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Extruded corn gruels containing linden flowers: quantitation of phenolic compounds and selected quality characteristics

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Abstract: Extrusion-cooking of plant materials may enhance antioxidant activity and improve health benefits. Selected antioxidant polyphenols in extruded corn gruels enriched with different amounts of linden flowers were determined by LC-ESI-MS/MS and quality characteristics were determined.

Phenolic content increased with *Tiliae inflorescentia* addition and was not decreased by high-temperature extrusion. Linden flower incorporation into instant gruels should be limited to 10% to retain acceptable sensory properties.

Keywords: *Tiliae inflorescentia*, extrusion-cooking, corn gruels, polyphenols, LC-ESI-MS/MS analysis

1 Introduction

Extrusion-cooking has become a popular high temperature, short time processing method for vegetable raw materials in snacks, pasta, flat bread, instant gruels, baby foods, confectionery, modified starches and pet food [1]. Grits, gruels and instant cereals are basic carbohydrate sources in baby and infant diets. These products should be processed to improve their dispersibility and digestibility, since a 3-4 month-old baby's pancreas has a limited ability to digest starch. Extrusion-cooking is much more efficient than traditional methods of infant cereals processing, *e.g.* drum or roll drying [2, 3].

Extrusion-cooking temperature, screw speed, moisture content, feed rate and residence time distribution are crucial for extrudate nutritional characteristics and antioxidant activity. The process changes texture, gelatinizes starch, cross-links proteins, and creates flavors [4]. It may inactivate antinutritional factors, denature undesirable enzymes or reduce lipid oxidation [5].

Extrusion-cooking may enhance food antioxidant activity and form resistant starch or insoluble fiber [6-8]. It retains more nutrition and active compounds than other thermal treatments, especially slow cooking or deep frying, but it is comparable to blanching, freezing or fermentation [9]. Analysis of extruded product extract showed higher antioxidant activity than in the raw materials; phenolics may be released [10-14].

Khanal *et al.* [15] reported the effects of extrusion-cooking on procyanidin monomers and dimers in grape seed and pomace. It appears to increase the level of low molecular weight bioactive compounds (procyanidins *etc.*) and biologically important monomers and dimers from polymer chains [16]. Extruded product enrichment with bioactive compounds is limited by secondary plant metabolites' low thermal stability and their susceptibility to changes during processing [5,17].

Fruits and vegetables are the most popular components added to cereal extrudates, but some waste products or functional compound isolates may be used [8,13,18-20]. Linden flower (*Tiliae inflorescentia)* is one of the herbal supplements most often used. Its main constituents include flavonoids (quercetin glycosides, kaempferol

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glycosides, tiliroside), phenolic acids, essential oils, phytosterols, organic acids, tannins, mucilage, minerals, niacin, and vitamin C [21-23]. Its pharmacological activity is in feverish colds, infectious diseases, and bronchitis, but it also has sedative and diuretic actions [24,25]. It is used as extracts or infusions (teas), but it may also be a pro-health component in infant or baby food.

Our aim was to determine selected polyphenols in extruded corn gruels enriched with different amounts of linden flower by LC-ESI-MS/MS and to evaluate their quality characteristics.

2 Experimental procedures

2.1 Chemicals

Analytical grade standards of gallic, protocatechuic, gentisic, 4-OH-benzoic, vanillic, caffeic, syringic, p-coumaric, ferulic, salicylic, veratric, synapic, 3-OH-cinnamic, and rosmarinic acids as well as rutin, hyperoside, isoquercetin, quercitrin, and apigenin-7-O-glucoside were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA). Tiliroside, kaempferol-3-rutinoside, astragalin were from Carl Roth (Karlsruhe, Germany). LC grade acetonitrile was purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA), LC grade methanol and analytical grade ethanol were purchased from J.T. Baker (Phillipsburg, USA). LC grade water was prepared using a Millipore Direct-Q3 purification system (Bedford, MA, USA). The SPE columns were Bakerbond C18, 3 mL containing 500 mg end-capped (17.5% C) 40 µm reversed phase packing (J.T. Baker, Deventer, Netherlands; catalog No. 1218800017).

2.2 Plant Materials

Tiliae inflorescentia (series 096/2011) was purchased from "*Kawon-Hurt"* herbal industry (Gostyń, Poland). Corn grits was purchased in the local market (Vegetus, Poland).

