

Article

Development of Dried Pasta with Improved Nutritional and Biological Value Using Sustainable Duckweed Flour

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Abstract

The transition toward sustainable and nutrient-dense food systems requires novel plant-based ingredients and multifunctional formulations. In this study, gluten-free pasta was developed from millet (*Panicum miliaceum*) and psyllium husk (*Plantago ovata*), enriched with duckweed (*Lemna minor*) flour at 0–20% inclusion levels, with and without egg addition. The study aimed to evaluate the combined effects of duckweed incorporation and processing (drying, cooking, and in vitro digestion) on nutritional quality, bioactive compound stability, and volatile aroma composition. Duckweed enrichment (5–10–12–50% based on millet flour) contributed to strengthening the protein content, total polyphenol levels, and antioxidant capacity across all formulations. Processing steps markedly influenced compound stability and bioaccessibility: thermal treatments reduced extractable antioxidant activity, whereas simulated gastrointestinal digestion enhanced the bioaccessibility of bioactive compounds. Technological properties were moderately affected, with increasing duckweed levels resulting in longer cooking times and higher water absorption capacity. GC–MS–olfactometry analysis revealed that duckweed flour is characterized by a complex volatile profile dominated by hydrocarbons, aldehydes, alcohols, and sulphur-containing compounds, contributing primarily green, fatty, and herbaceous odor notes. During pasta processing, a substantial shift in aroma composition was observed, with ketones and alcohols becoming dominant, while key duckweed-derived odor-active compounds (e.g., carotenoid degradation products and green leaf volatiles) were partially retained in the final products. Olfactometric evaluation confirmed that although processing reduces aroma intensity, duckweed-related volatile markers remain detectable at higher incorporation levels. Overall, millet–psyllium–duckweed pasta represents a promising gluten-free functional food system combining improved nutritional value with plant-based protein enrichment and antioxidant potential. However, aroma modification during processing remains a critical factor for sensory optimization and consumer acceptance. The results highlight the importance of integrating nutritional, technological, and GC–MS–olfactometry approaches in the development of next-generation sustainable cereal-based foods.



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1. Introduction

The development of sustainable and nutritionally adequate food systems has become a critical global priority, led by environmental challenges and the increasing prevalence of chronic non-communicable diseases (NCDs). International studies consistently show that diets with lower environmental impact largely overlap with healthy dietary patterns [1,2]. In particular, reducing the consumption of animal-based products—especially red meat—can significantly decrease environmental burdens while also improving health outcomes [3].

In Europe, current dietary patterns remain heavily dependent on animal-derived proteins, with approximately 59% of total protein intake from meat, fish, and dairy products, while plant-based proteins account for approximately 41%. Furthermore, plant protein consumption is often dominated by a limited number of staple crops, contributing to dietary imbalance and increased risk of NCDs such as cardiovascular diseases, cancer, and type 2 diabetes [4]. These trends highlight the urgent need to diversify protein sources and promote sustainable dietary alternatives.

One of the most widely consumed and accessible plant-based foods globally is pasta or noodle, making it an ideal candidate for nutritional improvement and protein diversification.

Pasta and noodles differ primarily in their cultural origin and technological processing. Pasta is traditionally associated with Italian cuisine and is typically produced through extrusion-based processes, most commonly using durum wheat semolina, resulting in a firm structure and a characteristic “al dente” texture after cooking. In contrast, noodles represent a broader and more diverse category of starch-based products widely used in Asian cuisines, which may be produced through various technological methods such as dough lamination, stretching, steaming, frying, or extrusion. These differences in processing result in a wider variability in noodle textures, hydration levels, and consumption formats, including soups, stir-fried dishes, and fresh preparations. While pasta is generally designed to maintain structural integrity during boiling and provide a firm bite, noodles are often characterized by higher elasticity or softness depending on the specific product type and culinary application. Noodles are typically produced from a wide range of raw materials, including cereals (e.g., wheat, rice, maize, millet), pseudocereals (e.g., quinoa, amaranth, buckwheat), legumes, and various starch sources such as mung bean, sweet potato, and tapioca, reflecting their broad and heterogeneous product category, allowing for the development of gluten-free alternatives [5,6].

The nutritional composition of pasta and pasta-like products (noodles) varies significantly depending on the raw material used. Since pasta and noodles are primarily carbohydrate-rich staple foods (cereals/pseudocereals 65–75%, legumes 40–60%) with moderate protein content (cereals/pseudocereals 5–18%, legumes 18–30%) and limited essential amino acid profiles (limited amino acid in cereals: lysine; in pseudocereals: methionine and cysteine, but higher lysine content than in cereals; in legumes: methionine and cysteine), furthermore they often lack other essential nutrients, such as dietary fiber, vitamins, and bioactive compounds [7]. So, their nutritional quality can be improved through protein fortification strategies (e.g., with duckweed). The incorporation of protein-rich plant-based ingredients represents a promising approach to enhance both the overall protein content and the biological value of these products, thereby contributing to the development of more balanced and functional staple foods.

Millet (*Panicum miliaceum*), for example, is a gluten-free cereal with moderate protein content (~10–12%) and with a favorable nutritional profile, including complex carbohydrates, minerals, and bioactive compounds, as well as a relatively low glycemic response [8,9]. However, as with most cereals, its protein quality is limited by low levels of certain essential amino acids, particularly lysine, which restricts their overall protein quality.

To address this limitation, protein-rich and sustainable ingredients such as duckweed (Lemnaceae family) flour offers a valuable strategy for nutritional enhancement [10]. Duckweed is an emerging alternative protein source with high protein content (up to 16–43% in dry matter), and a well-balanced amino acid profile compared to cereal proteins, including higher lysine levels, and rapid growth under a wide range of environmental conditions [11]. Unlike conventional crops, duckweed cultivation does not require arable land and can be carried out in aquatic systems, thereby significantly reducing land use and environmental impact. Besides its protein, other primary metabolites (as carbohydrate 17.6–45%, lipids 2.9–16.81%, and secondary metabolites (such as phenolic components), along with the vitamin (C, E, B12) and mineral (17–35%, Na, K, P) contents, play a distinct role in the plant's overall nutritional profile and its potential benefits for human health. Biological value is influenced by a number of factors, such as vitamin and mineral content, limiting amino acids, and amino acid composition [12], and it is worth to optimize the quantity of compounds, which can be directed by environmental influence/cultivation way [13]. Beside nutritional value, duckweed-based nutrients have bioactive properties such as antibacterial/antifungal, anticancer, antioxidant, and immunomodulatory activities. Its cell wall contains cellulose and hemicellulose, which contribute to its dietary fiber fraction and can modulate gut microbial activity. Processing (e.g., drying, milling, enzymatic treatment) improves nutrient bioaccessibility [11,13].

The use of duckweed in human nutrition within the European Union is regulated under the Novel Food framework [14,15]. Authorizations are granted for specific products, defined not only by plant species but also by production method and applicant (only for 5-year data protection/exclusive distribution period). Consequently, the use of duckweed is subject to clearly defined conditions, including maximum permitted levels in different food categories (specified safety limits related to heavy metals, trace elements, cyanotoxins, microbiological and pesticide contamination). Furthermore, only products placed on the market by the authorized applicant, or produced according to the approved specifications, may be used in food applications. These regulatory requirements must be carefully considered when developing new food products containing duckweed (such as beverage, soups, bakery products as pasta or noodles, snacks, salads, omelets, vegetable curries, flavored milk products, duckweed extract as sweetener enhancer, duckweed protein isolate as egg replacement in bakery products or methylcellulose replacement, and meat replacements) [10,13,16]. Fresh plants of *Wolffia arrhiza* and/or *Wolffia globosa* (manufactured by GreenOnyx Ltd., City of Tel Aviv, Izrael) have been authorized in the EU since 2021 by ref [17]. On 9 April 2024, the EU Commission authorized the marketing and use of protein concentrates derived from *Lemna gibba* and *Lemna minor* (manufactured by ABC Kroos BV, City of Raalte, The Netherlands) for human consumption [18].

The incorporation level of duckweed in pasta formulations is not defined by a single fixed value, but rather depends on technological, sensory, and regulatory considerations: (i) low inclusion (2–5%): primarily used to introduce functional components with minimal impact on taste, color, and texture; (ii) moderate inclusion (5–10%): the most commonly applied range, providing a meaningful increase in protein and bioactive compounds while maintaining acceptable technological and sensory properties; (iii) high inclusion (10–20%): allows for substantial nutritional enhancement, particularly in protein content, but may

negatively affect texture, cooking quality, and sensory acceptance due to intensified color and characteristic flavor. Higher inclusion levels are generally limited by factors such as changes in dough structure, increased water absorption, potential cooking losses, and the development of off-flavors. Therefore, moderate incorporation levels are typically preferred to balance nutritional benefits with product quality. Although limited data are available specifically for duckweed-enriched pasta, studies on plant-based fortification systems and emerging research on *Wolffia globosa* suggest that from low to moderate inclusion levels are generally preferred to balance nutritional improvement with technological and sensory acceptability [19]. Seephuea and co-workers [10] used 1%, 3%, 5% duckweed flour supplementation (*Wolffia globosa*) in the rice-based flour noodle, where tapioca starch was also used.

Psyllium husk is primarily composed of highly soluble, viscous, gel-forming dietary fiber, with minimal protein content. Its main role in food systems is functional rather than nutritional, as it contributes to water binding and structure formation. From a physiological perspective, recent studies have shown that psyllium intake is associated with improved bowel regularity, reduces LDL cholesterol levels, and moderated attenuation of postprandial glucose response [20,21].

