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Determination of Phenolic Compounds in Gluten Free Pasta Fortified with Vegetal Powders

Ioana TALOS[1], Elena-Suzana BIRIŞ-DORHOI[1], Floricuta RANGA[1], Vanda BABALAU–FUSS[2] and Maria TOFANA[1]*

[1] Faculty of Food Science and technology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Street Calea Manastur No.5, Cluj-Napoca, Romania
[2] INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath Street, 400293, Cluj-Napoca, Romania
** Corresponding author: M. Tofana e-mail: maria.tofana@usamvcluj.ro*

RESEARCH ARTICLE

Abstract

Food is necessary for human development. Several compounds from different food or food products possess bioactivities which help improve human health. In this study phenolic content of gluten free pasta and their ingredients were analysed using HPLC-DAD-ESI system. The phenolic compounds from the five ingredients (flours and powders), the control pasta and other five variants of gluten free pasta were identified. The highest content of phenolic compounds was identified in nettle powder (NP) and all the analyzed samples have a statistically significant content higher than the control.

Keywords: corn flour; gluten free pasta; phenolic content; vegetable powder.

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INTRODUCTION

Food is a natural or processed product, which aims to bring a contribution to the development of the human body (Tofană, 2006). Celiac disease, also called gluten intolerance, is becoming more widespread worldwide. Gluten consumption by celiacs induce the production of antibodies against these proteins by the immune system. This process leads to inadequate nutrition because the small intestine being affected, the absorption of nutrients cannot take place (Larrosa, et al., 2013). Except for gastrointestinal problems reported by celiac patients, they can also accuse anemia, fatigue, arthralgia, infertility and last but not least weight loss (Jones et.al, 2016). Unfortunately, the variety of foods available for celiac patients is limited because wheat is an ingredient found in a wide range of foods. For this reason, technologists from the food industry are trying to help people with celiac disease by developing gluten free alternatives of different types of food with similar sensory properties like the glutenic form of the same product. Moreover, they try to create gluten free products enriched in bioactive compounds (phenols, vitamins, minerals, fibers) that offer a superior nutritional intake and various benefits to the body. Phenols are chemicals that have as their structure one or more aromatic rings with one or more hydroxyl groups (Sheng, et al., 2018). Phenolic compounds, considered to be secondary metabolites of plants, beyond the varied structure, they also show different activity (Barba et al., 2014). They are the richest compounds with antioxidant potential in the human diet (Diaconeasa, et al., 2020). The increasing consumers` interest in clean labeled products and functional foods motivated the food industry to reorient and

rediscover medicinal plants that are so rich and varied in bioactive compounds. For these reasons, after long scientific studies, it was decided to lead medicinal plants not only to the medical and pharmaceutical industry but also to the food industry as primary or auxiliary ingredients (Chakravartula et al., 2021).

The increased antioxidant activity of cereals, plants and herbs is very beneficial, as it can be used successfully as a substitute for synthetic preservatives. The incorporation of aromatic plants in different foods helps to increase its stability, because they are generous and convenient resources of bioactive substances and natural antioxidants with proven beneficial effects for health (Filipcev, 2020).

Corn (Zea mays L.) cultivation is preferred both due to its ecological plasticity and due to its high production capacity (approximately 50% higher than in the case of other cereal crops) (Domuța and Domuța, 2010).

Nettle (Urtica dioica L.) is a plant rich in bioactive compounds. Both in nettle leaves and in their extract phytochemicals such as phenolic compounds (flavonoids) and organic acids that produce anti-inflammatory, anti-diabetic and diuretic effects have been determined (Chakravartula, et al., 2011)

Lucerne (Medicago sativa L.) It is the most widespread fodder plant in the world, being considered as a "possible vegetable" in the daily diet (Mielmann et al, 2017).

Studies have shown that lucerne used as a supplement in human nutrition has beneficial effects on digestive problems, malnutrition and the immune system, increasing the amount of hemoglobin in the blood (Gawel, 2017).

The beneficial effects of lucerne on apoptosis, breast adenocarcinoma, anemia, diabetes, gastric ulcer, low bone density, etc. were also highlighted. In 2009, the European Food Safety Authority (EFSA) approved the use of lucerne concentrates as a food supplement in human nutrition with rich nutritional value, due to the content of phytochemicals (isoflavones, chlorophyll, carotene), vitamins, minerals (Ca, Fe), phytoestrogens (lignans) (Igual et al., 2021).