2.3 Extrusion-cooking procedure

Blends of corn grits and ground linden flowers were prepared by mixing dry components in weight ratios of 100:0, 99:1, 97:3, 95:5, 90:10 and 80:20. The blended samples were conditioned to 15% moisture by spraying with water and mixing continuously for 10 minutes. Blends were processed in a TS-45 single screw extrusion-cooker (ZMCh Metalchem, Gliwice, Poland) with barrel length / screw diameter = 12, and screw compression ratio 3:1. The barrel temperatures were 120-135-140°C in the first zone, second zone, and forming die. The screw speed was 125 rpm and a 3 mm circular open die shaped the extrudate [29,30]. After cooling (extrudate moisture content was 6.0%) extrudates were ground in an iG5A laboratory grinder (TestChem, Poland) to less than 1 mm for instant gruels [3,29]. Samples were stored in polyethylene bags at room temperature.

2.4 Extraction procedure

Before extraction dry plant material was milled and sieved. Three 2 g portions were each extracted 3 times with 40 mL 80% aqueous ethanol in a Sonorex RK 100H ultrasonic bath (Bandelin Electronic, Germany) (20 kHz, 100 W) for 30 min at 60°C [26]. Extracts were filtered, combined and evaporated to dryness. Residues were dissolved in 10 mL of methanol.

2.5 Solid phase extraction (SPE)

Crude extracts were purified by SPE. 5 mL was passed through a previously conditioned Bakerbond C18 SPE column. Polyphenols were eluted with 5 mL 60% aqueous methanol followed by 10 mL of 30% aqueous methanol. The combined extracts were evaporated to dryness and dissolved in 10 mL of methanol in a volumetric flask. The procedure was repeated three times.

2.6 LC-ESI-MS/MS analysis

Analysis was performed using reversed-phase high-performance liquid chromatography with electrospray ionization mass spectrometry (LC-ESI-MS/MS). An Agilent 1200 HPLC system (Agilent Technologies, USA) equipped with a binary gradient solvent pump, degasser, autosampler and column oven connected to a 3200 QTRAP Mass spectrometer (AB Sciex, USA) was used.

Phenolic acid separations were slightly modified from the procedure of Nowacka *et al.* [27] They were carried out at 25°C on a Zorbax SB-C18 column (2.1 × 50 mm, 1.8-µm particles; Agilent Technologies, USA) with a mobile phase of 0.1% HCOOH in water (solvent A) and 0.1% HCOOH in methanol (solvent B), using 3 µL injections. The flow rate was 450 µL min^{-1} and the gradient was: 0–0.8 min = 5%

Table 1: LC-ESI-MS/MS phenolic acids results.

Compound	Peak no.	TR [min]	[M-H]-	Fragment ions	Collision energy [eV]
Gallic acid	1	0.75	168.7	124.9	- 14
				78.9	- 36
Protocatechuic acid	2	1.73	152.9	107.8	- 38
				80.9	- 26
Gentisic acid	3	2.73	152.8	107.9	- 36
				81	- 30
4-OH-benzoic acid	4	3.40	136.8	92.9	- 18
Vanillic acid	5	4.72	166.8	107.9	- 18
				123	- 12
Caffeic acid	6	4.92	178.7	134.9	- 16
				88.9	- 46
Syringic acid	7	5.57	196.9	181.9	- 12
				122.8	- 24
p-Coumaric acid	8	6.01	162.7	119	- 14
				93	- 44
Salicylic acid	9	6.20	136.8	93	- 16
				75	- 48
Ferulic acid	10	6.28	192.8	177.9	- 12
				133.9	- 16
Synapic acid	11	6.33	222.8	148.9	- 20
				121	- 36
Rosmarinic acid	12	6.60	358.7	160.8	- 20
				132.6	- 44

Table 2: LC-ESI-MS/MS flavonoid glcosides results.