In gluten-free systems, psyllium acts as a hydrocolloid, improving dough elasticity, water retention, and structural integrity. Additionally, its partial fermentation in the gut supports short-chain fatty acid (SCFA) production, contributing to microbiota health [22].

Therefore, in the present study, gluten-free pasta was developed using millet as the primary carbohydrate base, supplemented with psyllium husk for structural functionality and duckweed flour for nutritional enhancement. We hypothesized that millet flour would provide the basic starch matrix of the pasta, psyllium husk would improve water binding, dough cohesiveness, and structural stability in the absence of gluten, while duckweed flour would enhance the protein content and bioactive compound profile of the product. This formulation aims to combine sustainability, improved protein quality, and functional health benefits within a widely accepted food matrix.

The cooking properties of the pasta were evaluated, followed by analysis of samples collected during the pasta production stages and of the *in vitro* digested cooked pasta. In these samples, antioxidant capacity, total polyphenol content, and protein content were determined in order to assess the impact of processing and digestion on the stability and bioaccessibility of these compounds.

Importantly, the main analytical focus of the study was the characterization of volatile compounds using GC–MS–O analysis, which provided detailed insight into aroma formation and the impact of processing on sensory quality of duckweed-enriched pasta.

While the nutritional composition [13,23–25] and its utilization as an alternative food or feed source [13,26–30] are more intensely researched topics, the aroma profiling of duckweed receives much less attention [31,32]. Accordingly, one of the objectives of this study is to map the volatile composition of duckweed and certain food products made using it. Since duckweed can be processed in various ways and can be the raw material of several foodstuffs in the future, it is essential to characterize its organoleptic properties in detail. Namely, volatile organic compounds (VOCs) with undesirable odors diminish the organoleptic properties of duckweed-containing foodstuffs and can limit the consumer acceptance of these products. Consequently, an optimal supplementation level of duckweed was anticipated to improve nutritional quality while maintaining acceptable technological and sensory characteristics. The knowledge of the volatile fraction may contribute to the development of novel foods and can reveal the main sources of potential off-odors in the products as well.

2. Materials and Methods

2.1. Plant Materials

Millet flour (*Panicum miliaceum* L.): is a whole-grain product manufactured by Dénes-Natura Ltd. (Pécs, Hungary); label text: energy 1532 kJ (3652 kCal), fat 3.2%, carbohydrate 58.5% (including sugars 0.03%), protein 11.2 g, salt 0.03 g.

Psyllium husk flour: was produced by Caleido IT-Outsource, and purchased from BioMenü Ltd. (Budapest, Hungary); label text: energy 931 kJ (222 kcal), fat 1.3%, carbohydrates 16.4% (including sugars 0.4%), fiber 67.8 g, protein 3.6 g, salt 0.3 g.

Duckweed flour: *Lemna minor* was chosen for the experiments due to its high availability and ease of large-scale cultivation, making it a suitable and sustainable raw material for food application studies. Duckweed (*Lemna minor*) used in the experiment given from the University of Debrecen, Faculty of Agricultural and Food Sciences, and Environmental Management, Fish Biology Laboratory, where it was cultivated in a plastic greenhouse. Test environment: The duckweed (*Lemna minor*) was reared in a 10 m × 4 m × 0.5 m tank, where the ammonia concentration was 0.53 ± 0.02 mg/L, nitrite 0.021 ± 0.003 mg/L, and nitrate 0.1 ± 0.03 mg/L. The unit was illuminated with natural light (min. 7204–max. 33,462 Lux). The nutrient content of the water was maintained with NPK fertilizer, with 50 g added every two weeks. Furthermore bream larvae were reared in the water, which provided additional organic enrichment. After harvesting, the duckweed was pressed using a manual grape press until all the water had drained out (approx. 10 min), then dried in an air-circulating drying cabinet at 37–40 °C for 2 days. Composition data for the duckweed (dry matter 93.69 m/m%, moisture 6.31 m/m%, protein 33.96%, fiber 16.8 m/m%, fat 1.59%, ash 18.05% m/m, nitrogen-free extract (essentially carbohydrates) 23.29% m/m) were provided by the University of Debrecen. The dried duckweed was grounded to a particle size of 200 µm (Reisch Grindomix GM2000, REISCH Maschinenbau GmbH, Frastanz, Austria, 4-speed; 1000 rpm) and then stored at room temperature.

2.2. Pasta Samples

The composition and labeling of the pasta samples are shown in Tables 1 and 2.

Table 1. Composition of the pasta samples.

	Millet Flour (g)	Psyllium Husk Flour (g)	Duckweed Flour (g)	Duckweed Conc. (% w/w, Based on Millet Flour)	Weight of 4 Eggs (g)	Tap Water (mL)
Pasta With Egg (+E)	10	5	0	0 (control)	1.8	16.7
	9.5	5	0.5	5	1.8	16.7
	9	5	1	10	1.8	16.7
	8.5	5	1.5	15	1.8	16.7
	8	5	2	20	1.8	16.7
Pasta Without Egg	10	5	0	0 (control)	0	18
	9.5	5	0.5	5	0	18
	9	5	1	10	0	18
	8.5	5	1.5	15	0	18
	8	5	2	20	0	18

Table 2. Code of pasta samples in different processing stages, with duckweed flour incorporation at levels of 0, 5, 10, 15, and 20% by replacing millet flour on a weight basis.

	Raw Pasta Dough (R)	Dried Pasta (D)	Cooked Pasta (C)	Cooking Water of Cooked Pasta (CW)	Intestinal Phase of In Vitro Digested Cooked Pasta (IVD)
Pasta With Egg (+E)	R 0% + E	D 0% + E	C 0% + E	CW 0% + E	IVD 0% + E
	R 5% + E	D 5% + E	C 5% + E	CW 5% + E	IVD 5% + E
	R 10% + E	D 10% + E	C 10% + E	CW 10% + E	IVD 10% + E
	R 15% + E	D 15% + E	C 15% + E	CW 15% + E	IVD 15% + E
	R 20% + E	D 20% + E	C 20% + E	CW 20% + E	IVD 20% + E
Pasta Without Egg	R 0%	D 0%	C 0%	CW 0%	IVD 0%
	R 5%	D 5%	C 5%	CW 5%	IVD 5%
	R 10%	D 10%	C 10%	CW 10%	IVD 10%
	R 15%	D 15%	C 15%	CW 15%	IVD 15%
	R 20%	D 20%	C 20%	CW 20%	IVD 20%

Dried pasta: In this study, pasta was produced using an extrusion-based process inspired by traditional Italian pasta-making technology; however, instead of durum wheat semolina, gluten-free raw materials and functional plant-based ingredients were used to develop a nutritionally enhanced pasta system. Dried pasta was made either with eggs or without eggs (tap water was used instead), and enriched with duckweed in various concentrations (0%, 5%, 10%, 15%, and 20%) replacing an equivalent proportion of millet flour. The dough ingredients were mixed homogeneously, then tap water was added, and the dough was allowed to rest for 15 min. The dough was rolled with a rolling pin into thin sheets and cut into long, narrow strips, then dried under batch-drying conditions in a laboratory-scale convection oven at 80 °C for 60 min. Dried pasta was made three times and used for further processing.

Note: Drying at 80 °C for 60 min may appear relatively intensive. However, modern industrial pasta production commonly employs high-temperature (HT) and very-high-temperature (VHT) drying technologies. Industrial HT drying typically operates at approximately 60–80 °C, whereas VHT drying uses temperatures above 80–100 °C, depending on the drying system and pasta type. These drying technologies have largely replaced the traditional low-temperature (<60 °C), long-time drying processes because they improve process efficiency, microbiological stability, and cooking quality. Therefore, although our laboratory-scale batch drying does not reproduce the continuous industrial process, the applied drying temperature of 80 °C falls within the temperature range commonly used in contemporary industrial pasta production and was considered appropriate for preparing dried pasta samples [33].

Cooked pasta: Dried pasta was cooked in 350 mL tap water until it was soft.

Intestinal phase of in vitro digested pastas: cooked pasta was submitted under in vitro digestion according to Section 2.3, and samples from the intestinal phase of the digestion were used for further measurements.

2.3. Static Human In Vitro Digestion Simulation

Cooked pasta was used for the in vitro digestion experiments because it corresponds to the form in which the product is normally consumed, following household cooking, and therefore provides the most physiologically relevant assessment of nutrient bioaccessibility.

Cooked pasta (1 g) was submitted under human static in vitro digestion simulation according to the protocol of [34,35]. Simulated oral (pH 7.0), gastric (pH 3.0), and intestinal (pH 7.0) digestion fluids were used via the digestion; all contained an electrolyte solution with an appropriate concentration, digestive enzymes with appropriate activities, CaCl_2 with the appropriate concentration, and distilled water, according to as characteristic to the sections of the digestive tract. Fluids were adjusted according to the assumable physiological circumstances: human salivary alpha-amylase 75 U/mL in the final oral bolus, pepsin 2000 U/mL in the final gastric bolus, pancreatin 100 TAME U/mL—based on trypsin, and bile extract (10 mM) both in the final intestinal bolus. Oral, gastric and intestinal phases were done at 37 °C for 2 min, 2–2 h, respectively. After digestion process, the samples were put in the freezer and later used for all mentioned measurements.

