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Powdered *Calendula officinalis* Petals Incorporated into Fresh Pasta: Nutritional and Chemical Evaluation Before and After Processing

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Abstract: The sustainability of the agri-food chain is part of the current agenda through the investigation of alternative sources of ingredients and/or enriched foods. Following the current consumer trends for healthy foods with underlying sustainable principles, this work aimed to develop fortified fresh pasta incorporating powdered calendula petals. A chemical assessment was performed to determine the effect of incorporating calendula petals (5%) on the sensory characteristics (color, flavor, appearance), phytochemical content, and antioxidant activity of fresh and cooked pasta. The incorporation of calendula petals remarkably increased ash (64%), fat (24%), and crude protein (18%). Similarly, there was a considerable increase in total phenolics, total flavonoids, and anthocyanins. As expected, antioxidant activity increased significantly with the addition of calendula in pasta (88%). The sensorial evaluation revealed that pasta with 5% calendula powder was as accepted as the control by the sensory panel. Cooking affected the nutritional and chemical constituents of the pasta. These findings suggest that powdered calendula petals can be employed as a functional food ingredient due to the large increase in protein and minerals, bioactive chemicals, and antioxidant activity, which remains after the integration procedure in typical fresh pasta.

Keywords: edible flower; *Calendula officinalis*; polyphenolics; cooking effects; sensory analysis

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1. Introduction

Over the last few years, the continued and alarming rise in world population, as well as the associated increase in food demand, has presented a problem for global food security [1]. Furthermore, the increased knowledge of the health benefits associated with enriched foods has resulted in a shift in consumer habits around food intake [2]. In this regard, improving the foods that are currently most consumed around the world with the goal of enhancing their sensory characteristics and nutritional value is a new challenge [3]. Consuming nutritional food products may help to enhance overall diet quality while reducing the risks linked to malnutrition [4]. Currently, given the complexity of processing procedures and increasing obstacles in the food chain, industries supply consumers with high-quality foods, including a wide range of enhanced foods [5]. Pasta is one of the most popular foods among consumers due to its palatability, convenience, nutritional quality, and low cost [6]. However, although this food product is considered an adequate carbohydrate and energy food, it is low in essential amino acids, fiber, vitamins, and antioxidants [3,7]. According to the International Pasta Organization (IPO) [8], a total of 19.5 million metric tons of pasta were consumed in 2023, with an average consumption of 7.0 kg, and it is expected to grow annually by 6.56%. As a result, in addition to its high consumption, pasta can be supplemented with several ingredients that may have a positive impact on health. In this context, several studies have investigated pasta enrichment by adding different ingredients, including vegetables and fruits [7,9,10], microalgae [11–13], insects [14–16], and agro-industrial by-products, such as olive pomace [17,18], onion skin [19], and watermelon rind [20].

Edible flowers have been part of the human diet for centuries, mainly for their health benefits [21]. Parts of flowers including petals are rich in vitamins, minerals, and biologically active compounds [21]. In general, flowers are rich in phytochemicals, with antioxidant, anti-inflammatory, prebiotic, anticancer, anti-diabetic, and cardioprotective properties [21–23]. *Calendula officinalis*, popularly known as pot marigold or calendula, is a widespread flowering plant that can be found in Europe, Asia, and Australia [24]. Recently, calendula has taken on the role of an edible flower, improving the organoleptic characteristics, flavor, and aesthetic value of foods. This flower is commonly used in the preparation of soups and salads, and it is a substitute for saffron, providing rice, soups, and other foods with sensory qualities [25]. It is also used medicinally to treat dermatological diseases and rheumatology diseases [24,26]. Several pharmacological activities of *C. officinalis* are related to the presence of several classes of secondary metabolites. The most prevalent carotenoids found in flower petals are luteoxanthin, neoxanthin, violaxanthin, 9Z-violaxanthin, 9Z-neoxanthin, auroxanthin, 9Z-anthroxanthin, mutatoxanthin, 13/13′Z-lutein, α-cryptoxanthin, z-cryptoxanthin, 9/9′Z-lutein, α-carotene, β-carotene, and β-cryptoxanthin, recognized for their ability to protect against oxidative diseases through antioxidant activity [27]. Terpenoids, including erythrodiol, lupeol, calenduloside, and cornulacic acid acetate, are described as responsible for calendula's anti-inflammatory activity [28] and the prevention of several oxidative disorders such as Alzheimer's disease and diabetes-related complications [28]. Flavonoids (rutin, quercetin, calendoflavoside, isoquercitrin, narcissin, and isorhamnetin), in addition to their antioxidant activity, possess anti-gingivitis and anti-plaque properties [29]. Additionally, quercetin and rutin exhibit anti-depressive properties [27]. Coumarins are another class of secondary metabolites described in the flowers of *C. officinalis*. Umbelliferone, scopoletin, and esculetin are the main important ones, related to spasmolytic, phlebotonic, and anti-thrombotic properties [27]. Furthermore, some researchers have observed the beneficial effects of coumarins in the inhibitory process of specific enzymes implicated in the formation of cancers [30]. Also, Zeinsteger et al. [31] demonstrated that calendula extracts prevent mitochondria from lipid peroxidation in rat brains. In fact, the biological mechanism of neuronal cell loss in Parkinson's disease may be significantly influenced by lipid peroxidation. Similarly, this species' richness of phytochemicals promotes higher blood and lymphatic circulation, helping in the body's detoxification [23] and eliminating the superoxide and hydroxyl radicals generated during riboflavin photoreduction [32].