Compound	Peak no.	T_R [min]	$[M-H]^-$	Fragment ions	Collision energy [eV]
Rutin	1	9.62	608.7	299.6	- 46
				270.9	- 60
Hyperoside	2	11.40	462.7	299.7	- 28
				254.7	- 42
Isoquercetin	3	11.65	462.7	299.7	- 30
				270.7	- 44
Kaempferol-3-rutinoside	4	12.41	592.7	284.8	- 38
				226.7	- 68
Astragalin	5	14.03	446.7	254.8	- 40
				226.8	- 54
Quercitrin	6	14.32	446.8	299.7	- 30
				270.7	- 40
Apigenin-7-glucoside	7	14.32	430.7	267.7	- 38
				116.9	- 84
Tiliroside	8	17.50	592.8	284.8	- 30
				254.7	- 30

B; 2–3 min = 20% B; 5.5–8 min = 85 % B; 9.5–12 min = 5% B. The ESI operated in the negative ion mode: capillary temperature 600°C, curtain gas 25 psi, nebulizer gas 60 psi, source voltage – 4500 V. Nitrogen was the curtain and collision gas.

Flavonoid glycoside separations were carried out at 25°C on an Eclipse XDB-C18 column (4.6 × 150 mm, 5-µm particles; Agilent Technologies, USA) with a mobile phase of 0.1% HCOOH in water (A) and 0.1% HCOOH in acetonitrile (B), using 5 µL injections. The flow rate was 400 µL min^{-1} and the gradient was: 0–1 min = 18% B; 1.5–5.5 min = 20% B; 7–10 min = 25% B; 13–15 min = 60% B, 17–22 min = 18% B. The ESI operated in the negative-ion mode: capillary temperature 500°C, curtain gas 20 psi, nebulizer gas 50 psi, source voltage – 4500 V. Nitrogen was the curtain and collision gas.

For each compound the optimum multiple reaction mode (MRM) conditions were determined in the infusion mode. The data was acquired and processed using Analyst 1.5 software (AB Sciex, USA). Triplicate injections were made for each standard and sample. Analytes were identified by comparing retention time and m/z values obtained by MS and MS^2 with those of standards obtained under the same conditions (Tables 1 and 2). The calibration curves obtained in MRM mode were used for quantitation; peak areas were compared with calibration curves generated by three repeated injections of known standards at seven concentrations (0.005–50 ng μL^{-1}) [27]. Linearity ranges for calibration curves were determined. The limits of detection (LOD) and quantitation (LOQ) for phenolic compounds were determined at signal-to-noise ratios of 3:1 and 10:1 by injecting a series of dilute solutions with known concentrations. Calibration parameters, LOD and LOQ values are in Table 3.

Table 3: LC-MS/MS polyphenol analytical parameters.

Compound	LOD [ng μL^{-1}]	LOQ [ng μL^{-1}]	R^2	Linearity range [ng μL^{-1}]	Recovery [%]	Regression equation
Gallic acid	0.050	0.100	0.9985	0.05–10.00	93.7	y = 312 x - 1.59e+004
Protocatechuic acid	0.010	0.020	0.9995	0.05–25.00	98.2	y = 48.5 x + 9.41e+003
Gentisic acid	0.008	0.015	0.9997	0.025–25.00	102.3	y = 409 x - 3.16e+004
4-OH-benzoic acid	0.040	0.080	0.9992	0.05–5.00	95.3	y = 597 x + 2.1e+004
Vanillic acid	0.050	0.100	0.9992	0.1–50.00	93.2	y = 73.1 x + 3.67e+003
Caffeic acid	0.040	0.060	0.9985	0.05–1.00	94.5	y = 1.39e+003 x + 3.28e+004
Syringic acid	0.050	0.100	0.9994	0.1–50.00	93.3	y = 1.39e+003 x + 3.28e+004
p-Coumaric acid	0.050	0.100	0.9988	0.125–2.50	95.4	y = 794 x + 8.47e+004
Salicylic acid	0.020	0.050	0.9991	0.05–1.00	94.4	y = 3.23e+003 x + 2.83e+005
Ferulic acid	0.010	0.025	0.9996	0.05–5.00	93.1	y = 380 x + 2.01e+004
Synapic acid	0.010	0.025	0.9980	0.025–5.00	93.3	y = 119 x - 226
Rosmarinic acid	0.010	0.020	0.9996	0.025–12.50	94.8	y = 284 x - 1.65e+003
Rutin	0.005	0.010	0.9983	0.02–2.5	98.5	y = 280x-8.49e+003
Hyperoside	0.010	0.020	0.9987	0.05–2.5	95.1	y = 354x - 185
Isoquercetin	0.008	0.020	0.9991	0.05–2.5	101.9	y = 353x - 498
Kaempferol-3- rutinoside	0.001	0.003	0.9975	0.05–2.5	99.1	y = 639x - 1.11e+004
Astragalin	0.002	0.004	0.9992	0.01–2.5	93.1	y = 935x + 1.15e+004
Quercetrin	0.002	0.004	0.9994	0.05–2.5	101.8	y = 574x + 98.4
Apigenin-7-glucoside	0.0005	0.001	0.9992	0.005–1.00	95.3	y = 3.02e+003x + 1.4e+004
Tiliroside	0.0005	0.001	0.9972	0.005–1.00	92.9	y = 782x - 7.28e+003

