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Enhancing composition and functionality of jelly candies through apple and beetroot pomace flour addition

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The functionalization of food products with agri-industial residues is of great interest. Apple and beetroot pomace flour, abundant in dietary fiber and antioxidants, were incorporated into jelly candies using agar, pectin, or gelatin. Three functional formulations were devised for each flour type at the pilot scale, resulting in jelly candies with desirable sensory properties and texture. The high content of total polyphenolics, flavonoids, betacyanins, and betaxantines was determined upon in vitro digestion. The influence of different matrices on these bioactives, crucial for exerting antioxidant activity, was evaluated using DPPH and FRAP assays on both fresh and nine-month stored jelly candies, showcasing good bioavailability and retention. Enrichment with APF and BPF also led to reduced postprandial glucose levels, glycemic index, and load determined in vivo. These findings affirm that compositionally optimized innovative formulations of jelly candies facilitate the efficient delivery of compounds with anti-obesity effect from upcycled raw materials.

Recommended dietary fiber (DF) intakes of 25–38 g/day[1], required for the prevention of non-communicable diseases (NCD) is not easily reached in modern diet based on processed food. The link between diabetes and the obesity epidemic and the decrease in DF and dietary phenolics (DP) intake over the past decades was noticed[2]. Lower risk of hyperglycemia and improved glucose homeostasis through multiple mechanisms in the liver, muscle, adipocytes, and pancreatic β-cells are associated with DP[3]. The transportation of DP through the gastrointestinal tract is an essential function of DF. As carriers of DP, DF is responsible for the production of metabolites such as short-chain fatty acids and an antioxidant environment in the colon[4].

The effect of agri-industrial residues rich in DF and DP on endothelial function known to be related to obesity, diabetes, and metabolic syndrome was also reported. The bioactives profile and efficacy of fruit and vegetable pomace in various pathological conditions related to endothelial dysfunction were investigated[5].

Low-energy food rich in DP and DF can be obtained by reintroducing the valuable part of fruits and vegetables that remain after juice production into the food chain. Fruit and vegetable pomace obtained by simple pressing, without thermal treatment or enzyme application, represents an extremely rich source of DF and DP, depleted of a major part of easily available sugars. A recently developed technology for the production of pomace flour from minimally processed pomace was disclosed[6]. Good stability of pomace flour during storage and at elevated temperatures was reported[7]. Supplementation with apple and beetroot pomace flour (APF and BPF) obtained using the disclosed technology decreased glycemia, improved glucose tolerance, and decreased body weight gain in mice exposed to a high-fat and sucrose diet, as well as a standard diet[8]. The introduction of APF and BPF in cereal or dairy products resulted in an increase in DF and DP content and a reduction of the carbohydrates:fiber ratio[9–11].

As gluten-free flour, APF and BPF can be used in the development of a range of bakery, confectionery, and dairy products[12]. The transformation of nutritionally empty sweets into beneficial treats could be achieved by incorporating APF and BPF into various food matrices. Natural thickening and gelling agents sweetened with sucrose represent a matrix that can facilitate the delivery of DP and DF present in APF and BPF. Production of jelly candies enriched by natural DP and DF, as an alternative to products burdened with artificial additives, which would concomitantly retain desirable sensory properties, is a challenge[13]. In addition, these products are expected to have a lower glycemic index (GI) compared to conventional products.

Within the scope of this study, innovative jelly candies with APF and BPF incorporated into various matrices based on three common gelling agents were developed as alternative to energy dense product. The objective was to develop formulations with appealing sensorial properties and texture, and increased DF and DP content, as known contributors to glycemic

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response lowering power. Immediately after production and after nine months of storage total content of polyphenolics and flavonoids (TPC and TFC), along with antioxidant (AO) activity (FRAP, DPPH) were determined in all 9 developed jellies formulations subjected to in vitro digestion in order to enable insight into changes that occur during shelf life. Betacyanines and betaxanthines content in three samples with BPF was analyzed in parallel. The reduction of the carbohydrates to fiber ratio was calculated and discussed as the potential indicator of product perspective in body weight management and glucose control. A postprandial glucose response known to be associated with decreased risk of obesity and type 2 diabetes, has been followed in the early stages of product development. Thus, the relationship between pomace flour incorporation into jellies sweetened with sucrose and a decrease of postprandial glucose was established by in vivo trial.