2.4. Moisture Content Measurement

The moisture content of dried pasta was measured using a Sartorius MA 50 rapid moisture analyzer (Lab Instruments GmbH & Co. KG., Götting, Germany). The device calculates moisture content based on weight loss by evaporating the water content from the samples at 105 °C until a constant weight is reached. According to [36], the moisture content of duckweed flour and dried pasta may not exceed 13%.

2.5. Color Measurement

Color measurement is particularly important for foods where quality is a concern and where color significantly influences consumer perception and preference. Minolta CR-310 colorimeter (Konica Minolta, Inc., Tokyo, Japan) (CIELAB color coordinates) was used to measure the color of duckweed, millet, plantain seed husk flours, and raw, dried, and cooked pasta. In case of pasta the goal was to determine how the color changes relative to the control (0%) as the duckweed concentration increases.

The total color difference (ΔE^*) can be calculated as:

$$\Delta E = \sqrt{[(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]}, \quad (1)$$

L^* is the lightness factor; a measure that indicates the lightness or brightness of food colors; a^* value, ranges from green to red, where a negative value corresponds to green and a positive value to red; b^* value, ranges from blue to yellow, where a negative value corresponds to blue and a positive value to yellow.

Calibration was performed using a standard ceramic reference. The device determines the L^* , a^* , and b^* values of the samples after placing them on a white sheet. It was performed in 3–3 parallel measurements per sample.

2.6. Water Activity Measurement

Water activity plays a role in shelf life. It differs from moisture content in that water activity does not indicate the total moisture present in the sample, but only the amount of water that is available. Foods contain varying amounts of bound water, which can only be extracted using certain physical or chemical methods; therefore, microorganisms cannot utilize it. For microorganisms, free water is what they can use for their metabolic processes and reproduction; this is what examines when measuring water activity (a_w). a_w is a number between 0 and 1.00, which means the lower this number, the less water is available for microorganisms in the product, thereby extending its shelf life.

For the measurement, pasta was grounded into flour, then spread in the Novasina MS1 water activity meter's container.

2.7. Preparation of Extract

To 0.1500 g of sample (duckweed flour, raw-, dried-, cooked pasta), two spatulas of quartz sand were added and then the samples were ground for 2 min. A total of 1500 μ L of a methanol and distilled water solution (2:1 *v/v*) was pipetted into each sample and the grinding process was repeated for 5 min, and then the solution was centrifuged (6000 rpm, 15 min, 4 °C). Supernatants were stored at −20 °C until further analyses (see Sections 2.8–2.10). During the extraction process, three parallel measurements were performed.

2.8. Antioxidant Capacity Measurement

Supernatant samples prepared according to Section 2.7 were used for the measurement.

The antioxidant capacity of duckweed, millet flour, and raw, dried, and cooked pasta, as well as the cooking water was determined through the FRAP (Ferric Reducing Antioxidant Power) method, which is based on iron-reducing capacity [37].

The FRAP reagent consists of components (prepared freshly) including: 50 mL sodium acetate buffer (0.3 M; pH 3.6), 20 mL $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (20 mM), and 20 mL 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ, $\text{C}_{18}\text{H}_{12}\text{N}_6$) (20 mM) in 40 mM HCl.

A total of 1500 μ L of FRAP reagent was pipetted to 50 μ L of supernatant. After 5 min of incubation at 25 °C, they were measured at $\lambda = 595$ nm.

The antioxidant capacity was evaluated based on the calibration curve (prepared with 1 mM ascorbic acid stock solution) in ascorbic acid equivalents (AAEs, ascorbic acid equivalents/mg/g dry weight).

2.9. Total Polyphenol Measurement

Supernatant samples prepared according to Section 2.7 were used for the measurement.

The total polyphenol content of supernatants from duckweed, millet flour, and raw, dried, and cooked pasta, as well as the cooking water was determined according to the protocol from [38].

To 1250 μ L of Folin–Ciocalteu solution (10 mL of Folin–Ciocalteu reagent and 100 mL of distilled water), 200 μ L of diluent (80 mL of methanol and 20 mL of distilled water) and 50 μ L of sample were added. After 1 min of incubation at 25 °C 1000 μ L Na_2CO_3 solution (0.7 M) was added to the mixture, followed by further 5 min of incubation in a 50 °C water bath. After incubation, the reaction mix was cooled to room temperature, then its absorbance was measured at $\lambda = 760$ nm.

The total polyphenol content was evaluated based on the calibration curve (prepared with 0.3 mM gallic acid stock solution) in gallic acid equivalents (GAE, gallic acid equivalents/mg/g dry weight).

2.10. Protein Content Measurement

The protein content of the cooking water was directly determined through the Biuret method according to the protocol from [39].

The Biuret reagent consists of components including: 3 g $\text{CuSO}_4 \times 5 \text{H}_2\text{O}$ and 9 g $\text{KNaC}_4\text{H}_4\text{O}_6 \times \text{H}_2\text{O}$ dissolved in 1000 mL NaOH-buffer (0.2 M, pH 12).

To 300 μ L of supernatant 1500 μ L of Biuret reagent and 700 μ L of distilled water were pipetted, then the solution was incubated at 37 °C for 30 min and then cooled to room temperature. The absorbance of the samples was measured at $\lambda = 580$ nm.

The protein content was evaluated based on the calibration curve (prepared with 5 mg/mL BSA stock solution) in mg/g of dry matter.

2.11. Determination of Cooking Properties According to [40]

2.11.1. Cooking Time Measurement

A total of 25 g of unbroken dried pasta was cooked in 250 mL of tap water and stirred regularly with a glass rod to prevent it from sticking together. The cooking time was measured from the moment the water returned to a boil, and the cooking process was regarded to be completed when no white, floury streaks were visible inside the pasta. The cooking time was determined in min.

2.11.2. Water Uptake Capacity Measurement

Pasta was cooked according to Section 2.11.1. When the pasta reached the desired cooked state, it was rinsed with lukewarm water, then let the water drain. The weight of the cooked pasta was measured and the water uptake capacity was expressed using the following formula:

$$(w/w\%) = ((a - b)/a) \times 100, \quad (2)$$

where a = weight of cooked pasta (g), and b = weight of dried pasta (g). If the pasta's water absorption was adequate, the value was 100% or higher.

2.11.3. Cooking Loss Measurement

Pasta pieces that had stuck together and those that were overcooked (and caused stickiness) were selected, and their weight was calculated relative to the total weight of the cooked pasta and expressed as a percentage.

2.12. Gas Chromatography–Mass Spectrometry–Olfactometry (GC-MS-O) Analysis

Samples and chemicals: The mixture of n-alkanes (C10–C40) (Sigma-Aldrich, Steinheim, Germany) was applied as an external reference for the calculation of retention indices (RI) of volatiles.

Sampling: For the volatile analysis, 0.5 g of dried duckweed flour and cooked, chopped pasta were placed into a 4 mL sampling vial equipped with a screw-cap syringe valve. The samples were conditioned at 40 °C for 20 min, followed by a 20 min HS-SPME extraction with a 2 cm DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA, USA).

Instrumental analysis: The volatile analysis of the samples was performed using a GC-MS instrument (Shimadzu GCMS-QP2010SE, Shimadzu Corporation, Kyoto, Japan) coupled with an olfactometer (Phaser Pro Olfactory GC Port, GL Sciences B.V., Eindhoven, The Netherlands). After headspace extraction, the analytes were desorbed in the injector port for 5 min at 250 °C in splitless mode with a 6:1 split ratio. The volatiles were separated on a MEGA-WAX fused silica capillary column (60 m × 0.25 mm × 0.25 µm) (Mega S.r.l., Legnano, Italy). The initial oven temperature was 40 °C and it was increased to 230 °C at a rate of 5 °C·min^{−1}. The detector was run in electron impact mode (70 eV) at 250 °C. Scanning was performed in the 35–500 mass range at 1666 mass/s scan speed. Helium gas (6.0) was used as the carrier gas at a flow rate of 1.20 mL·min^{−1}. The chromatograms and mass spectra were analyzed using the LabSolutions software, version 4.52 (Shimadzu Corporation, Kyoto, Japan). Volatile compounds were tentatively identified through the comparison of each mass spectrum with spectra in reference libraries NIST05 and NIST05s and by comparing calculated retention indices. Internal normalization as a standard method in flavor analysis [41] was applied for the interpretation of the data. The results of the measurements were expressed as peak area% regarding the sum of peak areas as 100%. All analyses were performed in triplicate and the average of their results was reported.

The olfactometer was applied for the identification of odoriferous regions in the chromatograms. The previously described gas chromatographic parameters were applied for

the olfactometric examination as well. The volatile compounds eluted from the column were split in a 3:1 ratio between the olfactory port and the mass spectrometer. Humidified air ($5 \text{ mL} \cdot \text{min}^{-1}$) was applied during the olfactometric analysis to avoid the dehydration of the nasal mucosa. During the measurement, the odor character and the intensity of the volatiles (weak, middle, strong) were recorded. The data were processed using Olfactory Voicegram software Ver.2.30 (GL Sciences B.V., Eindhoven, The Netherlands). All measurements were repeated in triplicate.

Statistical analysis: All the measurements were carried out in triplicate and the mean values, standard deviations (SDs) and relative standard deviations (%RSDs) were calculated. For the quantification of the volatile compounds, internal normalization was applied and the results were expressed as peak area% of the analytes [36]. In data tables, the mean values of the parallel measurements were reported.