2.7 Physical properties

Extrudate expansion, which varies with processing and composition, is an important quality factor and should be as great as possible. The radial expansion index is the ratio of extrudate to die diameter [30]. Ten replicate measurements were done for each recipe. The water absorption index (WAI) is the ratio of gel to dry sample. It was determined as described previously [31] with some modification. Ground extrudates (0.7 g) were suspended in 7 mL room temperature water in plastic tubes and mixed. After gentle stirring for 10 min the tubes were closed and centrifuged for 10 min at 12500 x g in a T24 centrifuge (VEB MLW MEDIZINETECHNIK, Leipzig, Germany) at ambient temperature (21°C). WAI was the ratio of gel remaining after supernatant removal to the weight of original dry solids. The supernatant was dried in an air oven at 105°C to constant weight. The water solubility index (WSI) is the percentage of dry matter recovered after the supernatant is evaporated from the WAI determination. WAI and WSI determinations were replicated in triplicate.

The dry gruel color was tested using a Lovibond CAM-System 500 Colour and Appearance Measurements System (The Tintometer Ltd., UK). 20 replicate measurements were performed for each sample. CIE-Lab scale was used to evaluate L^* for lightness, a^* for (+)redness(-)greenness and b^* for (+)yellowness(-)blueness [32]. Background color values were: L^* = 94.0, a^* = 2.7 and b^* = -0.4. Parameters for reference corn gruels were: L^* = 93.42, a^* = -3.94, b^* = 24.43. The color change index ΔE was calculated following Carrini *et al.* [33].

For sensory tests gruels were prepared by mixing with 45°C water (20:80 gruel:water) and served. Sensory characteristics include the taste, color, flavor, consistency, and overall quality. A 15-member semi-trained panel judged gruels on a 5-point scale (1 = weak, 5 = very good). Acceptability was evaluated on a 9-point hedonic scale,

Table 4: Polyphenols content (n = 3).

Compound	Yield ± SD[a] [μg g^{-1} of dry weight]				
	Corn gruel	Linden inflorescence	Corn gruel with 5% addition of linden inflorescence	Corn gruel with 10% addition of linden inflorescence	Corn gruel with 20% addition of linden inflorescence
Gallic acid	-	68.10 ± 0.78	3.22 ± 0.02	4.18 ± 0.06	8.76 ± 0.05
Protocatechuic acid	-	373.67 ± 2.89	12.83 ± 0.03	21.05 ± 0.20	68.80 ± 0.20
Gentisic acid	-	6.60 ± 0.01	0.90 ± 0.01	0.94 ± 0.00	1.92 ± 0.02
4-OH-benzoic acid	BQL[b]	19.13 ± 0.09	0.53 ± 0.00	0.98 ± 0.01	2.45 ± 0.04
Vanillic acid	-	5.68 ± 0.06	BQL	BQL	0.36 ± 0.00
Caffeic acid	-	2.54 ± 0.01	BQL	0.20 ± 0.01	0.44 ± 0.01
Syringic acid	-	0.28 ± 0.00	BQL	BQL	BQL
p-Coumaric acid	1.21 ± 0.01	11.06 ± 0.45	2.89 ± 0.13	6.20 ± 0.06	7.42 ± 0.12
Salicylic acid	0.51 ± 0.02	4.80 ± 0.09	0.57 ± 0.02	0.61 ± 0.00	1.26 ± 0.03
Ferulic acid	0.43 ±0.01	4.57 ± 0.12	0.57 ± 0.03	0.69 ± 0.02	0.82 ± 0.02
Synapic acid	BQL	0.72 ± 0.06	-	-	BQL
Rosmarinic acid	-	1.13 ± 0.08	-	-	-
Rutin	5.60 ± 0.00	166.00 ± 3.00	7.50 ± 0.10	14.72 ± 0.10	35.05 ± 0.10
Hyperoside	-	62.90 ± 0.10	BQL	3.21 ± 0.10	6.50 ± 0.10
Isoquercetin	0.30 ± 0.00	1256.72 ± 28.90	3.11 ± 0.40	42.01 ± 0.70	126.51 ± 3.50
Kaempferol-3-rutinoside	0.20 ± 0.00	63.90 ± 0.60	3.32 ± 0.00	5.63 ± 0.20	15.54 ± 0.20
Astragalin	0.20 ± 0.00	1083.34 ± 11.50	29.60 ± 0.10	31.60 ± 0.30	75.43 ± 0.60
Quercetrin	-	448.32 ± 1.50	11.73 ± 0.20	13.54 ± 0.10	32.01 ± 0.00
Apigenin-7-glucoside	0.10 ± 0.00	0.80 ± 0.00	0.21 ± 0.00	0.31 ± 0.00	0.82 ± 0.00
Tiliroside	-	499.33 ± 4.70	6.70 ± 0.11	17.60 ± 0.20	35.31 ± 0.26

