respective values (316.89 mg/Kg, 77.07 mg/Kg, 124.19 mg/Kg, 91.41 mg/Kg). We can say that the formula with the highest teff content is richer in mineral components, as is the case with F4 and this is normal due to teff flour contains a higher amount of calcium than rice (1.53 and 0.78 mg per 100 g) and buckwheat flours (2.51, 2.86, and 12.4 mg per 100 g), as well as substantial amounts of iron and zinc (315, 9.58, and 11.41 mg per 100 g, respectively), according to studies (Hager et al., 2012b; Zhu, 2018; Sofi et al., 2023).

The antioxidant activity of the pasta samples was also determined (**Table 7**) through DPPH and ORAC assays. Antioxidants are essential for reducing oxidative stress, which is connected to cellular damage and several chronic illnesses (Lobo et al., 2010). The ORAC and DPPH tests are popular techniques for determining a food's antioxidant content by evaluating how well it can scavenge free radicals (Shahidi & Ambigaipalan, 2015). DPPH inhibition values ranged from 82.19% (F4_DW) to 85.89 (F9_TW), with no difference across the samples. Similarly, ORAC varied between 36.35 µmol Trolox eq/g and 38.72 µmol Trolox eq/g, also did not differ one from another. This suggests that the antioxidant capacity ofthe samples is not significantly affected using thermal water or by the different flour composition.

Table 7. Antioxidant activity of final formulations.

Antioxidant activity	**F4_DW**	**F4_TW**	**F9_DW**	**F9_TW**
DPPH (%inhibition)	82.19±1.00	82.72±0.00	83.14±0.18	85.89±1.44
ORAC (µmol Eq Trolox/g)	36.35±1.27	37.71±1.51	38.721±0.76	38.60±3.52
TPC (mg GAE/g)	1.03±0.02	1.11±0.05	1.33±0.04	1.30±0.06

The antioxidant activity of food samples is commonly attributed to phenolic compounds. Moreover, these compounds are linked to anti-inflammatory, anti-cancer, and cardiovascular effects (Del Rio et al., 2013). The TPC test is essential for screening the phenolic content of foods (Singleton et al., 1999). **Table 4** indicates that F9 (1.33 ± 0.04 mg GAE/g and 1.30 ± 0.06 mg GAE/g) had slightly higher TPC´s content than F4 (1.11 ± 0.05 mg GAE/g and 1.03 ± 0.02 mg GAE/g), implying a greater phenolic content in these samples. Buckwheat and teff are rich in phenolic compounds such as rutin and luteolin, which may have contributed to the total phenolic content response assayed (Zhu, 2018).

4.4 Microbiological analysis

The results for microbiological analysis are shown in **Table 8** and compared with the limit values from C.e.I.R.S.A. (Centro interdipartimentale de Ricerca e documentazione sulla Sicurezza Alimentare, Italy).

Table 8. Microbial stability of the developed formulations during 7 days at 4°C.

Time (days)	Microorganism	Sample (log UFC/g)			
		F4_DW	**F4_TW**	**F9_DW**	**F9_TW**
0	**TAM**[a]	4.18±0.03^{A}	4.33±0.15^{A}	4.14±0.06^{A}	4.20±0.03^{A}
	Coliforms	3.70±0.12^{A}	3.90±0.08^{A}	3.74±0.17^{A}	3.56±0.09^{A}
	Yeasts	ND	ND	3.52±0.09^{A}	3.31±0.16^{A}
	Molds	ND	ND	ND	ND
	B. cereus	ND	ND	ND	ND
3	**TAM**	4.11±0.08^{bcA}	4.50±0.15^{aA}	3.92±0.02^{cA}	4.22±0.03^{bA}
	Coliforms	3.68±0.05^{bA}	4.28±0.15^{aB}	3.67±0.07^{bA}	4.28±0.09^{aB}
	Yeasts	3.78±0.05^{bA}	4.32±0.05^{aA}	3.89±0.04^{bA}	4.36±0.16^{aB}
	Molds	ND[C]	ND	ND	ND
	B. cereus	ND	ND	ND	ND
7	**TAM**	5.43±0.06^{aB}	5.35±0.04^{aB}	4.41±0.02^{bB}	5.44±0.02^{aB}
	Coliforms	5.39±0.10^{bB}	5.18±0.03^{aC}	5.44±0.01^{bB}	5.45±0.02^{bC}
	Yeasts	5.65±0.03^{cB}	6.14±0.00^{aB}	6.03±0.01^{bB}	6.15±0.01^{aC}
	Molds	ND	ND	ND	ND
	B. cereus	ND	ND	ND	ND

Reference values according to C.e.I.R.S.A (log UFC/g)