Table 1 | Proximate composition (g/100 g) of APF and BPF produced at the industrial scale level by the recently disclosed technological process that includes dehydration and grinding

g/100 g	APF	BPF
Fat	0.60 ± 0.05^{a}	0.62 ± 0.07^{a}
Proteins	6.6 ± 0.2^{b}	12.0 ± 0.3^{a}
Total carbohydrates	86.0 ± 0.8^{a}	75.3 ± 1.4^{b}
Sugars	44.0 ± 0.7^{b}	50.6 ± 0.8^{a}
Glucose	10.6 ± 0.2^{a}	1.88 ± 0.18^{b}
Fructose	27.8 ± 0.3^{a}	0.85 ± 0.15^{b}
Sucrose	5.6 ± 0.1^{b}	47.9 ± 0.5^{a}
Total fiber	30.1	26.7
Insoluble	21.5 ± 0.4^{a}	16.1 ± 0.5^{b}
Soluble	8.52 ± 0.31^{a}	7.13 ± 0.34^{b}
Fructans	0.10 ± 0.02^{b}	3.50 ± 0.25^{a}
Moisture	4.90 ± 0.08^{a}	5.01 ± 0.07^{a}
Energetic value (kJ)	1326	1267
Energetic value (kcal)	316	301

The values are represented as mean ± SD ($n = 3$), different superscripts within the same row indicate a significant difference of means, according to t-test ($p < 0.05$).

Results

Manufacturing of jelly candies

The proximate composition of APF and BPF produced within the scope of this study confirms that both flour possess very high content of DF (Table 1) and thus can contribute to reaching the recommended intake of 14 g DF per each 1000 calories (4189 J)[1]. In this study, six samples combining three gelling agents (agar, pectin, or gelatin) with ABF and BPF as source of DF were produced at the laboratory level and analyzed for proximate composition and content of total polyphenolics, flavonoids, betacyanins, and betaxantines, as well as texture and sensorial properties, upon production and nine months of storage. For comparative reasons three respective controls based on agar, pectin, or gelatin as the gelling agents and sucrose as sweetener without flour addition were produced and analyzed in parallel.

Proximate composition of jelly candies - relation of total carbohydrates to total fiber

The proximate composition of jelly candies is shown in Table 2. The composition of jellies with the addition of APF and BPF, including moisture, protein, fat, carbohydrate, and fiber content reflected distinct differences in comparison to control samples and also enable valuable comparison to commercially available products. Observed disparity can serve as a competitive advantage for growing consumer groups, such as vegans, individuals avoiding gluten, and/or those steering clear of artificial additives. In jellies with APF and BPF, the content of DF was higher than 3 g per 100 grams of product.

Relation of total carbohydrates to total DF was calculated to enable a more comprehensive insight into the effect of APF and BPF addition on nutritional quality enhancement. Significant reduction achieved by APF and BPF incorporation, in comparison to control jelly candies without flour, was observed. Controls without APF and BPF based on gelatine and agar, being almost depleted of DF, exhibited high values for carb:fiber ratio, reaching 379 and 134, respectively, while pectin-based jelly had 37. As seen in Fig. 1a, the significant reduction of carb:fiber ratio has been achieved in all jelly candies with APF and BPF. The ratio obtained (from 19 to 15) did not approached the recommendation for processed food (less than 10:1)[14] but indicated significant functionality improvement.

The histogram shows the absolute value of carb:fiber ratios for controls and samples with APF and BPF (Fig. 1a) while the circles represent the reduction (%) of carb:fiber associated with the incorporation of pomace

Table 2 | Proximate composition (g/100 g) of jelly candies with APF and BPF based on gelatin (G-APF and G-BPF), agar (A-APF and A-BPF) and pectin (P-APF and P-BPF) sweetened with sucrose in comparison to respective control samples without flour (G, A and P)