[a] SD - standard deviation (n=3), [b] BQL - peak detected, concentration lower than the LOQ but higher than the LOD

where: 1=dislike extremely, 9=like extremely. Gruels were deemed acceptable if their mean acceptability scores were above 5 [34].

3 Results and discussion

3.1 Determination of polyphenols content

Our objective was the qualitative and quantitative analysis (LC-ESI-MS/MS) of phenolic extracts of corn gruels containing linden flower. Ultrasound assisted extraction [26] followed by SPE was precise and accurate (Tables 2 and 3). Ethanol, 80% aqueous ethanol, methanol, and 80% aqueous methanol were extractants; 80% aqueous ethanol gave higher yields of all the polyphenols because of their relatively polar nature.

Precision was evaluated by intra-day and inter-day tests. Intra-day experiments were performed by replicate analysis of six aliquots of the same sample within one day. Inter-day tests were carried out on three consecutive working days in the same way as intra-day experiments. Three peak area measurements for each component were carried out. The standard deviations are in good agreement with the requirements for a developed method (Table 4). Good linearity was obtained for all compounds. The correlation coefficients for all calibration curves were $R^2 > 0.9972$. Example chromatograms are shown in Figs. 1 and 2.

Recovery studies were performed to assess accuracy. Crude extracts were spiked with standard solution (three concentration levels) and SPE and analysis were carried out as for real samples. The experiment was repeated three times. Recoveries ranged from 92.9% (tiliroside) to 102.3% (gentisic acid), demonstrating the method's accuracy.

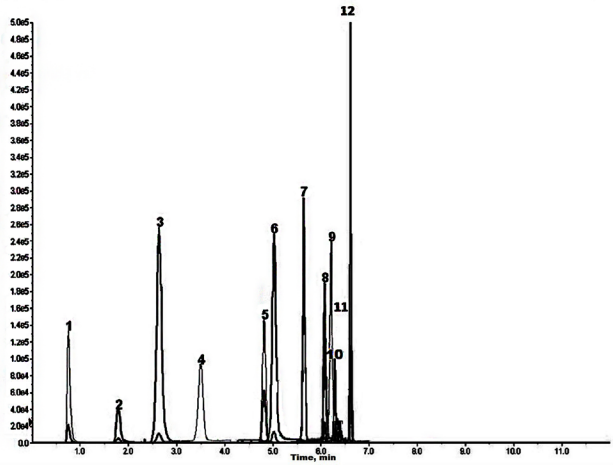

Figure 1: Exemplary LC-ESI-MS/MS chromatogram of analyzed phenolic acids. For compound numbers see Table 1. See Experimental section for details.

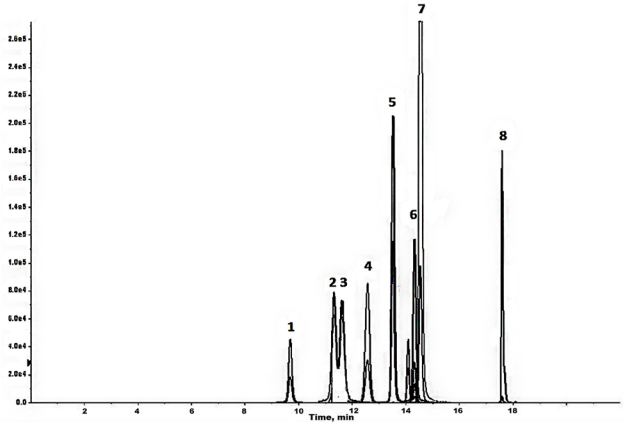

Figure 2: Exemplary LC-ESI-MS/MS chromatogram of analyzed flavonoid glycosides. For compound numbers see Table 2. See Experimental section for details.