Grape pomace powder contains a large number of polyphenols, which are distributed as follows: 10% in the pulp, 28-35% in the shell and 60-70% in the seeds (Zhang et al., 2017). The high phenol content is due to incomplete extraction during the technological process of obtaining wines (Mohamed, 2020), but these compounds are extremely beneficial to the organism having an effective role as inhibitors in the oxidation of low-density lipoproteins (Vodnar et al., 2017).

MATERIALS AND METHODS

Materials

Gluten free pasta products in six variants control (C) and other five samples codified as S1, S2, S3, S4, S5 were prepared with a mixture of corn flour, extruded corn flour, nettle powder, lucerne powder, grape pomace powder and tapioca starch. The control pasta was obtained only from the two-corn flour mixture, and for the other five samples the corn flour mixture was enriched with all the 3 vegetal powders in different concentration. The ingredients and their concentrations are presented below in Table 1.

Table 1. Ingredients of pasta samples (%)

	CF (Corn Flour)	ECF (Extruded Corn Flour)	NP (Nettle Powder)	LP (Lucerne Powder)	GPP (Grape Pomace Powder)	TS (Tapioca Starch)
C	80	15	0	0	0	5
S1	72,5	15	2	3	2,5	5
S2	65	15	4	6	5	5
S3	57,5	15	6	9	7,5	5
S4	68,5	15	6	3	2,5	5
S5	67,5	15	2	3	7,5	5

Methods

The phenolic content of the ingredients and pasta samples were analyzed according to Diaconeasa et al 2014 using HPLC-DAD-ESI system. Analysis was carried out using a HP-1200 liquid chromatograph equipped with a quaternary pump, autosampler, DAD detector and MS-6110 singlequadrupole API-electrospray detector (Agilent-Techonologies, USA). The positive ionisation mode was applied to detect the phenolic compounds; different fragmentor, in the range 50-100 V, were applied. The column was an Eclipse XDB-C18 (5 µm; 4.5x150 mm i.d.) from Agilent. The mobile phase was (A) water acidified by acetic acid 0.1 % and (B) acetonitrile acidified by acetic acid 0.1 %. The following multistep linear gradient was applied: start with 5% B for 2 min; from 5% to 90% of B in 20

min, hold for 4 min at 90% B, then 6 min to arrive at 5% B. Total time of analysis was 30 min, flow rate 0.5 mL/min and oven temperature 25±0.5 0C.

Mass spectrometric detection of positively charged ions was performed using the Scan mode. The applied experimental conditions were: gas temperature 3500 C, nitrogen flow 7 L/min, nebulizer pressure 35 psi, capillary voltage 3000 V, fragmentor 100 eV and m/z 120-1200.

Chromatograms were recorded at wavelengths λ = 280, 340, 520 nm and data acquisition was done with the Agilent ChemStation software.

The calibration curves ar presented in Figure 1 and the chromatograms for each ingredient and pasta samples are presented below in Figure 2.