To the best of our knowledge, there have been no investigations performed on pasta with added calendula, an ingredient that is high in bioactive compounds. The aim of this work was to develop a health-promoting pasta enhanced with 5% calendula petals and investigate its nutritional and chemical contents, as well as the antioxidant activity of the product. The assessment of the phytochemicals' thermal stability during the pasta-cooking process was also part of this study. Considering the innovation of this work, a sensory analysis was carried out, establishing how the inclusion of calendula petals affected sensory pasta characteristics—appearance, flavor, aroma, and acceptability—among consumers.

2. Materials and Methods

2.1. Materials

Dried calendula petals (*Calendula officinalis* L.) were purchased from a local market, a food distributor Morais e Costa & Cª Lda (Porto, Portugal).

Wheat flour T65 "Espiga" with 73.0% carbohydrates, 9.6% protein, 1.3% fat, and 4.0% fiber, from Fábricas Lusitana, Lisboa, Portugal, and fresh eggs (Modelo Continente Hipermercados S.A., Oliveira do Hospital, Portugal), were purchased locally. All chemicals and reagents used were analytical grade.

2.2. Pasta Formulation and Processing

Pasta formulations were prepared by an expert in the field, more specifically, by the Chef of L'Artista Ristorante, located in Oliveira do Hospital (40.3425147, −7.8483763), Portugal. Briefly, two dough formulations were developed using wheat flour, water, and eggs. The control pasta was prepared using 100% wheat flour and the other formulation with the incorporation of dried calendula petals by mixing 5% dry powder petals with 95 g of wheat flour. For each pasta formulation, 1 egg was added. All the ingredients were placed in an electric pasta maker (Ariete Pastamatic 1581, Italy) and mixed for five minutes. After homogenization, they were left to rest for one hour. Then, a 2 mm wide rolling pin was used to form both pastas into Fettuccine. After cutting, the pasta rested for 10 min. To create cooked pasta samples, about 30 g of fresh control pasta (FCP) and fresh pasta enriched with 5% calendula petals (FPC), were boiled in 400 mL of water at 100 °C for 10 min. Both fresh and cooked pasta samples were stored separately at −80 °C, and subsequently lyophilized (48 h, −80 °C, 0.015 mbar) in a Telstar Cryodos-80 freeze dryer (Terrassa, Barcelona, Spain) and ground in a mill (Knife mill, GM 200, Retsch, Haan, Germany) to obtain a fine dry powder, which was then kept under controlled humidity and light conditions until further investigation. Calendula petals were also subjected to the same milling and freeze-drying process. Three replications were used to study dried calendula (CP) and fresh and cooked pasta samples (control—CCP; with 5% calendula petals—CPC) to analyze their nutritional and physicochemical characteristics.

2.3. Proximate Analysis

The nutritional analysis of the five samples analyzed followed the AOAC's procedures [33]. Briefly, moisture was determined using an infrared balance (Scaltec model SMO01, Scaltec Instruments, Heiligenstadt, Germany) (AOAC, 925.09). The ashes were determined after incineration at 500 °C (AOAC, 935.42). The Soxhlet and the Kjeldahl methodologies were used to calculate the total lipids and crude protein (AOAC, 989.05 and AOAC, 991.02, respectively). Protein content was determined based on food nitrogen concentration using a conversion factor of 6.25.

Total carbohydrates were determined by difference. Results were expressed as g/100 g of dry weight (dw).

2.4. Phytochemical Analysis

2.4.1. Extracts Preparation

To evaluate total phenolics, total flavonoids, total anthocyanins, and antioxidant activity, an aqueous extract was used, following the protocol described by Costa et al. [34]. The experimental dried samples (~1 g) were macerated with 20 mL of distilled water and stirred at 40 °C for 30 min. Aqueous extracts were filtered through Whatman No. 4 filter paper before freeze-drying. The dried extracts were then reconstituted with distilled water (5 mL), transferred to 10 mL tubes, and stored at −20 °C for the subsequent analysis of total phenolics, flavonoids, anthocyanins, and antioxidant activity. To evaluate the carotenoid content, another extraction approach was performed, as further detailed in Section 2.4.5.

2.4.2. Total Phenolic Content

The Folin–Ciocalteu assay, a standard method for measuring total (poly)phenols in foods, was used according to the procedure provided by Vinha et al. [35], adapted from the methodology described by Costa et al. [34]. Briefly, 150 μL of Folin–Ciocalteu reagent (1:10) and 120 μL of Na_2CO_3 aqueous solution (7.5%, *w*/*v*) were combined with 30 μL of each extract. After 15 min of initial incubation at 45 °C, the mixture was incubated for 30 min at room temperature, without light. Absorbance measurements (765 nm) were performed using a Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA). A gallic acid calibration curve was used to calculate the amount of total phenolic content, and the results are displayed in milligrams of gallic acid equivalents (GAE) per g of dry weight (dw).