Phenolic content increased with the addition of linden flower (Table 4). In gruels without linden addition only ten phenolic compounds were identified. These were 4-OH-benzoic, p-coumaric, salicylic, ferulic, and synapic acids as well as rutin, isoquercetin, kaempferol-3-rutinoside, astragalin, and apigenin-7-glucoside. In *Tiliae inflorescentia* extract twenty polyphenols were found. The additional ones were gallic, protocatechuic, vanillic, caffeic, syringic, gentisic, rosmarinic acids along with hyperoside, quercetrin, and tiliroside. In gruel with 5% and 10% linden addition eighteen phenolic compounds were identified, and nineteen were found in gruel with 20% *Tilia*.

Most polyphenols increased proportionally to the linden addition, but some phenolic acids (*e.g.* p-cumaric and ferulic acids) did not increase significantly with additional flower. This may be due to limited solubility of these acids in the extractant or acid content variation in the linden.

Extrusion process conditions preserve the antioxidant compounds; high-temperature extrusion-cooking does not deactivate polyphenols in raw *Tilia inflorescentia*. This agrees with Özer and coworkers [35] who found that extrusion-cooking had no effect on total phenolic content in extrudates containing chickpea, corn, oat, carrot and hazelnut.

3.2 Physical properties of enriched snacks

Radial expansion indices are in Table 5. Increased linden decreased expansion (R^2 = 0.954). The difference between reference corn and extrudates with the highest linden addition was 30.8%, which may reflect increased fiber in the products.

WAI measures granule or starch water absorption after swelling in excess water. WSI is the free polysaccharide or polysaccharide released after water addition [8,31]. The WAI decreased significantly as additive increased (R^2 = 0.9715), attributed to decreasing starch content with increasing linden and less water absorption by starch. WAI (Table 5) ranged from 5.51 g g^{-1} for corn gruels to 4.65 g g^{-1} for extrudates containing the most linden. Replacement of starchy raw materials by vegetables, fruits, or high-fiber additives reduces the starch undergoing swelling and gelatinization during processing so WAI is usually much lower with more additives and can be temperature-dependent [36]. Testing barley-tomato pomace extrudates, Altan and coworkers [37] reported that WAI decreases from 7.03 to 6.10 g g^{-1} with increasing temperature and pomace level.

WSI is often used as an indicator of starch degradation. It depends mostly on starch granule disruption, amylose and amylopectin depolymerization, starch gelatinization and the consequent starch solubilization generated in the extruder by pressure, heating, shearing and residence time [1,8,38]. WSI results (Table 5) showed decreased solubility with linden enrichment (from 15.32% for corn extrudates to 7.86% for the highest additive level). Increasing additives increases fiber content and decreases starch, reducing solubility.

Linden addition decreased L^* values (R^2 = 0.953, Table 5). Green-red balance varied from -3.94 for a sample without additives to -2.66 for 10% and 0.28 for 20% linden; the product became more red with increasing additive. The nature of the linden used may be key to the redness

Table 5: Selected properties of corn gruels enriched with linden flower.