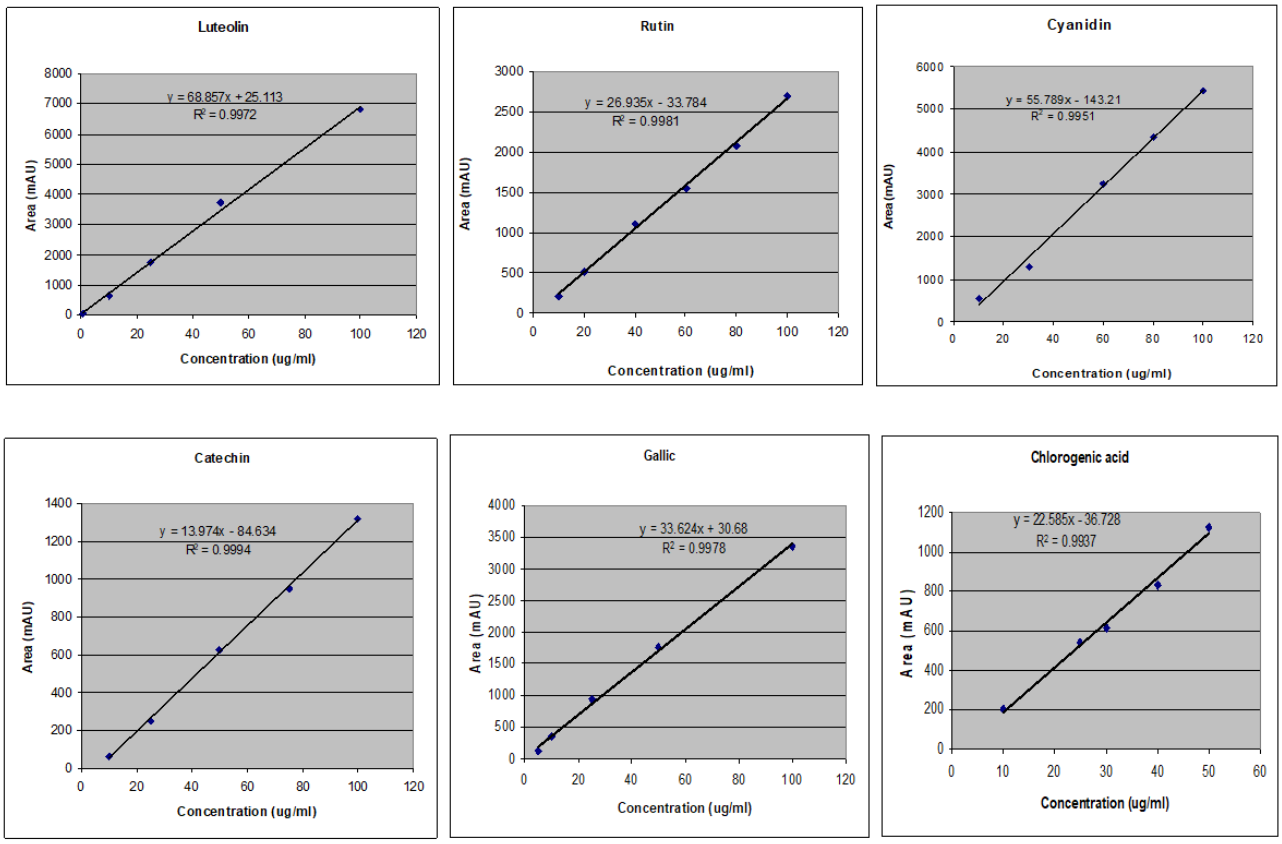
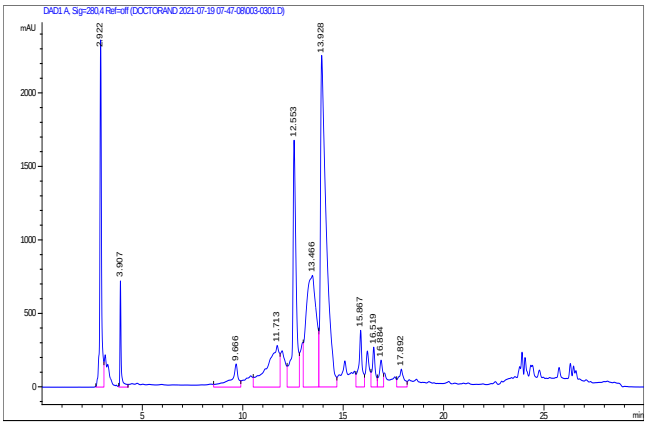
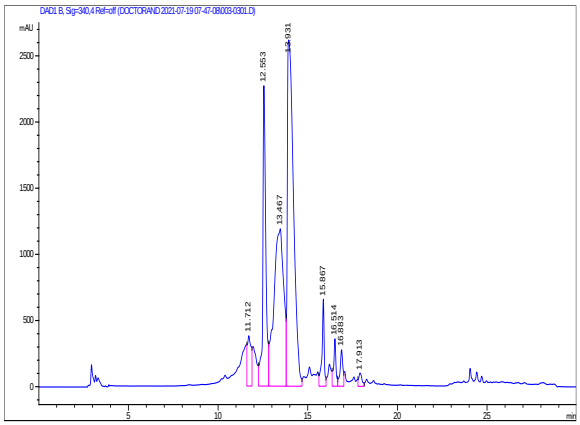