2.4.3. Total Flavonoid Content

The total flavonoid content was ascertained using a colorimetric technique, previously described by Costa et al. [36]. An amount of 1 mL of each sample extract was mixed with 4 mL of distilled water and 300 μL of $NaNO_2$ (5%). After 5 min, 300 μL of $AlCl_3$ (10%) was added, and one minute later, 2 mL of NaOH (1 M) and 2.4 mL of distilled water were added. The absorbance was determined at 510 nm versus a blank using a Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA), and catechin was used as the standard for the calibration curve. The results were presented in mg of catechin equivalents (CE)/g of dry weight (dw).

2.4.4. Total Anthocyanin Content

The anthocyanin content was determined via pH differential, based on a spectrometry test according to the methodology described by Zhao et al. [37] with slight modifications. Briefly, two solutions of each sample were prepared: (i) 1 mL of sample extract + 10 mL of ethanol with 0.1% concentrated HCl + 10 mL of buffer solution (Na_2HPO_4 (0.2 M) with $C_6H_8O_7$ (0.1 M) at pH = 3.5); (ii) 1 mL of sample extract + 10 mL of ethanol with 0.1% concentrated HCl + 10 mL of 2% HCl (pH = 0.6). Absorbance was measured at 520 nm and 700 nm and calculated as Abs = $(A_{510\,nm} - A_{700\,nm})\,pH_{0.6} - (A_{510\,nm} - A_{700\,nm})\,pH_{3.5}$ using a molar extinction coefficient for cyanidin 3-glucoside of 26,900. Total anthocyanin content was calculated using the following equation and expressed as mg/g.

$$\text{Total Anthocyanin content (mg/g)} = [(\text{Abs}/e\text{L}) \times \text{MW} \times \text{D} \times (\text{V}/\text{G})] \quad (1)$$

where Abs represents the absorbance, *e* represents the cyanidin 3-glucoside molar absorbance [26,900 mL/(mmol·cm)], L represents the cell path length (1 cm), MW represents the molecular weight of anthocyanin (449.2 Da), D is a dilution factor, V the final volume (mL), and G represents the sample mass (mg).

2.4.5. Carotenoid Content

The methodology described by Vinha et al. [38] was used to evaluate the contents of β-carotene and lycopene. In short, 10 mL of acetone/hexane (4:6, *v*/*v*) was used to extract approximately 0.5 g of each sample, which was then centrifuged for 30 min at 5000 rpm. The absorbance of the supernatants was measured at 453, 505, 645, and 663 nm using a BioTek Synergy HT microplate reader (GEN5, Winooski, Vermont, USA). The carotenoid contents were determined using the following equations: β-carotene (mg/g = $0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$; Lycopene (mg/g) = $-0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$. Further, the sum was expressed in mg/g of dry weight (dw).

2.5. Antioxidant Activity

The antioxidant activity was determined using a 2,2-diphenyl-1-picryl-hydrazyl (DPPH) technique, previously described by Costa et al. [34]. To summarize, 270 µL of the $DPPH^{\bullet}$ solution (6.1×10^{-5} M) was combined with 30 µL of Trolox standard (562 µg/L), blank solution, or sample. A Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA) was used to observe the kinetic reaction at 525 nm at equal intervals of 10 min, with termination at 20 min. The calibration curve was prepared using Trolox as a standard and the $DPPH^{\bullet}$ scavenging activity was determined in milligrams of Trolox equivalents (TE)/grams of sample (dw).

2.6. Sensory Analysis

Consumer perception was assessed through visual, aroma, and taste assessments. Cooked control pasta (CCP) and cooked pasta enriched with 5% calendula petals (CPC) were presented to an untrained panel consisting of 67 members (23 men and 44 women, aged between 18 and 74 years), who were recruited across the university campus. Prior to participation in this study, each voluntary participant read and validated the free and informed consent form. After, each participant received two samples (control pasta and pasta enriched with calendula), presented in white containers and containing ~10 g of each cooked pasta. For collecting consumer impressions, a questionnaire was used. In the first section, participants were asked to identify their gender and age. In another section, participants were asked to indicate their impression using a 9-point hedonic scale (range from 1 = "Extremely unpleasant" to 9 = "Extremely pleasant"), firstly for appearance and aroma and in closing concerning mouthfeel aspects, namely the flavor. Finally, in the last section, the volunteers were asked about the overall acceptability. The buying intention was also evaluated with a 5-point hedonic scale, ranging from 1 = "Would not buy" to 5 = "Would certainly buy". The highest score represents the greatest level of acceptability (5 = extremely pleasant; 1 = extremely unpleasant).

2.7. Statistical Analysis

The analyses were conducted in triplicate, and the results are presented as the mean $\pm$ standard deviation. Statistical analyses were performed using Minitab software, version 17.1.0 (LEAD Technologies, Inc., Charlotte, NC, USA). The Shapiro–Wilk test was applied to assess the normality of the data. Subsequently, one-way ANOVA was used to identify significant differences between samples, followed by the post hoc Tukey test for pairwise comparisons of means. For many sensory attributes, the citation frequency values for the groups did not follow a normal distribution. Therefore, the Kruskal–Wallis test was employed to determine the presence or absence of statistically significant differences ($p \leq 0.05$) among the groups.