Parameters/ samples	G	G-APF	G-BPF	A	A-APF	A-BPF	P	P-APF	P-BPF
Fat	0.08 ± 0.02^{b}	0.19 ± 0.05^{a}	0.19 ± 0.03^{a}	0.10 ± 0.02^{b}	0.23 ± 0.04^{a}	0.23 ± 0.04^{a}	0.10 ± 0.02^{b}	0.22 ± 0.05^{a}	0.22 ± 0.05^{a}
Proteins	1.8 ± 0.2^{d}	5.4 ± 0.1^{b}	6.2 ± 0.1^{a}	0.6 ± 0.1^{f}	1.9 ± 0.2^{d}	2.7 ± 0.2^{c}	0.06 ± 0.02^{g}	1.2 ± 0.1^{e}	2.0 ± 0.2^{d}
Total carbohydrates	93.1 ± 1.4^{a}	72.6 ± 1.0^{cd}	72.0 ± 1.0^{d}	94.0 ± 1.2^{a}	77.0 ± 1.1^{a}	75.7 ± 1.1^{bc}	92.6 ± 1.2^{a}	76.1 ± 1.1^{b}	75.5 ± 1.5^{bc}
Total sugars	61.8 ± 0.8^{c}	69.6 ± 0.6^{a}	69.2 ± 0.7^{a}	61.9 ± 0.6^{c}	69.5 ± 0.7^{a}	68.3 ± 0.9^{a}	65.3 ± 0.8^{b}	69.9 ± 0.8^{a}	69.7 ± 0.9^{a}
Glucose	10.1 ± 0.2^{d}	16.0 ± 0.3^{b}	14.7 ± 0.2^{c}	10.2 ± 0.2^{d}	16.8 ± 0.2^{a}	14.9 ± 0.2^{c}	10.9 ± 0.2^{d}	16.8 ± 0.3^{a}	15.1 ± 0.4^{c}
Fructose	2.7 ± 0.2^{c}	6.6 ± 0.2^{a}	3.5 ± 0.2^{b}	2.5 ± 0.1^{c}	6.8 ± 0.3^{a}	4.0 ± 0.2^{b}	2.9 ± 0.3^{c}	6.6 ± 0.2^{a}	4.1 ± 0.3^{b}
Sucrose	49.0 ± 0.4	47.0 ± 0.2^{d}	51.0 ± 0.3^{b}	49.2 ± 0.3	45.9 ± 0.5^{e}	49.4 ± 0.5^{b}	51.5 ± 0.3^{a}	46.5 ± 0.4^{de}	50.5 ± 0.2^{c}
Total fiber	0.10 ± 0.04^{b}	3.7 ± 0.3^{a}	4.3 ± 0.6^{a}	0.50 ± 0.16^{b}	4.1 ± 0.6^{a}	4.4 ± 0.6^{a}	2.0 ± 0.4	4.3 ± 0.7^{a}	4.9 ± 0.9^{a}
Insoluble	0.10 ± 0.04^{b}	2.7 ± 0.3^{a}	2.6 ± 0.3^{a}	0.4 ± 0.1^{b}	3.0 ± 0.3^{a}	2.9 ± 0.3^{a}	0.11 ± 0.05^{b}	3.1 ± 0.5^{a}	3.2 ± 0.4^{a}
Soluble	<0.1	1.0 ± 0.3^{b}	0.9 ± 0.2^{b}	0.10 ± 0.05^{c}	1.1 ± 0.3^{b}	0.9 ± 0.2^{b}	1.9 ± 0.3^{a}	1.2 ± 0.3^{b}	1.0 ± 0.4^{b}
Fructans	<0.1	<0.1	0.8 ± 0.1^{a}	<0.1	<0.1	0.6 ± 0.2^{a}	<0.1	<0.1	0.7 ± 0.2^{a}
Moisture	4.1 ± 0.2^{h}	21.1 ± 0.2^{bc}	20.8 ± 0.1^{cd}	5.1 ± 0.2^{g}	20.2 ± 0.2^{e}	20.5 ± 0.2^{de}	7.0 ± 0.2^{f}	21.8 ± 0.2^{a}	21.4 ± 0.2^{ab}
Ash	0.9 ± 0.1^{a}	0.7 ± 0.2^{a}	0.8 ± 0.2^{a}	0.2 ± 0.1^{a}	0.7 ± 0.1^{a}	0.9 ± 0.2^{a}	0.2 ± 0.1^{a}	0.7 ± 0.1^{a}	0.9 ± 0.2^{a}

The values are represented as mean ± SD, different superscripts within the same row indicate a significant difference in means, according to Tukey's HSD test ($p < 0.05$).