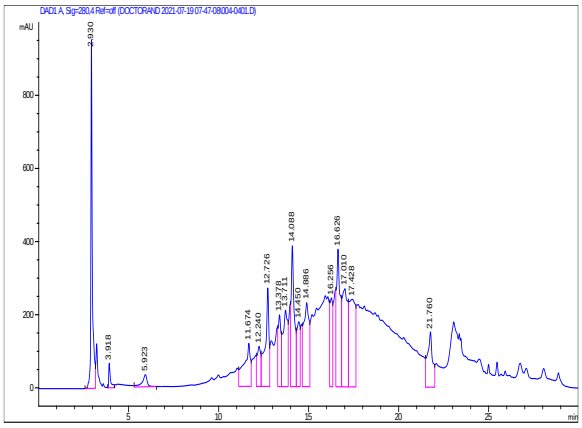
Figure 1. Calibration curves of phenolic compounds



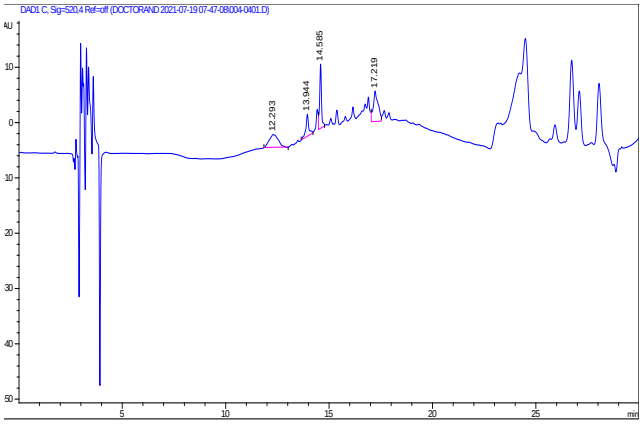
Nettle Chromatogram – 280 nm



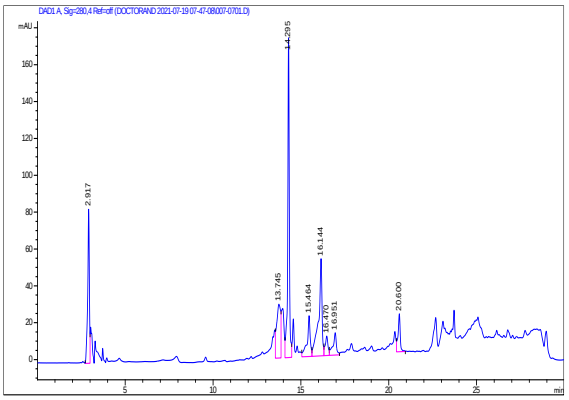
Nettle Chromatogram – 340 nm



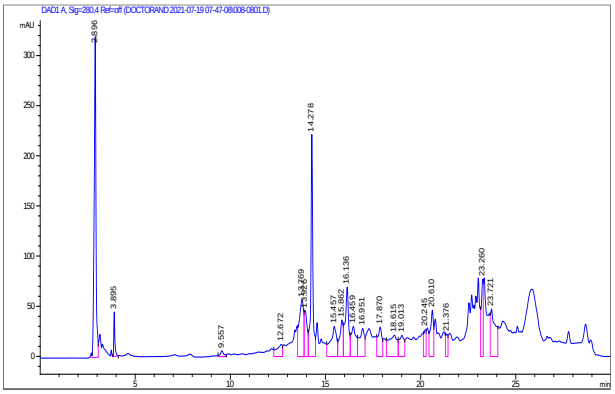
Grape pomace powder Chromatogram – 280 nm



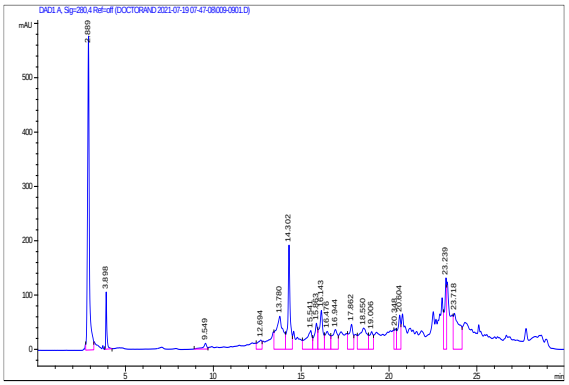
Grape pomace powder Chromatogram – 520 nm