3. Results

The development of food products using composite flour has increased and is attracting much attention from researchers, especially in the production of pasta [3,7,9–14,16,18,39]. Pasta fortification might offer an approach to address nutrient deficits and malnutrition. Table 1 shows the proximate composition of freeze-dried calendula petals (CP), as well as freeze-dried uncooked and cooked control pasta (FCP and CCP, respectively), and freeze-dried uncooked and cooked enriched pasta with 5% calendula petals (FPC and CPC, respectively).

Table 1. Nutritional composition of calendula petals, uncooked and cooked control pasta, and enriched pasta (5% calendula petals). Results are expressed in g/100 g dry weight (dw).

Nutritional Composition	CP	FCP	FPC	CCP	CPC
Ash	5.77 ± 0.14 [a]	0.69 ± 0.11 [e]	1.95 ± 0.19 [c]	1.49 ± 0.34 [d]	3.64 ± 0.23 [b]
Total Fat	6.09 ± 0.21 [a]	4.47 ± 0.05 [c]	5.89 ± 0.15 [b]	3.79 ± 0.49 [d]	6.51 ± 0.27 [a]
Crude Protein	4.64 ± 0.08 [d]	9.38 ± 0.22 [c]	11.47 ± 0.15 [b]	9.68 ± 0.03 [c]	12.87 ± 0.13 [a]
Total Carbohydrates	81.02 ± 0.17 [b]	83.07 ± 0.50 [a]	78.32 ± 0.27 [c]	82.68 ± 0.77 [a]	76.49 ± 0.63 [d]

Results expressed in g/100 g dry weight. Values are presented as mean ± standard deviation (n = 3). Values within each line that do not share the same letter are significantly different ($p < 0.05$), determined by ANOVA followed by the Tukey HSD test. CP—calendula petals; FCP—uncooked pasta control; FPC—uncooked enriched pasta with 5% calendula petals; CCP—cooked pasta control; CPC—cooked enriched pasta with 5% calendula petals.

Considerable nutritional differences were observed in both pastas (control and enriched with 5% calendula petals). Cooking significantly influenced the nutritional value of pasta, mainly in the nutrient content of calendula-enriched pasta.

Regarding the results of the bioactive compounds and antioxidant activity (Table 2), the highest amounts of total phenolics and flavonoids were observed in the calendula petals. The addition of calendula at 5% was necessary to significantly increase the total phenolics, flavonoids, anthocyanins, and antioxidant capacity, as well as carotenoids (0.039 mg/g), in raw pasta. The carotenoid contents shown in Table 2 represent the average sum of the content of lycopene and β-carotene. The cooking process influenced the content of all phytochemical compounds evaluated, decreasing its antioxidant profile by 50.7, 3.89, 12.2, and 14.1% for total phenolics, total flavonoids, anthocyanins, and DPPH$^{\bullet}$, respectively; however, the resultant values remained significantly higher than cooked control pasta.

Table 2. The quantification of bioactive compounds and antioxidant activity (DPPH$^{\bullet}$) of calendula petals, uncooked and cooked control pasta, and enriched pasta with 5% calendula petals. Results are expressed in mg/g dry weight (dw).

	CP	FCP	FPC	CCP	CPC
Total phenolics (mg GAE/g)	8.36 ± 0.59 [a]	0.15 ± 0.01 [e]	2.23 ± 0.06 [b]	0.46 ± 0.06 [d]	1.10 ± 0.03 [c]
Total flavonoids (mg CE/g)	5.17 ± 0.72 [a]	0.09 ± 0.04 [d]	3.86 ± 0.32 [b]	1.85 ± 0.21 [c]	3.71 ± 0.16 [b]
Total anthocyanins (mg/g)	0.49 ± 0.03 [a]	0.06 ± 0.00 [b]	0.49 ± 0.04 [a]	0.04 ± 0.00 [c]	0.43 ± 0.01 [a]
Carotenoids (mg/g)	0.081 ± 0.001 [a]	ND	0.039 ± 0.001 [b]	ND	0.054 ± 0.018 [b]
DPPH$^{\bullet}$ (mg TE/g)	110.33 ± 2.53 [a]	10.33 ± 1.53 [d]	90.67 ± 3.51 [b]	7.77 ± 0.25 [e]	77.93 ± 0.31 [c]

Results expressed in mg/g dry weight. Values are presented as mean ± standard deviation (n = 3). Values within each line that do not share the same letter are significantly different ($p < 0.05$), determined by ANOVA followed by the Tukey HSD test. CP—calendula petals; FCP—uncooked pasta control; FPC—uncooked enriched pasta with 5% calendula petals; CCP—cooked pasta control; CPC—cooked enriched pasta with 5% calendula petals. ND—Not Detected; GAE—gallic acid equivalent; CE—catechin equivalent; TE—Trolox equivalent.