3. Results

3.1. Laboratory Experiment to Develop Dried Pasta with 0–5–10–15–20% of Duckweed Flour (*Lemna minor*) Supplementation on the Base of Millet Flour and Its Basic Characteristics

The development of pasta based on millet (*Panicum miliaceum*), psyllium husk (*Plantago ovata*), and duckweed (e.g., *Lemna* spp.) represents a multifunctional food design strategy integrating nutritional enhancement. Such formulations may be relevant for gluten-free and plant-based dietary applications. Dried pasta prototypes were made from millet flour and psyllium husk flour, both with and without eggs, which was enriched with 5%, 10%, 15%, and 20% duckweed flour by replacing millet flour on a weight basis (Figure 1).

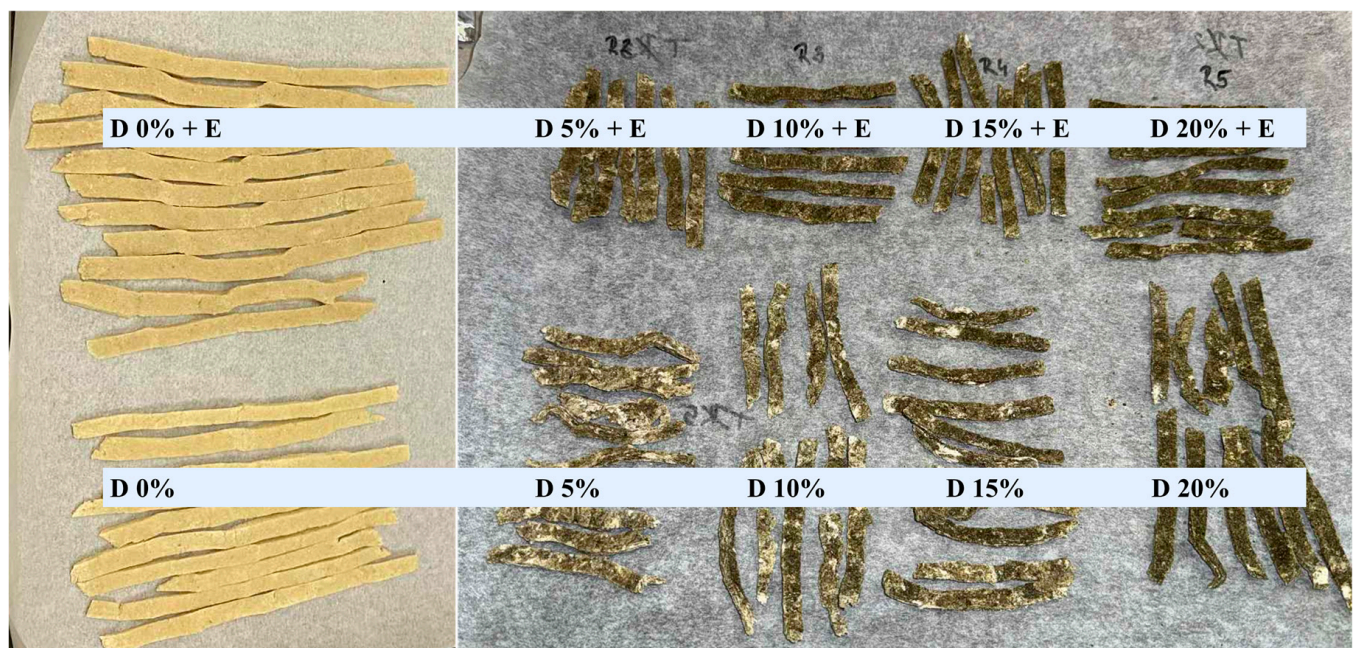


Figure 1. Developed dried pasta (D—dried; +E = made with egg; 0–5–10–15–20%—duckweed supplementation in different concentration by replacing millet flour on a weight basis).

It is generally observed that the moisture content of egg-containing pasta is slightly higher (D0% + E: 10.220%; D5% + E: 9.900%; D10% + E: 9.350%; D15% + E: 4.450%; D20% + E: 5.770%) than that of egg-free pasta (D0%: 12.560%; D5%: 6.380%; D10% 4.910%; D15% 6.220%; D20% 7.030%), which can be explained by the increase in moisture content resulting from the addition of eggs. The addition of duckweed flour to the dough at various concentrations did not show a strong correlation with changes in moisture content.

The moisture content of dried pasta must be a maximum of 13%, which complies with the regulations.

By calculating ΔE^* , it was determined how the color changes relative to the control dried pasta (0%) as the duckweed concentration increases. A slight deviation from the trend is clearly visible; that is, the ΔE^* value does not change proportionally with an increase in the duckweed content, which can be attributed to the drying method. (ΔE^* of pasta made with eggs: D5% + E:9.91; D10% + E:12.5; D15% + E: 19.2; D20% + E:20.4; ΔE^* of pasta made without eggs: D5%: 11.3; D10% 12.7; D15% 19.6; D20% 23.1). In the case of cooked pasta, we assume that cooking time and temperature could influence changes in color parameters.

3.2. Cooking Properties of Cooked Pasta [40]

3.2.1. Cooking Time of Pasta

Cooking time ranged from 5 to 9 min (Figure 2). A gradual increase in cooking time was observed with increasing duckweed supplementation, which may be attributed to changes in the pasta matrix structure. The samples without duckweed showed the shortest cooking time, while 20% duckweed-enriched samples required the longest cooking time. Except for the 0% formulations, egg-free pasta samples generally exhibited slightly longer cooking times than egg-containing samples. This may be attributed to differences in matrix structure and hydration behavior. In the absence of egg proteins, the network was primarily formed by millet starch, psyllium husk, and duckweed components. The high water-binding capacity of psyllium and the additional protein and fiber fractions introduced by duckweed may slow water penetration and starch hydration during cooking, thereby increasing the optimum cooking time. The more pronounced floury appearance observed in egg-free pasta may be related to the absence of egg proteins, which normally coagulate during cooking and contribute to matrix stabilization. Without this protein network, starch granules may be less effectively retained within the structure, leading to increased surface starch release and a flourier appearance after cooking.

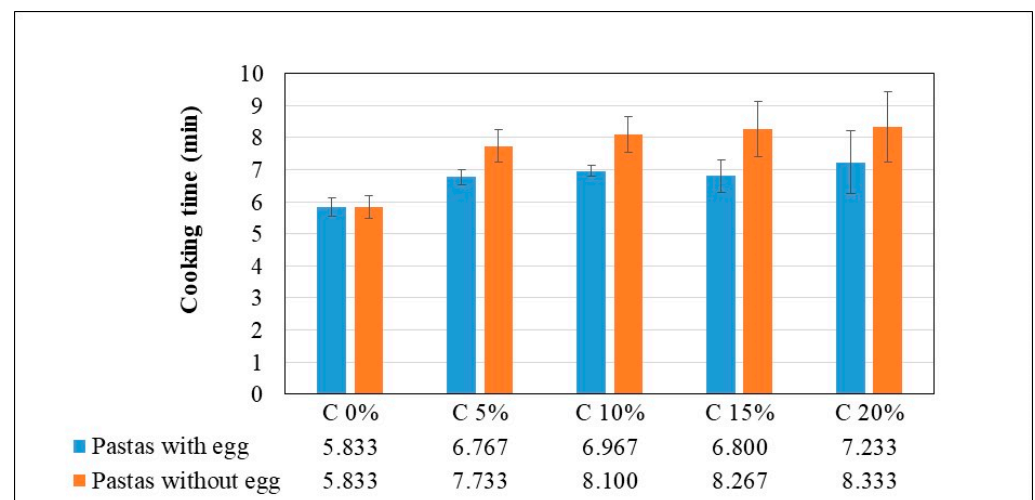


Figure 2. Cooking time of cooked pasta.

3.2.2. Water Uptake Capacity of Cooked Pasta

Water uptake capacity describes the ability of pasta to absorb and retain water during cooking, which influences final texture and yield. For both sample groups (with and without egg), a slight increase in water uptake capacity was observed with increasing duckweed supplementation (Figure 3). Egg-containing samples generally showed slightly higher values compared to egg-free formulations. This may be related to the functional properties of egg proteins, which can contribute to water binding and structural stabilization of the

dough matrix. The highest water uptake capacity was observed in the egg-containing 20% duckweed sample (D20% + E), reaching 181.2%, indicating the greatest hydration capacity among all formulations. Our results are similar to [10].