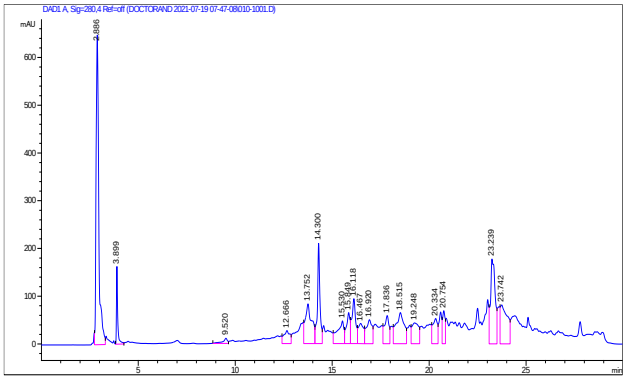
Control pasta Chromatogram – 280 nm



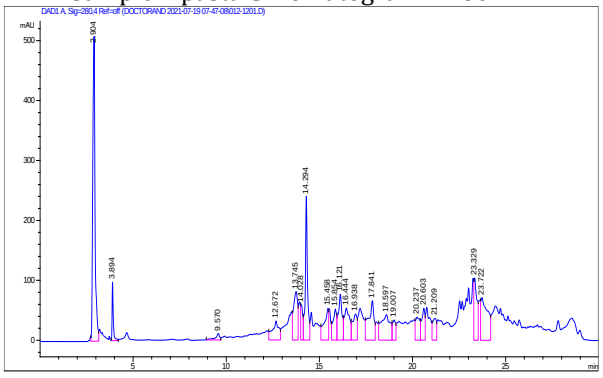
Sample 1 pasta Chromatogram – 280 nm



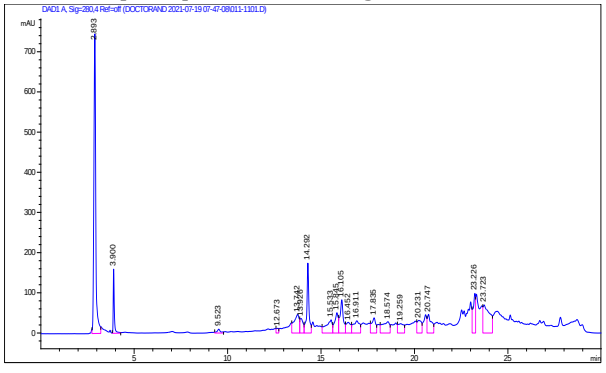
Sample 2 pasta Chromatogram – 280 nm



Sample 3 pasta Chromatogram – 280 nm



Sample 4 pasta Chromatogram – 280 nm



Sample 5 pasta Chromatogram – 280 nm

Figure 2. Chromatogram of the standard of phenolic compounds

RESULTS AND DISCUSSIONS

The phenolic compounds identification of the raw materials and final products are described in the tables below. In corn flour caffeic acid derivative presented the highest concentration with 221.338 μg/g and ferulic acid the lowest concentration with 23.153 μg/g (Table 2).

Table 2. Identification of phenolic compounds in corn flour and extruded corn flour

Peak No.	Retention Time R_t (min)	$[M+H]^+$ (m/z)	UV λ_{max} (nm)	Compound	Subclass	Corn Flour μg/g
1	2.92	155	275	2,3-Dihydroxybenzoic acid	Hydroxybenzoic acid	90.631
2	13.74	181	322	Caffeic acid	Hydroxycinnamic acid	89.923
3	14.29	438, 181	323	Caffeic acid derivative	Hydroxycinnamic acid	**221.338**
4	15.48	369	324	Feruloylquinic acid	Hydroxycinnamic acid	79.828
5	16.18	165	323	*p*-Coumaric acid	Hydroxycinnamic acid	60.877
6	16.49	225	323	Sinapic acid	Hydroxycinnamic acid	32.894
7	16.98	195	324	Ferulic acid	Hydroxycinnamic acid	**23.153**
8	20.61	517	323	3,5-Dicaffeoylquinic acid	Hydroxycinnamic acid	33.957
				Total Phenolics		***632.601***

In nettle powder caffeic acid presented a value of 20.839 mg/g this being the highest value and kaempferol-glucoside, which presented the lowest value of 0.375 mg/g (Table 3).