A Pearson correlation was performed between all bioactive compound contents and antioxidant activity studied in the five samples and the correlations are presented in Table 3.

Table 3. Pearson's correlation coefficient for bioactive compounds and antioxidant capacity in calendula petals and enriched pasta with 5% calendula petals (uncooked and cooked).

r *	Phenolics	Flavonoids	Anthocyanins	DPPH•	Carotenoids
Phenolics	1.00	1.00	0.62	0.97	0.82
Flavonoids	1.00	1.00	0.84	0.95	0.58
Anthocyanins	0.62	0.84	1.00	0.80	0.05
DPPH•	0.97	0.95	0.80	1.00	0.64
Carotenoids	0.82	0.58	0.05	0.64	1.00

* r—Pearson correlation coefficient.

As shown in Table 3, the parameters studied exhibit a strong correlation, with values exceeding 0.50. As anticipated, flavonoids display the highest correlation with phenolics, reflecting their status as a subgroup of these compounds.

The addition of novel ingredients might affect the appearance, flavor, and aroma of pasta, and few studies have been conducted on the sensory analysis of enriched pasta. Currently, no research has been carried out on the inclusion of petals of calendula in fresh pasta.

To examine the previous requirements, a sensory analysis was conducted using an untrained taster panel (Table 4). Since this was considered an innovative study, an assessment of the product's acceptance was included (Figure 1).

Table 4. Kruskal–Wallis test to check the difference between tasters in relation to an age range for each sample.

Attributes	Age	Rank			Test Statistics		
		N	Mean Rank	Z-Value	Chi-Square	DF	*p*-Value
Control							
Appearance	<25	26	36.1	0.71	0.79	2	0.675
	25–50	33	31.9	−0.86			
	>50	8	35.7	0.26			
Aroma	<25	26	36.4	0.81	0.85	2	0.654
	25–50	33	33.0	−0.40			
	>50	8	30.0	−0.60			
Flavor	<25	26	35.3	0.45	0.55	2	0.758
	25–50	33	34.0	0.00			
	>50	8	29.6	−0.68			
Purchase probability	<25	26	30.5	−1.16	3.14	2	0.208
	25–50	33	34.8	0.34			
	>50	8	41.8	1.21			
Enriched pasta with 5% calendula							
Appearance	<25	26	32.4	−0.54	0.99	2	0.610
	25–50	33	33.8	−0.08			
	>50	8	40.1	0.94			
Aroma	<25	26	28.8	−1.75	4.49	2	0.106
	25–50	33	35.7	0.70			
	>50	8	44.0	1.55			
Flavor	<25	26	25.2	−2.95	10.02	2	0.007
	25–50	33	38.3	1.77			
	>50	8	45.0	1.70			
Purchase probability	<25	26	32.0	−0.68	2.65	2	0.266
	25–50	33	33.9	−0.04			
	>50	8	41.0	1.08			

N—sample size; DF—degrees of freedom.

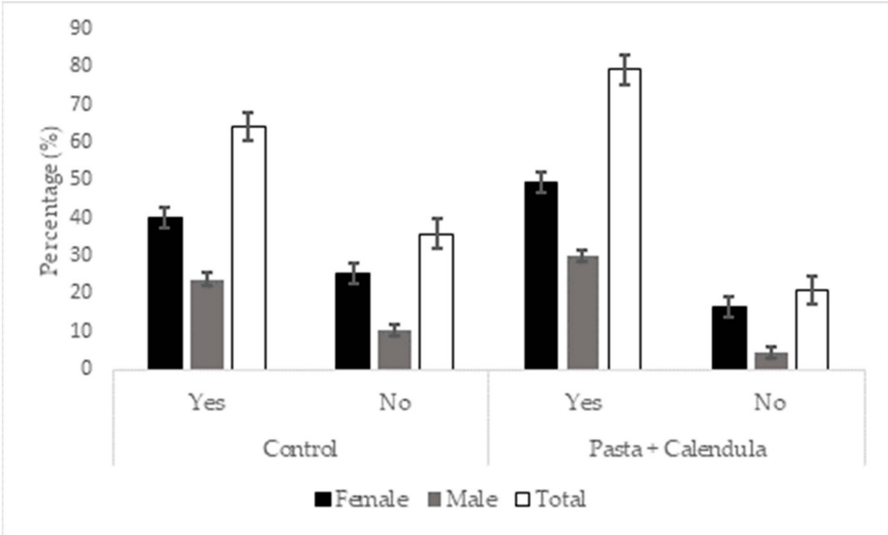

Figure 1. Acceptability estimation of the enriched pasta based on the tasters' gender and age.

Based on the sensory analysis, enriched pasta with calendula petals and control pasta showed no significant differences. However, in general, all sensory attributes were evaluated above the center point of the scale (5 = neither pleasant nor unpleasant), showing that calendula-enriched pasta was not rejected, observing good acceptance by the taster panel. In contrast, regarding Figure 1, the enriched pasta was highly accepted by tasters upon its acquisition.

4. Discussion

Consumer attitudes and acceptance of new food products, including enriched and fortified foods, are determining factors for their successful implementation. In this study, fresh pasta was enhanced with powdered calendula petals to increase its nutritional and phytochemical contributions while also ensuring an improvement in organoleptic characteristics and, consequently, promoting good acceptance by the final consumer.