	Satisfactory	Acceptable	Unsatisfactory
TAM	< 5	$5 \leq \log < 6$	≥ 6
Coliforms	< 3	$3 \leq \log < 4$	≥ 4
Yeasts	≤ 4	$4 \leq \log < 5$	≥ 5
Molds	< 3	$3 \leq \log < 4$	≥ 4
B. cereus[b]	< 2	$2 \leq \log < 4$	≥ 4

[a]Total aerobic meophiles; [b]Foodborne desease risk: ≥ 5 log UFC/g. [C]ND= not detected. Results are expressed as mean±standard deviation. Different lowercase letter in a row indicate statistical significance at 95% confidence level between formulations. Different capital letters in a column mean statistical difference in time for one formulation at 95% confidence level. F4_DW and F4_TW = formulation 4 (27.5 g teff, 27.5 g buckwheat flour) + distilled water (DW) or thermal water (TW). F9_DW and F9_TW = formulation 9 (36.3 g buckwheat, 9.35 g teff, 9.35 g rice flour) + distilled water (DW) or thermal water (TW).

The produced formulations' microbiological quality, both with and without TW, was examined over a 7-day storage period at 4°C, which is the normal shelf-life of handmade fresh pasta products (Manzo et al., 2021). The initial microbial load of the formulations was unaffected by the addition of TW, suggesting that TW is a viable high-quality component. This observation is supported by the microbiological investigation of TW (**Table 9**).

Table 9. Microbiological analysis of thermal water utilized in the formulations.

Parameter[a]	**Legal limit**[b]	**Results**
Viable microrganisms (22°C) (UFC/mL)	20	Nd[b]
Viable microrganisms (36°C) (UFC/mL)	5	nd
Escherichia coli (UFC/250 mL)	nd	nd
Coliforms (UFC/250 mL)	nd	nd
Spores of anaerobic sulfite-reducing bacteria (UFC/50 mL)	nd	nd
Pseudomonas aeruginosa (UFC/250 mL)	nd	nd
Enterococos (UFC/250 mL)	nd	nd

[a]Methods: ISO 6222:1999, ISO 6222:1999, ISO 9308-1:2014/Amd1: 2016, ISO 9308-1:2014/Amd1: 2016, ISO 6461/2:1986, ISO 16266:2006, ISO 7899-2:2000. [b]Portugal, nº 1220 of 29 December 2000. [b]ND = not detected.

Table 8 shows that the total mesophilic aerobic values obtained are satisfactory until the 3rd day of storage and acceptable on the 7th day, according to C.e.I.R.S.A. Regarding coliforms, the values obtained are acceptable by C.e.I.R.S.A up to the 3rd day, but they are unsatisfactory by the 7th day of storage. About yeast, the results obtained are satisfactory on the first day, acceptable on day 3 but unsatisfactory by the 7th day, and molds were absent throughout all the days. Regarding *Bacillus cereus*, the values obtained are considered satisfactory from T0 until T7 because the analyses does not detect any microorganisms of *B. cereus* throughout the storage period.

We postulated that the samples might become contaminated by the raw ingredients, and using **Table 10**, we can see that there is contamination in the flour used in the pasta formulation so the results confirmed this hypothesis, consequently, the inadequate microbiological results discovered on day 7 were probably caused in part by the initial degree of contamination in the flours.

Table 10. Microbiological analysis (log UFC/g) of rice, teff and buckwheat flours.

Sample	TAM	Coliforms	*B. cereus*	Yeasts	Molds
Rice	3.34±0.10	2.41±0.16	ND[a]	0.00±0	ND
Teff	4.98±0.01	4.06±0.12	ND	4.36±0.08	ND
Buckwheat	3.20±0.01	2.31±0.10	ND	2.94±0.08	ND

[a]ND = not detected.

5 Conclusions

This study has advanced the creation of gluten-free pasta by investigating new formulations based on buckwheat, rice, and teff, along with the use of thermal water (TW).

Teff, which is known for its strong nutritional and antioxidant qualities, has been shown in studies to be a useful ingredient in gluten-free goods, enhancing their health benefits and consumer appeal. This work addresses the growing demand for wholesome gluten-free alternatives by developing pasta with enhanced antioxidant characteristics, which helps to diversify and grow the gluten-free market. Furthermore, it showed the feasibility of using thermal waters, an important Portuguese endogenous resource, to produce added-value food products.

For my future study, first, I want to perform consumer and sensory research to find out how people feel about gluten-free pasta made from rice, buckwheat, and teff. By offering insightful information for upcoming product development and marketing plans, this analysis will assist in comprehending the market potential of these items. Furthermore, I intend to look into the wider uses of thermal water by analysing the potential distinct effects that various TW types which differ in their mineral compositions—may have on gluten-free pasta. The nutritional profile of the finished product could be improved if this study reveals more noticeable advantages in terms of mineral content and health characteristics.

Going forward, this study will investigate consumer acceptance of gluten-free pasta formulations and the effects of various thermal water types on nutritional enhancement, with the ultimate goal of developing innovative products that appeal to customers who are health-conscious and support regional resources.

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