Table 3. Identification of phenolic compounds in nettle powder

Peak No.	Retention Time R_t (min)	$[M+H]^+$ (m/z)	UV λ_{max} (nm)	Compound	Subclass	mg/g
1	2.92	155	275	2,3-Dihydroxybenzoic acid	Hydroxybenzoic acid	2.980
2	3.91	155	275	2,4-Dihydroxybenzoic acid	Hydroxybenzoic acid	0.661
3	9.66	155	280	Gentisic acid	Hydroxybenzoic acid	0.437
4	11.71	355	322	3-Caffeoylquinic acid (Neochlorogenic acid)	Hydroxycinnamic acid	1.907
5	12.55	355	322	5-Caffeoylquinic acid (Chlorogenic acid)	Hydroxycinnamic acid	8.830
6	13.46	343	323	Caffeic acid-glucoside	Hydroxycinnamic acid	15.261
7	13.92	181	322	Caffeic acid	Hydroxycinnamic acid	**20.839**
8	15.86	611	255,360	Quercetin-rutinoside (Rutin)	Flavonol	1.599
9	16.51	465	256,360	Quercetin-glucoside	Flavonol	0.900
10	16.88	596	256,361	Quercetin-apiosyl-glucoside	Flavonol	0.851
11	17.89	449	255,350	Kaempferol-glucoside	Flavonol	**0.375**
				Total Phenolics		***54.640***

In Lucerne powder apigenin-glucoside had the lowest value (0.018 mg/g). From all phenolic compounds

apigenin-glucuronide presented the highest value of 2.247 mg/g (Table 4).

Table 4. Identification of phenolic compounds in lucerne powder

Peak No.	Retention Time R_t (min)	$[M+H]^+$ (m/z)	UV λ_{max} (nm)	Compound	Subclass	mg/g
1	2.92	155	275	2,3-Dihydroxybenzoic acid	Hydroxybenzoic acid	1.525
2	3.91	155	275	2,4-Dihydroxybenzoic acid	Hydroxybenzoic acid	0.291
3	9.66	155	280	Gentisic acid	Hydroxybenzoic acid	0.036
4	12.79	433	255,340	Apigenin-glucoside	Flavone	**0.018**
5	13.97	417	254	Daidzein-glucoside (Daidzin)	Isoflavone	0.106
6	15.95	623	255,341	Apigenin-diglucuronide	Flavone	0.104
7	16.15	433	254	Genistein-glucoside (Genistin)	Isoflavone	0.290
8	16.98	463	253,345	Luteolin-glucuronide	Flavone	0.300
9	18.42	447	255,341	Apigenin-glucuronide	Flavone	**2.247**
10	18.71	639	260,342	Luteolin-diglucuronide	Flavone	0.237
11	19.21	799	255,341	Apigenin(feruloyl-glucuronyl)glucuronid	Flavone	0.726
12	20.32	256	254	Daidzein	Isoflavone	0.350
13	23.25	257	250,340	Liquirtigenin	Flavone	0.613
14	24.06	271	254	Genistein	Isoflavone	0.204
				Total Phenolics		***7.047***

Grape pomace powder presents different phenolic compounds and values. The lowest value was identified for pelargonodin-glucoside (0.024 mg/g) and the highest value for 2,3-dihydroxybenzoic acid (1.193 mg/g) (Table 5).

Table 5. Identification of phenolic compounds in grape pomace powder

Peak No.	Retention Time R_t (min)	$[M+H]^+$ (m/z)	UV λ_{max} (nm)	Compound	Subclass	mg/g
1	2.92	155	275	2,3-Dihydroxybenzoic acid	Hydroxybenzoic acid	**1.193**
2	3.91	155	275	2,4-Dihydroxybenzoic acid	Hydroxybenzoic acid	0.098
3	5.92	155	280	Gallic acid	Hydroxybenzoic acid	0.137
4	11.67	579	280	Procyanidin dimmer	Flavanol	0.614
5	12.24	493	280,530	Cyanidin-acetyl-glucoside	Antocianin	0.028
6	12.72	291	280	Catechin	Flavanol	1.091
7	13.37	867	280	Procyanidin trimmer	Flavanol	0.423
8	13.71	433	279,331	Pelargonodin-glucoside	Antocianin	**0.024**
9	14.08	291	280	Epicatechin	Flavanol	1.157
10	14.54	480	279,330	Petunidin-glucoside	Antocianin	0.029
11	14.88	867	280	Procyanidin trimmer	Flavanol	0.492
12	16.32	465	256,360	Quercetin-glucoside	Flavonol	0.157
13	16.62	443	280	Epicatechingallate	Flavanol	0.816
14	17.01	479	256,360	Quercetin-glucuronide	Flavonol	0.078
15	17.21	639	279,520	Malvidin-coumaroy-glucoside	Antocianin	0.031
16	21.76	303	256,360	Quercetin	Flavonol	0.405
				Total Phenolics		***6.772***