4.1. Calendula Petals Characterization

Regarding proximate analysis (Table 1), carbohydrates were the most predominant macronutrient presented in calendula petals (81.02%), followed by fat (6.09%), ash (5.77%), and crude protein (4.64%), respectively. Our results contradict the data provided by Miguel et al. [40], who reported lower amounts of total fat (5.6%) and crude protein (2.4%) in calendula petals. According to Pires et al. [41] the lipid fraction of calendula petals presents high contents of linolenic acid (37%), palmitic acid (22%), and linoleic acid (20%). Rop et al. [42] reported reduced crude protein content (0.673%) in calendula harvested in the Czech Republic. Despite the observed differences, which may be due to several factors such as plant variety, growing conditions, soil, and climatic conditions, calendula can be considered a functional ingredient to enrich pasta. Thus, according to Patil et al. [23], fifteen amino acids have already been identified in calendula, including alanine, arginine, aspartic acid, asparagine, valine, histidine, glutamic acid, leucine, lysine, proline, serine, tyrosine, threonine, methionine, and phenylalanine. Edible flowers may be used as an alternative protein supply, addressing current concerns about environmental stability, food accessibility, and food safety, while also meeting rising consumer demand and combatting protein-energy malnutrition. In this sense, some studies emphasize the protein content of other edible flowers. Slightly higher protein values were described in *Hibiscus sabdariffa* (5.5%) [43] and *Helichrysum italicum* (5.44%) [44], but lower values were reported in *Rosa damascena* Mill (0.5%) [45], *Viola wittrockkiana* (2.11%), and *Antirrhinum majus* (1.87%) [46].

The use of edible flowers as food and/or ingredients has nutritional benefits, including micronutrient supplementation. In fact, minerals perform multiple metabolic functions, such as bone formation, hormone synthesis, and the transmission of nerve impulses [47]. Thus, consumers are increasingly interested in food products rich in micronutrients, driving the edible flower market [42].

The ash content reflects the whole mineral content, and our results are in accordance with those described by Pires et al. [41], who reported similar values of ash in several edible flower petals, including *Rosa canina*, *Centaurea cyanus*, and *Dahlia mignon*. *C. officinalis* can be characterized as a value-added ingredient in an assortment of foods due to its nutritional composition and acknowledged biological properties.

Despite its nutritional benefits, pasta contains a limited level of bioactive compounds. Therefore, the content of bioactive compounds and the antioxidant activity of the calendula petals were analyzed, with the intent of measuring the enrichment of the pasta with its integration. The total phenolic, flavonoid, anthocyanin, and carotenoid contents of calendula petals were first calculated, as well as the antioxidant activity (Table 2). Furthermore, the bioactive compounds present in flowers have become relevant for improving the chemical and organoleptic profiles of enriched pasta. Regarding bioactive compounds, calendula petals contained higher contents of total phenolics (8.36 mg/g), followed by flavonoids (5.17 mg/g), anthocyanins (0.49 mg/g), and carotenoids (0.081 mg/g). Lower total phenolic contents were reported by Youssef et al. [48], through methanol (MeOH) and ethyl acetate (EtOAc) extracts (0.57 mg/100 g and 1.25 mg/100 g, respectively). Lower total phenolic contents have also been described in hydromethanolic [49] and hydroalcoholic calendula extracts [50]. As already mentioned, these differences can be due to a range of causes, including plant varieties, as well as the application of hydrophobic solvents, like methanol, hexane, and acetone. Calendula is recognized for its high carotenoid content; however, our results showed significantly higher anthocyanin content. These observations are in agreement with the results previously obtained by Vella et al. [51], who reported substantial concentrations of anthocyanins in calendula, primarily glycosides of cyanidin, delphinidin, malvidin, peonidin, pelargonidin, and petunidin.

The evaluation of antioxidant activity using the DPPH$^{\bullet}$ assay is widely used in food extracts. Calendula petals presented the highest antioxidant activity (110.33 mg TE)/g in all the samples analyzed. Calendula's bioactive components have been described as important antioxidant phytoconstituents [27,29,49].

Considering our findings, the aqueous extract of calendula petals represents a potential resource of natural antioxidants that can play a crucial role in protecting human health. Thus, it is recognized that the antioxidant activity of phenolic acids is mostly attributable to the number of substituents, their relative position (*para* > *meta* > *ortho*), and the substitution of hydroxyl groups with carboxylate.

Thus, adding edible flowers into the human diet, providing enrichment to a widely consumed food, could significantly contribute to overall daily nutrient requirements, as well as other bioactive and health-beneficial components [41].

4.2. Pasta Enriched with Calendula Petals

Ingredient-enriched products are growing in popularity, leading significant studies towards the production of novel enriched pasta products. However, according to the available knowledge, calendula petals have never been used to fortify a pasta product.