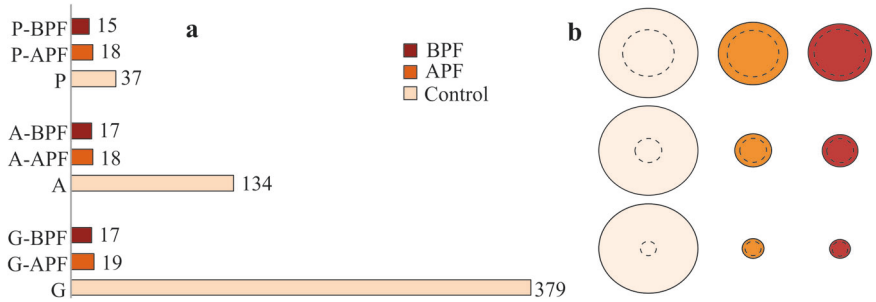

Fig. 1 | Carb: fiber ratio of jelly candies prepared with agar, pectin, and gelatin (P, A, G) without and with the addition of APF and BPF. a absolute values of carb:fiber ratio **b** relative values of carb:fiber ratio with inner circles (dashed line) corresponding to the recommended value (10:1).

Table 3 | Total phenolics (TPC) and flavonoids (TFC) content as well as AO activity determined by DPPH and FRAP test of fresh jelly candies digested in vitro according to INFOGEST procedure

Sample	TPC µg GAE/g	TFC µg QE/g	FRAP µmol TE/g	DPPH µmol TE/g
G	564 ± 29^{e}	289 ± 23^{c}	1.19 ± 0.15^{d}	1.47 ± 0.31^{d}
G-APF	1153 ± 133^{b}	1,464 ± 83^{a}	3.11 ± 0.21^{ab}	3.86 ± 0.41^{ab}
G-BPF	1476 ± 83^{a}	1,576 ± 61^{a}	3.62 ± 0.23^{a}	4.16 ± 0.47^{a}
A	255 ± 12^{f}	195 ± 10^{c}	0.70 ± 0.13^{d}	0.67 ± 0.19^{d}
A-APF	614 ± 28^{d}	1,242 ± 67^{b}	2.30 ± 0.24^{c}	2.56 ± 0.28^{c}
A-BPF	825 ± 18^{c}	1,125 ± 49^{b}	2.50 ± 0.14^{c}	2.60 ± 0.34^{c}
P	247 ± 13^{f}	228 ± 30^{c}	0.70 ± 0.18^{d}	0.80 ± 0.19^{d}
P-APF	810 ± 4^{c}	1,520 ± 65^{a}	2.72 ± 0.28^{bc}	2.99 ± 0.28^{bc}
P-BPF	869 ± 20^{c}	1,575 ± 73^{a}	3.03 ± 0.20^{b}	3.42 ± 0.38^{ab}

The values are represented as mean ± SD, different superscripts within the same column indicate a significant difference of means, according to Tukey's HSD test ($p < 0.05$).

flour (Fig. 1b). Values obtained for respective controls are taken as starting point (100%) (Fig. 1b). The recommended value of carb:fiber ratio 10:1 (inner circles) shown in Fig. 1b indicated the effectiveness of APF and BPF addition. As seen, remarkable functionality improvement was achieved in all gelling agents used. Incorporation of APF and BPF into gelatine and agar matrices reduced carb:fiber ratio ~95 and 87%, respectively, while in pectin approx. 60%.

Total content of polyphenolics, flavonoids, betacyanins and betaxanthins – bioavailability and retention survey

The content of DP and AO activity in fresh jelly candies with APF and BPF was compared with those without flour (controls) (Table 3). Control samples produced within this study, with apple juice addition but without APF or BPF, have moderate TPC, TFC and AO activity. The addition of APF and BPF caused a prominent increase in TPC, TFC and AO activity, determined upon in vitro digestion that were well maintained during storage (Table 3). Although a statistically significant decrease was observed, high values after storage confirmed good retention (Fig. 2). The values of blank (control jelly candies) have been subtracted from those obtained for the enriched samples to highlight the contribution of APF and BPF during the period of storage.