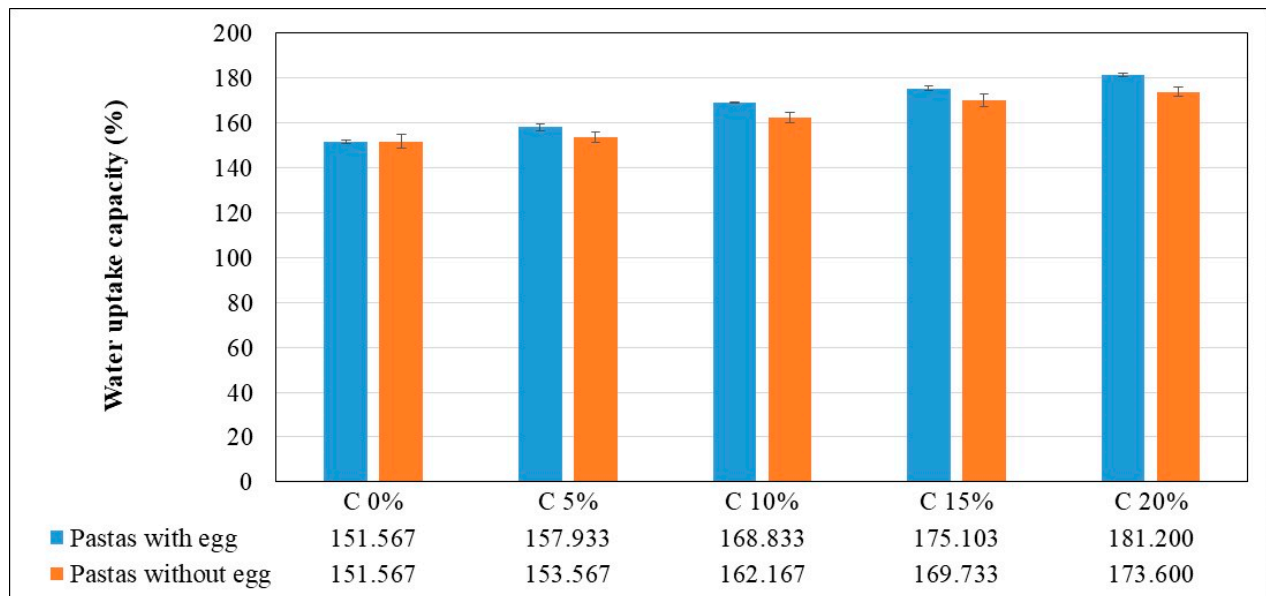


Figure 3. Water uptake capacity of cooked pasta.

3.3. Physicochemical Properties and Water Activity of Raw, Dried and Cooked Pasta

The water activity of raw, dried, and cooked pasta was examined (Figure 4). Raw pasta ($a_w = 0.82\text{--}0.93$) exhibited relatively high water activity, making it susceptible to microbial spoilage and therefore requiring refrigerated storage. In dried pasta ($a_w = 0.68\text{--}0.84$), bacterial growth is generally inhibited at water activity levels below approximately 0.85; molds may grow slowly within a water activity range of 0.7–0.8, and osmophilic yeasts are also capable of surviving in such an environment. Nevertheless, dried pasta is considered a microbiologically stable and shelf-stable product when stored under appropriate low-humidity conditions. Cooked pasta ($a_w = 0.84\text{--}0.92$) showed high water activity, indicating high perishability and the need for rapid cooling and refrigerated storage to limit the growth of spore-forming bacteria [42].

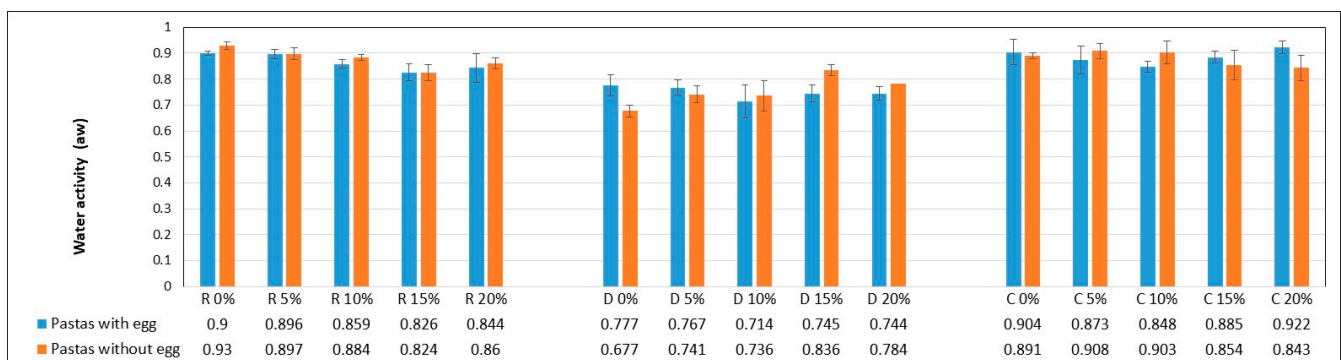


Figure 4. Water activity of raw (R), dried (D), and cooked (C) pasta with duckweed flour incorporation at levels of 0, 5, 10, 15, and 20% by replacing millet flour on a weight basis.

3.4. Monitoring Bioactive Components in Pasta Through Different Processing Stages and After In Vitro Digestion

The nutritional and functional properties of millet-, psyllium-, and duckweed-enriched pasta are dynamically influenced by processing steps, including drying, cooking, and gastrointestinal digestion. These stages do not merely reduce or increase nutrient levels but instead modify matrix structure, compound extractability, and bioaccessibility, which ultimately determines measured protein-related and antioxidant properties.

According to previous studies, watermeal enhances the nutritional and bioactive characteristics of food products [10,43–46].

3.4.1. Monitoring the Antioxidant Capacity in Pasta Through Different Processing Stages and After In Vitro Digestion

In general, in pasta at all levels of processing—both in the egg-containing and egg-free samples—a gradual increase in antioxidant capacity values was observed as the amount of duckweed flour was increased (Figure 5). The highest antioxidant capacity was found in the raw pasta made with eggs and enriched with 20% duckweed flour (R20% + E sample: 1.8002 ASE mg/g dry matter).

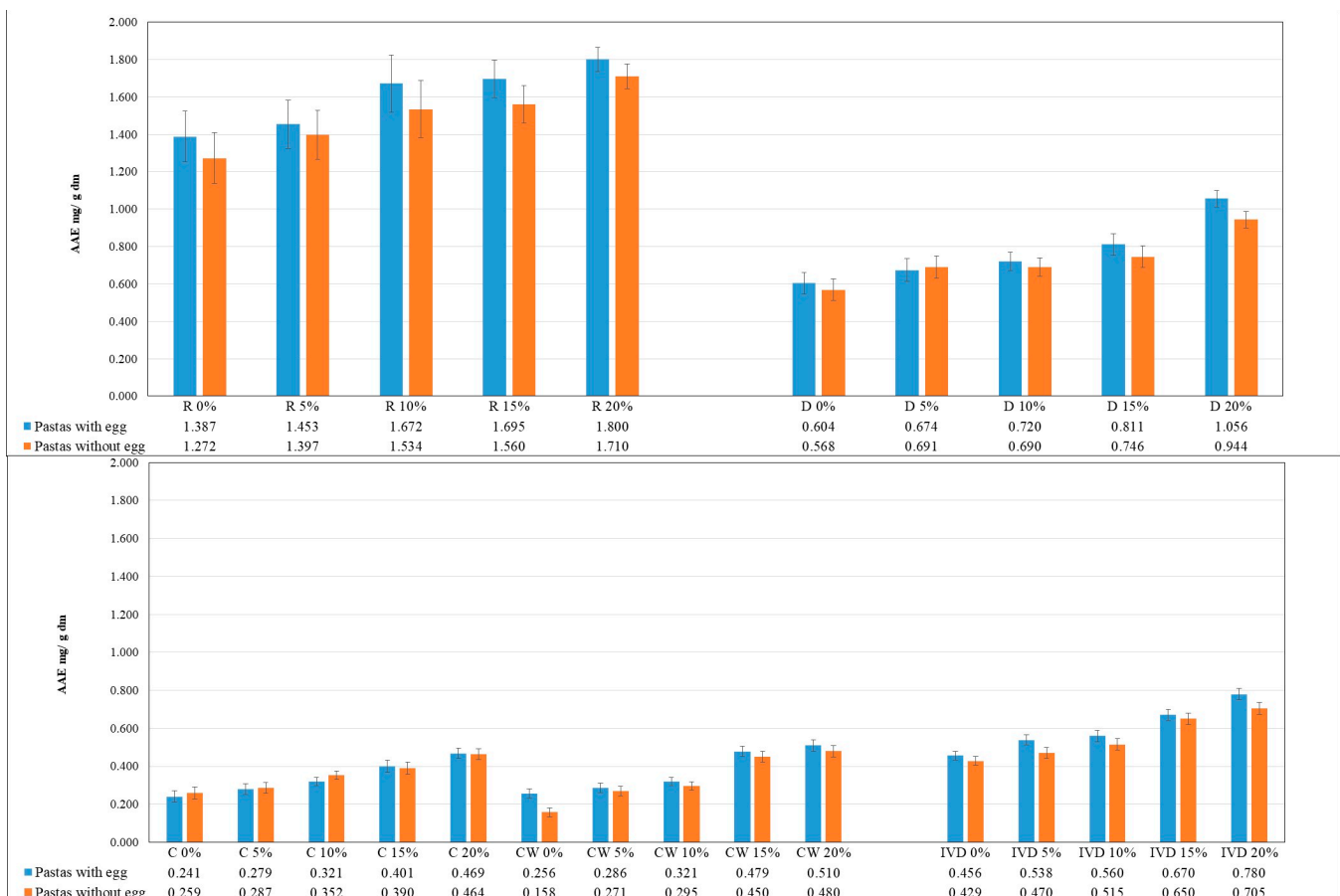


Figure 5. Antioxidant capacity of raw (R), dried (D), cooked (C), and intestinal digested cooked (IVD) pasta with duckweed flour incorporation at levels of 0, 5, 10, 15, and 20% by replacing millet flour on a weight basis and antioxidant capacity dissolved in cooking water (CW).

The antioxidant capacity of both egg-containing and egg-free dried pasta samples decreased compared to that of the raw pasta. This was because drying (at 75–80 °C) reduced antioxidant capacity values.