The incorporation of calendula petals elevated the ash content about 2.82 times in pasta compared to pure wheat-based pasta control. Thus, the variation in ash content may also be related to the degree of flour extraction and the yield during milling, in addition to influencing the size of the particles. In fact, the flour used in this analysis was obtained after the technological process of flour extraction, before the incorporation of 5% calendula petal-enriched powder. This element can also have a direct impact on the protein amount of the flour, as both are concentrated in the aleurone layer of the wheat grain. A significant increase in enriched pasta was observed in fat (4.47 to 5.89%) and crude protein (9.38 to 11.47%), with a consequent decrease in total carbohydrates (5.72%). In short, the incorporation of calendula into the pasta allowed a significant increase of 31.8% fat and 22.3% crude protein content. Thus, an increase of nearly 2.83 times the total amount of ash was observed. Previous studies have reported interesting mineral profiles in edible flowers [42,43,52], which might support this increase in calendula-enriched pasta. The addition of calendula to pasta also resulted in a significant ($p < 0.05$) increase in the fat content. One hypothesis for the increase in this content is the presence of carotenoids, which are nonpolar compounds that might affect lipid content. Nevertheless, it is known that calendula petals are a rich source of polyunsaturated fatty acids, mainly linolenic acid and linoleic acid [41]. However, analyzing the fatty acid profile might be useful for the additional validation of the fat content. Krupa-Kozak et al. [53] found that adding broccoli leaf powder to gluten-free bread considerably increased its protein, fat, and ash contents.

Plant proteins offer health advantages and critical nutrients unique to each species. In recent years, various foods enhanced with plant-based proteins have been developed as an ongoing solution to environmental and socioeconomic problems. Protein sources from algae [11–13], insects [14–16], and agroindustry byproducts [17–20], have been studied as ingredients for enriched foods. In terms of carbohydrate content, the enriched pasta presented lower values than the control pasta (78.32 and 83.07%, respectively). This difference can be related to the fact that calendula contains lower carbohydrates than control pasta, resulting in a reduction in carbohydrate content due to flour replacement. An identical observation was described in pasta enriched with olive pomace compared to the control [18]. Thus, it is possible to assert that calendula petals applied to high-carbohydrate foods could help sustain glycemic control by lowering carbohydrate content. In agreement with Ombra et al. [54], enriched pasta formulation may induce a low glycemic response and could be used to comply with diabetes control diets.

The contents of bioactive compounds, including natural pigment (anthocyanin and carotenoid) contents and antioxidant activity, are shown in Table 2. As expected, the addition of calendula to the formulation of pasta caused a significant increase ($p < 0.05$) of about 14.9 times for total phenolics, 8.2 times for anthocyanins, and 8.8 times for antioxidant activity. A remarkable increase was observed in flavonoid content (from 0.09 to 3.86 mg CE/g). The carotenoid content, measured as the total of β-carotene and lycopene, increased to 100%. Previous studies have shown that enriching pasta with additional plant ingredients enhanced the content of bioactive compounds [18,39,52,55]. As expected, the addition of 5% calendula changed the color of the pasta, ranging from the whitish color of the control pasta to the dark orange of the enriched pasta, reflecting the use of petals in the formulation. Regarding natural pigments, our results showed higher amounts of anthocyanins (0.49 mg/g) than carotenoids (0.039 mg/g). Antioxidant activity also increased from 10.33 to 90.67 mg TE/g, respectively. Similar observations were reported by Betrouche et al. [56] in a gluten-free pasta enriched with 10% and 15% tomato waste. Therefore, employing plant products as functional ingredients to enhance common foods might present a new challenge to the utility of edible flowers. Consequently, the results highlight the possibility of integrating 5% calendula into pasta formulas, significantly increasing antioxidant properties along with offering consumers additional health advantages.

4.3. Cooking Process

Cooking losses are one of the fundamental variables used to predict pasta cooking quality. As shown in Tables 1 and 2, the cooking process significantly affected the nutritional and chemical profile of the studied pastas. The control pasta exhibited a decrease in fat content (15.2%) and a significant increase in ash content (2.2 times more). One of the causes for these variances could be due to the cooking process, as the flour matrix absorbs water and the starch granules enlarge, shedding their hard structure and releasing soluble chemicals. Despite that, no differences ($p > 0.05$) were observed in protein and carbohydrate contents. Likewise, the cooking process affected calendula-enriched pasta by increasing the amount of ash (1.87 times), total fat (1.11 times), and crude protein (1.12 times). In this study, the highest cooking loss value was observed for total carbohydrates in fortified pasta (~2.3%), while control pasta presented significantly higher cooking losses in fat content (~15.2%). Cooking loss occurs when amylose leaks from starch granules during cooking, resulting in an unpleasant sticky texture. Thus, it is widely used as an indicator of overall cooking efficiency, demonstrating the resistance of disintegration during cooking pasta. Although cooking time optimization was not carried out in this study, the evaluation of the cooking process is one of the crucial factors in pasta, displaying how much solid material is lost in the boiling water. According to industry guidelines, nutritional losses in cooked pasta should not exceed a technological level greater than 8% [57], a level that this investigation was unable to obtain. Consequently, it is recommended that the cooking time should be optimized in a future investigation.