Table 6. Identification of phenolic compounds in pasta samples (μg/g)

R_t (min)	Compound	Control Pasta	S1	S2	S3	S4	S5
2.89	2,3-Dihydroxybenzoic acid	96.122	243.381	467.724	**729.325**	590.657	482.685
3.89	2,4-Dihydroxybenzoic acid	0	17.234	47.156	81.212	68.921	46.035
9.65	Gentisic acid	0	5.220	15.666	18.348	10.460	15.331
12.66	Chlorogenic acid Catechin	0	50.805	55.864	107.781	104.195	58.484
13.75	Caffeic acid	87.444	173.554	232.972	266.831	275.063	213.665
14.01	Epicatechin		103.342	107.941	136.004	91.226	140.297
14.28	Caffeic acid derivative Chicoric acid	201.324	284.554	285.931	335.765	239.641	233.063
15.45	Feruloylquinic acid	54.324	120.947	148.238	196.509	131.858	135.063
15.86	Quercetin-rutinoside		71.869	90.819	126.490	98.549	102.368
16.11	*p*-Coumaric acid Genistein-glucoside	63.180	162.507	178.103	231.930	77.400	186.509
16.45	Quercetin-glucoside Epicatechingallate Sinapic acid	27.758	84.808	93.731	121.539	126.270	126.836
16.95	Quercetin-apiosyl-glucoside Ferulic acid Luteolin-glucuronide	36.259	97.625	136.604	187.306	124.605	126.123
17.83	Kaempferol-glucoside	0	68.781	111.785	144.398	141.545	88.224
18.51	Apigenin-glucuronide	0	37.091	65.774	106.267	40.160	76.840
19.01	Apigenin-(feruloyl-glucuronyl)-glucuronide	0	20.527	26.021	32.001	26.273	20.630
20.32	Daidzein	0	12.129	27.672	45.043	13.382	15.374
20.61	3,5-Dicaffeoylquinic acid	38.384	44.668	50.654	43.188	44.990	44.147
21.72	Quercetin	0	52.313	106.980	132.313	57.816	51.734
23.25	Liquirtigenin	0	19.089	64.122	74.316	20.741	21.847
24.06	Genistein		14.315	35.987	**44.701**	12.442	12.829
	Total Phenolics	***604.795* μg/g**	***1684.758* μg/g**	***2349.744***	***3161.267* μg/g**	***2396.195* μg/g**	***2198.08***

In pasta samples genistein was found to have high values in all samples with the highest value for samples 3 (44.701 µg/g). This sample also showed the highest value for 2,3-Dihydroxybenzoic acid. Overall sample 3 presented the highest value for all phenolic compounds identified, except 3,5-Dicaffeoylquinic acid, quercetin-glucoside, epicatechingallate, sinapic acid and caffeic acid).