Cooking further reduced the antioxidant capacity of the samples. Cooking, like hot water extraction, causes the water-soluble components with antioxidant capacity to release [47,48].

When comparing pasta made with and without eggs, any difference in antioxidant capacity values was not noticed.

During *in vitro* human digestion of the cooked pasta, the results showed that digestive enzymes made the bioactive compounds in the pasta, including those with antioxidant properties, more accessible.

3.4.2. Monitoring the Total Polyphenol Content in Pasta Through Different Processing Stages and After *In Vitro* Digestion

The solubility of polyphenols depends largely on their chemical structure. In general, most polyphenols are highly soluble in polar solvents, such as water (to varying degrees), ethanol, methanol, and acetone–water mixtures. Water solubility of the following compounds is: phenolic acids (e.g., gallic acid)—good; chlorogenic acid—good; catechins—moderate/good; flavonols (e.g., quercetin)—poor; anthocyanins—good; high-molecular-weight tannins—variable. The usage of each solvent and their aqueous solutions resulted in higher TPC values compared to distilled water [49], but in the case of biscuit samples, Vegh and co-workers used distilled water as solvent (1 g/10 mL) to extract compounds that are water-soluble [50].

Sequential extraction and hydrolysis procedures may provide a more comprehensive quantification of total phenolic compounds, particularly including both free and bound phenolic fractions, but in the present study, our objective was to evaluate the extractable (free and readily soluble) phenolic fraction in raw duckweed flour and in processed pasta matrices (dried and cooked samples), rather than to determine the total bound phenolic content. For this purpose, a methanol:water (2:1, *v/v*) extraction system combined with mechanical disruption was selected, as it is widely used for the assessment of extractable phenolic compounds in plant-based food systems.

Bound phenolic forms associated with cell wall components may not be fully recovered using this approach. However, since the primary aim of the study was to compare processing-induced changes in the bioaccessible/extractable fraction, the applied method was considered appropriate and consistent across all sample types. Nevertheless, the method allows robust comparative evaluation across processing stages, which was the main objective of the study.

For raw, dried, and cooked pasta—in both egg-containing and egg-free samples—a gradual increase in total polyphenol content was observed as the pasta-making process progressed, due to the increased amount of duckweed flour (Figure 6). When comparing the sample groups, the polyphenol content of the egg-containing samples was higher than that of the egg-free samples.

The highest total polyphenol content was observed in the egg-containing pasta enriched with 20% duckweed flour across all sample groups.

The changes in total polyphenol content values following drying, hot water extraction, and *in vitro* digestion showed a similar trend to the antioxidant capacity values for the same reasons.

Drying generally maintained or slightly concentrated the measured polyphenol content due to water removal. However, matrix tightening may reduce immediate extractability of some phenolics, particularly those weakly bound within the starch–protein network. This behavior aligns with previous findings in cereal-based products, where dehydration stabilizes phenolics but may limit their immediate accessibility [51].

Cooking induced two opposing effects on polyphenol content: loss of water-soluble phenolics due to leaching into cooking water and release of bound phenolic compounds

due to structural disruption of the food matrix. As a result, cooked samples generally showed moderate polyphenol retention, with values depending strongly on duckweed concentration. The samples with higher duckweed content maintained higher post-cooking phenolic levels, suggesting a protective matrix effect. Similar trends have been widely reported in pasta and cereal systems, where thermal treatment decreases free phenolics but enhances extractability of bound fractions [51].

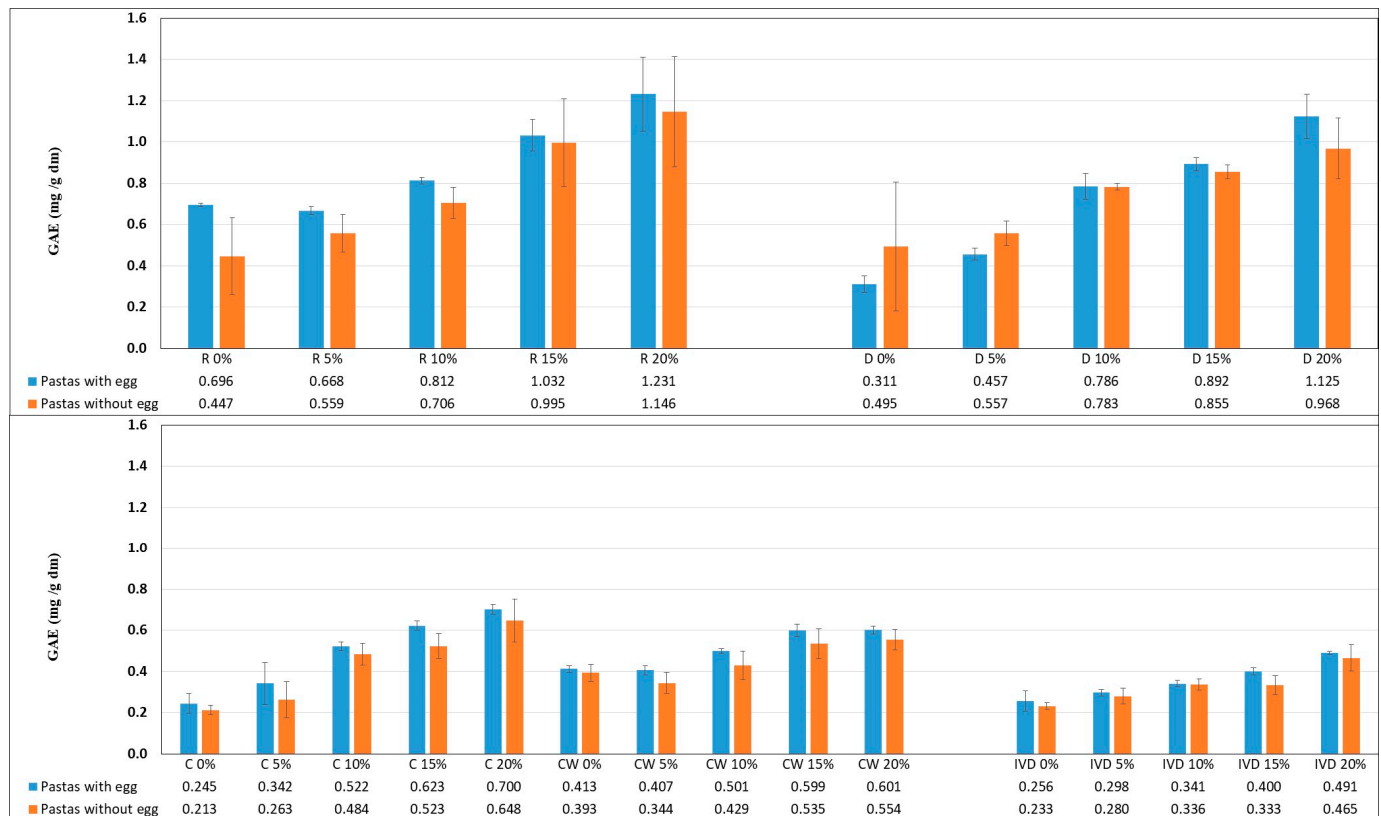


Figure 6. Total polyphenol content of raw (R), dried (D), cooked (C), and intestinal digested cooked (IVD) pasta with duckweed flour incorporation at levels of 0, 5, 10, 15, and 20% by replacing millet flour on a weight basis and total polyphenol content dissolved in cooking water (CW).

Standardized in vitro digestion models (INFOGEST) have shown that gastrointestinal processing increases polyphenol bioaccessibility in plant-based foods [29]. This effect is particularly pronounced in fiber-rich systems, where polysaccharide–phenolic interactions are disrupted during enzymatic digestion [52].

3.4.3. Monitoring the Protein Content Dissolved in Cooking Water Through Different Processing Stages

Figure 7 presents the protein content released into the cooking water of millet-based pasta formulations containing psyllium husk and increasing levels of duckweed flour as a partial replacement for millet flour. Protein concentration in the cooking water increased with increasing duckweed flour incorporation in both egg-containing and egg-free samples, indicating enhanced protein leaching during cooking. The protein content released into the cooking water increased from 1.025 to 2.367 mg g^{−1} dm in egg-containing pasta and from 0.933 to 2.225 mg g^{−1} dm in egg-free pasta as the substitution level increased from 0 to 20%. This trend can be primarily attributed to the higher protein content of duckweed flour compared with millet flour and the subsequent release of soluble or weakly bound protein fractions during hydration and thermal processing. At all substitution levels, egg-containing samples exhibited slightly higher protein concentrations in the cooking water,

reflecting the additional protein contributed by egg ingredients. From a technological perspective, the results suggest a reduced ability of the millet–psyllium matrix to retain proteins at higher duckweed incorporation levels, contributing to greater cooking losses. However, the increase in protein leaching was gradual and followed a consistent pattern across all formulations. From a nutritional standpoint, the release of proteins into the cooking water represents a loss of potentially valuable nutrients, as the cooking water is typically discarded before consumption. Nevertheless, the higher protein content provided by duckweed flour is expected to outweigh these losses, resulting in an overall increase in the protein value of the final product. Therefore, the observed protein leaching should be considered a predictable consequence of protein enrichment rather than a detrimental effect on the nutritional quality of duckweed-enriched pasta.