Additive amount [%]	Expansion ratio [-]	Water absorption index WAI [g g^{-1}]	Water solubility index WSI [%]	Lightness L^*	Redness-greenness balance a^*	Yellowness-blueness balance b^*	Color change index ΔE
0	6.00 ± 0.12	5.51 ± 0.11	15.32 ± 0.15	93.42 ± 0.49	-3.94 ± 0.79	24.43 ± 2.89	ref
1	5.77 ± 0.21	5.49 ± 0.15	14.84 ± 0.09	92.62 ± 0.47	-3.99 ± 0.62	21.10 ± 2.45	3.42
3	5.15 ± 0.20	5.35 ± 0.08	14.34 ± 0.13	92.42 ± 0,56	-3.38 ± 0.54	21.96 ± 2.56	2.72
5	4.88 ± 0.10	5.21 ± 0.12	13.89 ± 0.22	92.30 ± 0.69	-3.53 ± 0.72	23.74 ± 2.22	1.38
10	2.55 ± 0.86	5.10 ± 0.07	12.09 ± 0.67	88.96 ± 2.60	-2.66 ± 0.95	26.51 ± 3.56	5.08
20	1.85 ± 0.95	4.65 ± 0.01	7.86 ± 0.11	83.64 ± 3.65	0.28 ± 1.18	30.87 ± 3.37	12.45
R^2	0.9540	0.9715	0.9605	0.9537	0.9133	0.9765	0.9664
Regression equation	$y = -0.164x^2 + 0.272x + 5.903$	$y = -0.036x^2 + 0.093x + 5.441$	$y = -0.428x^2 + 1.685x + 13.659$	$y = -0.627x^2 + 2.680x + 90.7$	$y = 0.285x^2 – 1.286x – 2.699$	$y = 0.823x^2 – 4.326x + 27.429$	$y = 1.512x^2 – 10.061x + 18.022$

Table 6: Sensory assessment and acceptance results for corn gruels enriched with linden flower.

Added linden [%]	Color[a]	Flavor[a]	Taste[a]	Consistency[a]	Overall quality[a]	Acceptability[b]
0	4.05 ± 0.89	3.60 ± 1.43	3.70 ± 1.34	4.10 ± 1.21	3.86	7.85 ± 2.37
1	4.20 ± 0.89	3.50 ± 1.24	3.15 ± 1.27	4.20 ± 0.95	3.76	7.15 ± 2.28
3	3.40 ± 1.10	2.70 ± 1.08	2.65 ± 0.88	3.75 ± 0.91	3.12	6.17 ± 1.84
5	2.95 ± 0.89	2.20 ± 0.83	2.50 ± 0.95	3.40 ± 0.94	2.76	6.15 ± 1.63
10	2.65 ± 1.55	1.85 ± 1.34	2.10 ± 1.35	3.10 ± 1.50	2.45	5.45 ± 2.55
20	1.85 ± 2.30	1.50 ± 1.96	1.85 ± 2.25	2.85 ± 1.85	2.01	4.85 ± 1.72
R^2	0.9607	0.9722	0.9896	0.9578	0.9810	0.9716
Regression equation	$y = -0.049x^2 – 0.116x + 4.335$	$y = 0.009x^2 – 0.524x + 4.245$	$y = 0.033x^2 – 0.596x + 4.230$	$y = -0.020x^2 – 0.139x + 4.365$	$y = -0.006x^2 – 0.339x + 4.284$	$y = 0.028x^2 – 0.777x + 8.552$

[a] sensory profile in 5-point scale, [b] acceptability in 9-point hedonic scale

and yellowness improvements with increasing additive (R^2 = 0.9133 and 0.9765). b* increased from 24.43 for control gruels to 30.87 for extrudates with 20% linden. Yellowness was observed in all samples because carotenoids are present in both corn grits and linden.

Color, flavor, taste and consistency characteristics are presented in Table 6. The best colors were for corn snacks and gruels with 1% additive; increasing linden lowered the color results (R^2 = 0.9607). The panelists noted that linden flower addition darkened and gave a red tint to gruels, confirmed by instrumental measurements (Table 5). Flavor and taste notes also decreased with increased linden content (R^2 = 0.9722 and 0.9896), because of the distinctive linden odor and taste. Good sensory properties were found with linden addition limited to 5%, with overall quality notes 3.86–3.12. More additive significantly lowered the sensory notes to 2.45 and 2.01 for extrudates with 10 and 20% linden. These high proportions also lowered gruel acceptability (R^2 = 0.9716) (Table 6) due to the darker color, intense flavor and distinctive linden taste. Acceptability results were below 6.0 for those with 10% and 20% linden .

4 Conclusions

High-temperature extrusion-cooking did not decrease the polyphenolics present in both raw materials. Linden herb enriched instant gruels demonstrate the incorporation of nutritionally functional components into food products by extrusion-cooking. 10 and 20% linden incorporation lowered extrudate expansion, reduced WAI and WSI values and considerably lowered instant gruel acceptance due to intense herbal taste and flavor. Linden in instant gruels should be below 10% to improve nutritional characteristics and retain acceptable sensory properties.

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