Interpretation of the results

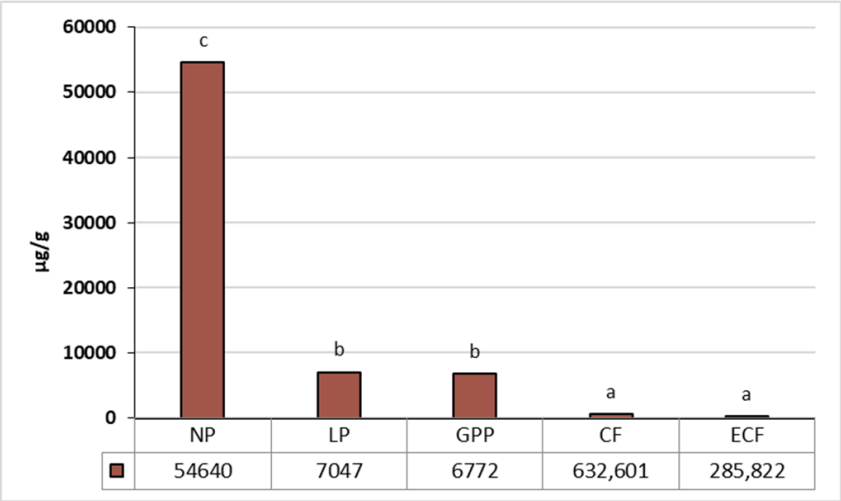


Figure 3. Total phenolic compounds identified in the ingredients

The values presented are average (n = 3). The letters above the columns indicate the differences between the averages.Samples that have one letter in common do not show statistically significant differences according to the multiple comparison test, Duncan ($p < 0.05$)

The results of the Duncan test show that NP has the highest statistically significant phenolic content compared to the other samples analyzed. Between the control, CF and ECF no statistically significant differences were found as in the case of LP and GPP samples.

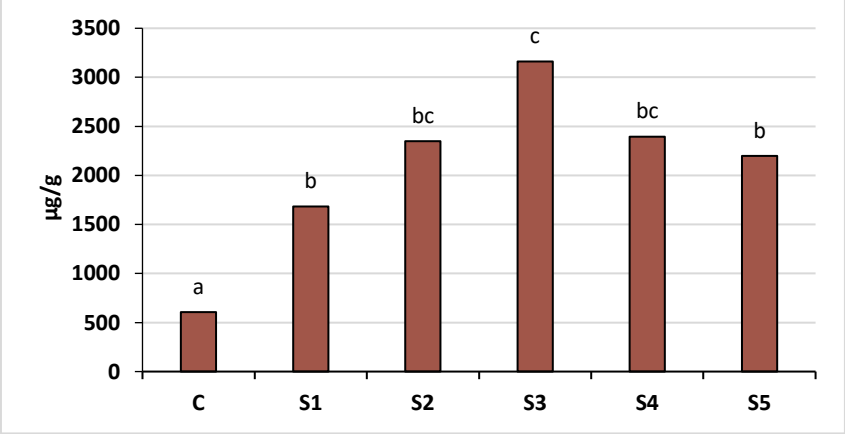


Figure 4. Total phenolic compounds identified in the final products

All the analysed samples have a statistically significant content higher than the control. However, no significant differences were detected between variants S1, S2, S4 and S5, as in the case of variants S2, S3 and S4.

Statistical analyses

All the data were analyzed using IBM SPSS Statistics 19 Software. One-way analysis of variance (ANOVA) was assessed at 95% confidence level to determine if there are any statistically significant differences between the

means of the variants. When the ANOVA null hypothesis was rejected, post-hoc multiple comparisons tests were performed using Duncan's multiple range test at $P<0.05$ to determine significant differences between the means. The values shown are means included in Tables or graphical representations chosen adequately for better representation of the data.

CONCLUSIONS

As a conclusion, the present study shows that nettle powder is a rich source of phenolic compounds and could be used in different products to enrich their biological potential and nutritional value. The results also indicate that there is a significant variation among the phenolic compounds contents of the ingredients and also between the pasta samples and the control pasta. The lower phenolic content was determined in control pasta because in all of the other five pasta variants, nettle powder was present as ingredient. In corn flour caffeic acid derivative presented the highest concentration with 221.338 μg/g and ferulic acid the lowest concentration with 23.153 μg/g. In pasta samples genistein was found to have high values in all samples with the highest value for samples 3 (44.701 μg/g). This sample also showed the highest value for 2,3-Dihydroxybenzoic acid. Sample 3 presented the highest value for all phenolic compounds identified, with the exeption of 3,5-Dicaffeoylquinic acid, Quercetin-glucoside, Epicatechingallate, Sinapic acid and Caffeic acid).

Author Contributions: S.E.B.D. and T.I. Conceived and designed the analysis; I.T. Collected the data; M.T., F.R., I.T. and V.B.F. Contributed data or analysis tools; I.T. Performed the analysis; I.T. and S.E.B.D. Wrote the paper.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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