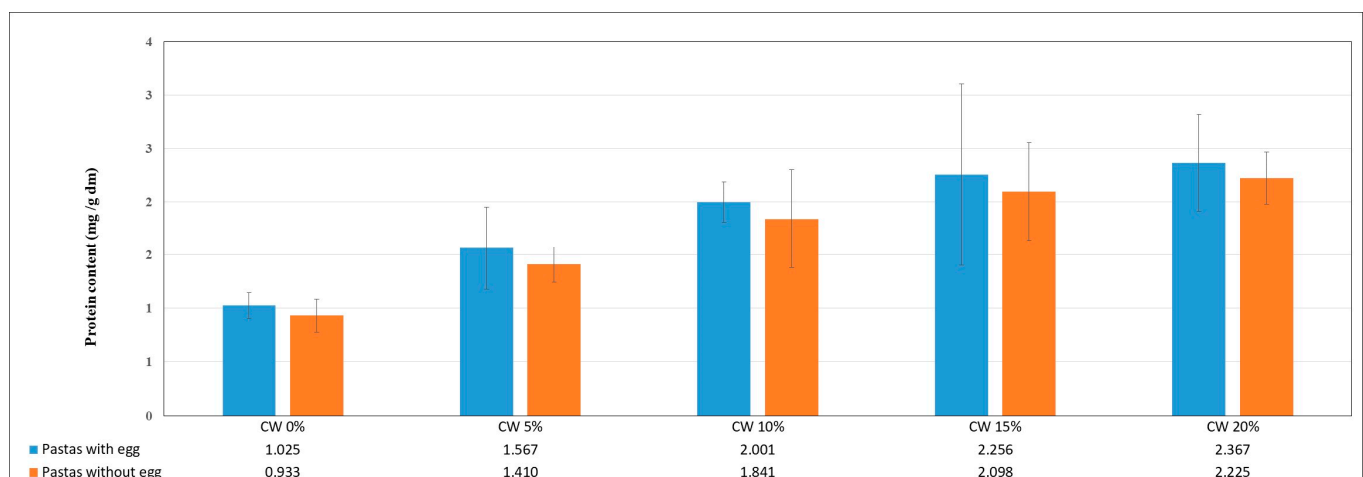


Figure 7. Protein content dissolved in cooking water (CW) of the cooked pasta (C) with duckweed flour incorporation at levels of 0, 5, 10, 15, and 20% by replacing millet flour on a weight basis.

3.5. GC-MS-O Analyses

Among the investigated parameters, the GC–MS–O analysis represented the central analytical approach of the study, providing the most detailed insight into the influence of duckweed supplementation and processing on the aroma profile of pasta products. Before the instrumental analysis, the panelists have smelled the samples. The intensity of the characteristic sharp, green, grassy odor of duckweed flour has diminished significantly in supplemented pasta. In the samples, some raw, herbal odor was slightly noticeable.

3.5.1. The Aroma Profile of Duckweed Flour

The volatile composition of duckweed flour was first characterized in order to establish a reference aroma profile for comparison with pasta samples. This step is essential for understanding the transfer and transformation of odor-active compounds during processing.

Thirty-nine VOCs and 16 aroma-active regions were identified in the volatile fraction of duckweed flour, where flavors could be detected, with varying intensities (Table 3). The members of eight groups of compounds constituted the aroma profile: terpenoids, sulphur compounds, alcohols, aldehydes, ketones, ethers, acids and hydrocarbons. The most intense compounds were hydrocarbons (48.78%), with decan as the dominant odorant (16.90%). Aldehydes (21.49%) followed by alcohols (9.77%) and acids (9.69%) were also abundant volatile groups.

Table 3. VOCs identified in the volatile fraction of duckweed.

RI	Compounds	Peak Area %	Aroma Character	Intensity
Terpenes and terpene derivatives		1.30		
1989	β -cyclocitral	0.74	Fruity	Strong
2675	β -ionone	0.34		
2764	β -ionone epoxide	0.22		
S-containing compounds		2.97		
638	Dimethyl sulfide	2.32	Sour, unpleasant	Weak
1955	Dimethyl sulfoxide	0.65		
Alcohols		9.77		
946	1-penten-3-ol	5.24	Mushroom	Middle
1077	2-butyloctanol	0.91		
1129	Pentanol	0.65		
1244	2-methyl-1-octanol	0.25		
1255	cis-2-penten-1-ol	0.36		
1928	2,6-dimethylcyclohexanol	2.17		
2504	2,3,6-trimethyl-7-octen-3-ol	0.19		
Aldehydes		21.49		
706	Isovaleraldehyde	17.62	Green, grass herbal	Strong
750	Pentanal	0.96		Strong
858	Hexanal	2.91		
Ketones		2.49		
1295	2,6,6-trimethylcyclohexanone	0.37	Mushroom	Middle
1333	6-methyl-5-hepten-2-one	1.39		
1482	.alpha.-isophorone	0.33		
1875	(E,E)-3,5-octadien-2-one	0.20	Sour, herbal	Weak
2137	4-oxoisophorone	0.20		
Ethers		0.44		
929	1-methoxy-2-propanol	0.44		
Acids		9.69		
1563	Acetic acid	3.37	Unpleasant, sour, rotten Sour, herbal	Weak
1741	Propanoic acid	0.52		
1805	Isobutanoic acid	1.14		
2039	Isovaleric acid	3.81		
2179	Pentanoic acid	0.57		
2449	Hexanoic acid	0.28		
Hydrocarbons		48.78		
614	Hexane	1.85	Green, raw	Weak
658	2,4-dimethylhexane	7.52		
725	2,4,6-trimethyloctane	2.36		
760	4-ethyloctane	6.81		
770	3,5-dimethyloctane	3.36		
796	3,6-dimethyldecane	5.11		
964	2,6,8-trimethyldecane	0.59		
980	3-methylundecane	0.57	Apple	Weak
1040	Decane	16.90		
1128	3,6-dimethyldecane	0.45	Raw fruit, green	Middle
1137	4,6-dimethyldodecane	1.10	Raw fruit, green	Middle
1455	Tetradecane	2.16		
Unknown compounds		3.07		

The characteristic 'green', 'grassy' and 'leafy' nature of plants, which also characterized the odor of duckweed are usually associated with the presence of C5 and C6 volatiles (Green Leaf Volatiles, GLVs). These odorants—mostly aldehydes and alcohols—are important VOCs in plants and were reported in plants by several authors [53–56]. GLVs are important contributors to communication between plants, since they are produced in wounded and damaged tissues as the products of hydroperoxide lyase (HPL) enzyme [54]. They also play an important role in attracting and repelling herbivores and their natural enemies [57]. These wound signal compounds had also been identified in the duckweed (10.12%), with 1-penten-3-ol (5.24%) and hexanal (2.91%) as most abundant odorants. The presence of these GLVs was observable formerly in nutraceuticals and spirulina and chlorella flours as well [57], and they are in accordance with the results in [28]. Among these VOCs in our duckweed flour, only cis-2-penten-1-ol and pentanal were aroma-active with their mushroom and herbal characters, respectively.

Although terpenoids are important odorants in most plants, duckweed contained only three terpene volatiles: β -cyclocitral, β -ionone and β -ionone epoxide. The first two terpenes were also reported in other *Lemna* species as well [32]. These carotenoid degradation products are the volatile metabolites of lutein, zeaxanthin and β -carotene, phytochemicals that are present in duckweed in large amounts [13,26,27,58]. Among the identified carotenoid metabolites, merely the strong fruity odor of β -ionone was perceived by the panelists.

Sulphur volatiles are significant contributors of plant odor, owing to their specific aroma and low odor thresholds [59,60]. Some of these VOCs may have chemotaxonomic significance as well [59]. Sulphur-containing odorants are formed in plants from sulphur-containing secondary metabolites and by the breakdown of amino acids, many of which serve protective purposes and are released when plant tissue is damaged [60,61]. In duckweed, flour dimethyl sulphide and dimethyl sulfoxide were identified, the latter with weak sour and unpleasant odor.

The group of acids in duckweed were quite intensive, and some of these volatiles, like isovaleric acid and pentanoic acid, exhibited unpleasant, sour character. Although acids are important constituents in the volatile fraction of a number of agricultural products, their odor contributes less to the formation of the aroma due to their high odor threshold.

Relatively high detection threshold also characteristic of hydrocarbons, which were the dominant volatile group in duckweed, regarding their total peak area. However, their role in the formation of flavor is probably not significant. Their aroma characteristics in duckweed flour were 'green', 'raw' (2,4-dimethylhexane, 3,6-dimethyldecane, 4,6-dimethyldodecane) and 'apple-like' (3-methylundecane). Alkanes are usually the enzymatic or nonenzymatic oxidation products of free fatty acids [62]. These latter precursors may derive from the cuticle of duckweed, which consists of cutin and waxes and has major role in protection. Namely, this waxy layer defends the plants against biotic and abiotic stresses like small organisms, UV light or water [63–65]. The predominant (48.78%) hydrocarbons in the volatile fraction of duckweed flour are therefore likely to be from the surface cuticular waxes.

Beyond the identified aroma-active volatiles in duckweed, some VOCs without peaks on the chromatograms were also perceived during the olfactometric measurement, due to their low odor detection thresholds. These aroma notes were 'boiled potato', 'green' and 'spicy, camphor'.

These results indicate that duckweed contributes characteristic green, fatty, and sulphur-related odor notes, which may influence the sensory acceptance of enriched pasta products.