The preservation of bioactive compounds, including natural pigments, after cooking is an important factor in the validation of pasta enrichment techniques, as they can interfere negatively due to losses caused by leaching or chemical hydrolysis degradation at high temperatures. Furthermore, color stability is one of the most important organoleptic attributes when it comes to colored food products. Thus, the color difference between raw and cooked pasta is one observation regarding organoleptic variables. As shown in Table 2, fresh calendula pasta was characterized by a high content of total phenolics (2.23 mg GAE/g) and flavonoids (3.86 mg CE/g) and a high antioxidant capacity, determined by DPPH assay. Likewise, cooking affected the contents of bioactive compounds in the enriched pasta, with a decrease in total phenolics (−50.7%) > anthocyanins (−12.2%) > flavonoids (−3.9%). Antioxidant activity also decreased (−14.1%). Therefore, it can be concluded that thermal processing is responsible for the variations in antioxidant activity and phenolic contents. In this study, it is more likely that the phenolic compounds released during the boiling process are compounds from calendula, and not from wheat, due to its high total phenolic contents (8.36 mg GAE/g). It is accurate to state that variances in phenolic content as well as the quantities of phenolic compounds may be linked to variations in antioxidant activity. Future research should focus on analyzing specific phenolic profiles to better understand the correlation between antioxidant activity and phenolic composition in calendula.

Remarkably, cooking increased the enriched pasta's carotenoid concentration by 38.5%. Large levels of carotenoids are accumulated in calendula flower petals, namely, β-carotene, lutein, luteoxanthin, flavoxanthin, violaxanthin, rubixanthin, γ-carotene, and lycopene, the predominant ones in this species. Heating is related to pigment degradation as well as to increasing their extractability due to the dissolution of protein–carotenoid complexes, which supports our findings. Abushita et al. [58] observed similar results on the effects of technological interventions in carotenoid contents in tomato, which were found to be congruent with those in the current study.

Pearson's correlation coefficient for bioactive compounds and antioxidant activity in calendula petals and both pastas (uncooked and cooked) was conducted (Table 3). As expected, anthocyanins and carotenoids do not have any correlation, thus there is no linear relationship (r ≈ 0), due to their belonging to different classes of compounds. Additionally, significant correlations were evident between all groups of compounds analyzed and their antioxidant activity, as indicated by the DPPH• assay results, aligning with expectations. It is worth noting that phenolics were the compounds that presented the highest correlation, since this technique also quantifies simpler phenolic compounds when compared to the quantification methodology of flavonoids and anthocyanins, more complex molecules. It is also observed that carotenoids are the group of compounds with the least influence on DPPH• due to having a moderate linear correlation relationship.

The results obtained indicate that calendula can be successfully used as an ingredient in pasta products, since its incorporation allows a clear improvement in several nutritional properties. However, further investigations into the stability and interactions of phytochemicals with other food ingredients during processing must be carried out in the future. Moreover, the content of fiber and gluten should be analyzed, since they may affect technological properties.

4.4. Sensorial Analysis

Table 4 displays the sensory evaluation results of the pasta samples supplemented with calendula petals in comparison to the control. In terms of the analyzed parameters (appearance, flavor, and aroma) the results were very similar for the control mass and the enriched mass. However, purchase intention was significantly higher for this new formulation. As shown in Table 4, the results for the control sample were consistent across all age groups, indicating that the control did not cause significant variations in the outcomes following the incorporation of calendula. Nevertheless, the analysis of the calendula-enriched pasta revealed that the flavor exhibited a *p*-value below 0.05, indicating that at least one age group had a significantly different median compared to the others. Additionally, the average ratings for aroma and flavor increased for the calendula-incorporated samples, with this increase being particularly pronounced in the age group over 50 years. According to our results, this prototype pasta (enhanced with 5% calendula) can serve as a substitute for other pasta varieties enriched with vegetables and cereals that are presently offered on the food market.

5. Conclusions

The incorporation of calendula petals as an ingredient in the manufacture of fortified pasta was investigated based on the analysis of the nutritional, functional, and sensorial properties of the developed product. The addition of *Calendula officinalis* at 5% promoted an increase in protein (22.3%) and fat (31.8%) contents. Similarly, an increase of approximately 2.83 times the amount of total ash was observed. Likewise, significant increases were observed in bioactive compounds, as well as antioxidant activity. After the cooking process, calendula-enriched pasta decreased its bioactive compounds by 50.7, 3.9, and 12.2% for total phenolics, flavonoids, and anthocyanins, respectively. Despite the observed decrease in antioxidant activity (14.1%), the resultant values remained significantly higher than the cooked control pasta. Furthermore, increases of 38.5% were noticed for total carotenoids.

This study demonstrated that the addition of calendula in a previously unknown formulation may produce pasta with potential health advantages by increasing nutritional value, phenolic content, antioxidant capacity, and pigments such as anthocyanins and carotenoids. The pasta incorporated with 5% calendula was as accepted as the control by the sensorial panel. Additional research is needed to investigate the rheological and sensory properties of this enriched food. Also, it would be interesting to investigate the bioactive compounds and antioxidant capacity of enriched pasta after in vitro *digestion*.

This analysis would provide more accurate information on the health benefits of goods supplemented with calendula petals.

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