3.5.2. The Volatile Composition of Pasta Containing Duckweed Flour

Processing significantly influences the aroma composition of foodstuffs. In the volatile fraction of pasta, the ratio of the aroma compound groups has altered significantly: ketones (12.79–76.87%) and alcohols (13.69–66.76%) became the dominant compounds groups with acetoin, isoamyl alcohol, 1-octen-3-ol and 2,3-butanediol as the most intense odorants. These volatiles can be released during microbial activities or lipid oxidation and other enzymatic processes. However, the peak area ratio of the dominant hydrocarbons in duckweed flour was greatly reduced in the pasta (0–3.80%): the other ingredients diluted the plant material in the products. The same is true for terpenes, as carotenoid degradation products were found in the pasta only in trace amounts. The total amount of flavor compounds and the number of identified VOCs did not show an increase with the rise in duckweed content. The characteristic ‘green’, ‘raw’ odor of duckweed was not intensely perceptible in the pasta made with it. The number of the identified volatiles in pasta was between 21 and 43, with the C 15% + E sample as the richest in flavor compounds. The aroma composition of the duckweed pasta is detailed in Tables S1 and S2. Regarding pasta without eggs, five odorants were present in the volatile fraction of duckweed flour and all pasta as well, indicating the presence of the plant in the product. These common aroma compounds were dimethyl sulphide, carotenoid degradation product 6-methyl-5-hepten-2-one, 1-methoxy-2-propanol, acetic acid and hexanoic acid. Other flavor compounds like β -ionone, 1-penten-3-ol, pentanol, isobutanoic acid, isovaleric acid and 2,4-dimethylhexane may also refer to the use of duckweed in the products, since they could be identified in the plant flour and in at least three types of pasta as well. The number of odor-active regions were lower in pasta compared with duckweed flour. The identified flavor-active volatiles and their aroma characters are indicated in Table 4.

With the addition of plant flour, terpenes and hydrocarbons appeared in the volatile fraction of the products, indicating duckweed application. C5 and C6 green leaf volatiles were also identified in pasta, although their characteristic odor was not detected during the olfactory measurements probably due to dilution during processing. In pasta with eggs, only two duckweed volatiles, namely 1-methoxy-2-propanol and acetic acid were present in all pasta samples. Carotenoid-derived 6-methyl-5-hepten-2-one, isobutanoic acid, isovaleric acid and 2,4-dimethylhexane may be additional volatile indicators of plant enrichment. Similar to the other type of pasta, the number of odorants with green-like character decreased compared to duckweed flour. Although, acetoin was the most intense volatile in the majority of the pasta samples, and in most cases it was not detected during the olfactometric analysis. The identified odor character in case of acetoin was ‘mushroom-like’. Overall, in pasta, ‘buttery’ and ‘baked’ markers were perceived more often, probably due to processing (pasta-making, cooking).

Similar to duckweed, aroma characters without peaks on the chromatograms have also been perceived in pasta. These odor marks were likely to be from very small amounts of compounds present which were not detected using the mass spectrometer.

Overall, the GC–MS–O analysis confirmed that aroma formation and degradation pathways are strongly influenced by both duckweed concentration and thermal processing, making volatile profiling a key determinant in evaluating product quality and consumer acceptability. This instrumental method usefully complements the organoleptic experiences and can identify the key volatiles responsible for the characteristic odor of the product.

Table 4. Odor-active VOCs in duckweed and cooked pasta.

Odorant	Odor Quaity ^a	Aroma Intensity ^b										
		D	C 0%	C 5%	C 10%	C 15%	C 20%	C 0% + E	C 5% + E	C 10% + E	C 15% + E	C 20% + E
2,4-dimethylhexane	Green, raw	1										
Isovaleraldehyde	Green, grass	3										
Ethyl alcohol	Herbal										1	
Pentanal	Herbal	3										
2,3-butanedione	Raw, green, sour, herbal		3	2	3		2		2	3	2	
2-pentanone	Raw, green, herbal		3						2	3		
3-pentanone, 2-methyl-	Sour cream, yogurt, butter, cheese			2		1	2			3	2	
Ethyl 2-methylbutanoate	Fruity										1	
Ethyl isovalerate	Fruity										1	
Isobutanol	Butter							2				
3-methylundecane	Apple	1										
Isoamyl alcohol	Sour, herbal									1	1	
Decane, 3,6-dimethyl-	Raw fruit, green	2										
4,6-dimethyldodecane	Raw fruit, green	2										
3-octanone	Green							1				
Acetoin	Mushroom			2			1			2	1	
cis-2-penten-1-ol	Mushroom	2										
2-methyl-1-pentanol	Mushroom								1			
2,6,6-trimethylcyclohexanone	Mushroom	2										
2-methyl-4-penten-1-ol	Mushroom							1				
Tetramethyloxirane	Baked, bakery								1	3	1	
Methyl glycidyl ether	Herbal, sour							1				
1,2-epoxy-3-methoxypropane	Baked, bakery									3		
Isobutyl 2-hydroxypropanoate	Baked								1			
3-octanol	Herbal					1						
2-nonanone	Unpleasant										2	
Dimethyl trisulfide	Steamed cabbage, herbal					1	1			2	2	

Table 4. Cont.

Odorant	Odor Quaity ^a	Aroma Intensity ^b										
		D	C 0%	C 5%	C 10%	C 15%	C 20%	C 0% + E	C 5% + E	C 10% + E	C 15% + E	C 20% + E
<i>trans</i> -2-nonenol	Steamed cabbage					1						
2,3-butanediol	Fruity			1								
Dimethyl sulfoxide	Sour, unpleasant	1										
Isovaleric acid	Sour, green, fermented	1		1		2	2	1		1	2	
Hexanoic acid	Sour, herbal, fruity		1	1	1							
4-oxoisophorone	Sour, herbal	1										
Anethole	Cinnamon		2					3				
Guaiacol	Herbal, spicy			1	1	1	2				1	1
Benzyl alcohol	Herbal, spicy					1	2					1
Phenylethyl alcohol	Floral									1	1	1
β -ionone	Fruity, floral	3					1					
Octanoic acid	Baked			1					1	1	1	1

^a: flavor character perceived at the olfactory detection port. ^b: 1—weak; 2—middle; 3—strong. D—duckweed flour; C 0%—cooked pasta without egg; C 5%—cooked pasta without egg and with 5% duckweed; C 10%—cooked pasta without egg and with 10% duckweed; C 15%—cooked pasta without egg and with 15% duckweed; C 20%—cooked pasta without egg and with 20% duckweed; C 0% + E—cooked pasta with egg; C 5% + E—cooked pasta with egg and with 5% duckweed; C 10% + E—cooked pasta with egg and with 10% duckweed; C 15% + E—cooked pasta with egg and with 15% duckweed; C 20% + E—cooked pasta with egg and with 20% duckweed.

4. Conclusions

- This study focused on the development of gluten-free pasta systems based on millet (*Panicum miliaceum*) and psyllium husk (*Plantago ovata*), enriched with duckweed (*Lemna minor*) flour as a novel plant-based protein and bioactive ingredient.
- The incorporation of duckweed significantly enhanced the nutritional profile of the products, particularly by increasing protein content, antioxidant capacity, and total polyphenol levels in a dose-dependent manner across all formulations.
- While processing steps (thermal treatments such as drying, cooking) generally reduced extractable antioxidant activity and phenolic content, simulated digestion significantly improved the bioaccessibility of these compounds.
- Technological properties were moderately affected by duckweed enrichment, with increased incorporation levels leading to longer cooking times and higher water absorption capacity, without compromising overall structural integrity within the tested range. These results indicate that duckweed can be successfully integrated into cereal-based pasta systems at functional levels while maintaining acceptable technological performance.
- A major contribution of this work lies in the comprehensive GC–MS–olfactometry characterization of duckweed and duckweed-enriched pasta systems. The aroma of duckweed flour composed of various components, dominated by ‘green’, ‘raw’, and ‘herbal’ notes, owing to the presence of C5 and C6 alcohols, aldehydes and long-chain hydrocarbons. In addition, some unpleasant aroma characteristics have also appeared, attributed to dimethyl sulfoxide and isovaleric acid. In pasta, ‘green’ aroma notes were less characteristic, instead, ‘buttery’ and ‘baked’ descriptors were more frequent with ketones and alcohols as the dominant VOCs in the samples. Duckweed enrichment could be confirmed by the appearance of terpenes—including carotenoid degradation products like β -cyclocitral and β -ionone—and green leaf volatiles. These findings demonstrate that processing significantly modifies the volatile and odor-active profile of duckweed-enriched pasta but does not completely eliminate plant-specific volatile signatures.
- Overall, millet–psyllium–duckweed pasta represents a promising multifunctional food system that combines improved nutritional quality with plant-based protein enrichment and bioactive compound delivery. The observed changes in volatile composition and odor-active compounds suggest that duckweed incorporation and processing may influence aroma characteristics. However, dedicated sensory evaluation is required to determine how these changes affect sensory perception and consumer acceptance.
- Future studies should focus on sensory evaluation to determine the effects of duckweed incorporation on aroma perception and consumer acceptance, as well as on formulation adjustments, flavor masking strategies, and optimization of duckweed processing methods while maintaining its nutritional and functional benefits. Additionally, broader consumer studies and in vivo bioavailability assessments would further support the application potential of duckweed as a sustainable ingredient.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su18147377/s1>, Table S1: Volatile composition of cooked pasta without egg; Table S2: Aroma compounds in pasta with eggs.

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