Since it was shown that the food matrix could improve the stability of betalains in the gastrointestinal tract, making them more accessible for metabolism and absorption, determination of Bx and Bc was also conducted upon digestion of either fresh or stored jelly candies. Surveying of Bc and Bx after nine months of storage of jelly candies confirmed their good retention. All values remained high while most of the Bx changes were not statistically significant (Table 4). In the case of gelatin as a gelling agent, no statistical difference was noticed in both Bc and Bx content. Their stability in the tested matrixes can facilitate BPF's promising application in various types of confectionery popular among children.

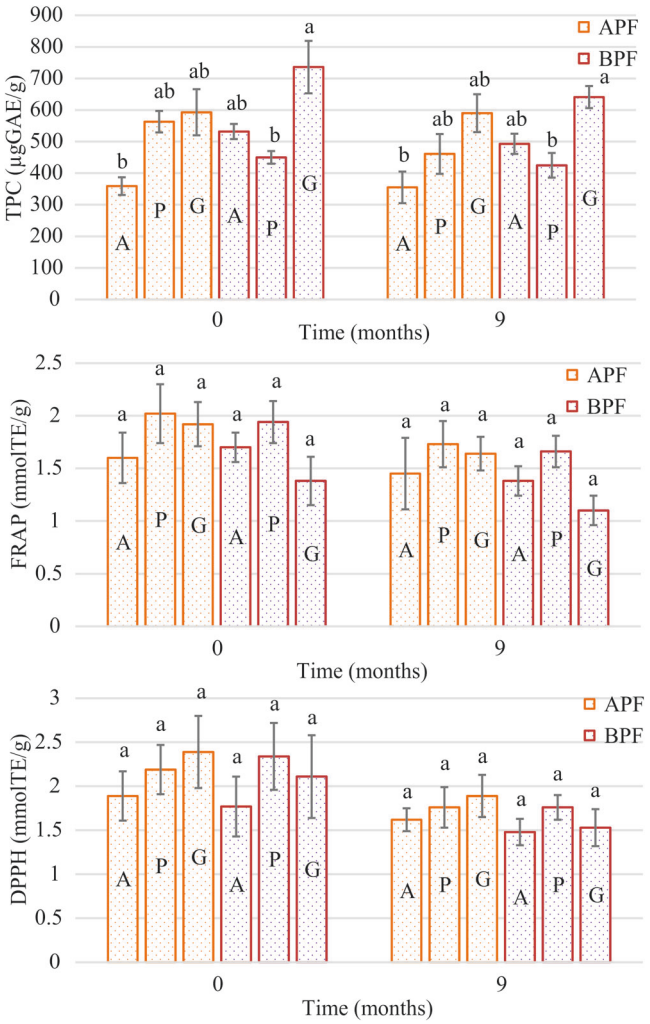

Fig. 2 | Decrease of total polyphenolics content (TPC) and AO activity (FRAP and DPPH) during nine months of storage of jelly candies based on agar (A), pectin (P), and gelatin (G), enriched with APF and BPF. Different lowercase indicates a significant difference of means, according to Tukey's HSD test ($p < 0.05$) ($n = 3$).

Sensorial analysis

Sensory analysis by the panel is employed to select the novel products with the most appealing properties. Regarding the property of appearance, all factors and their interactions (PFxGA, PFxT, GAxT, and PFxGAxT) had a statistically significant effect ($p < 0.05$) (Table 5). For texture, all factors and the interactions PFxGA and PFxGAxT had a statistically significant effect ($p < 0.05$), while the interactions PFxT and GAxT did not show a significant effect ($p > 0.05$). Regarding odor, only time had a statistically significant effect ($p < 0.05$). For taste, all factors and interactions had a significant effect

($p < 0.05$), except for the GAxT interaction ($p > 0.05$). In the case of Score, the interactions PFxT and PFxGAxT did not have a significant effect ($p > 0.05$), while other factors and interactions proved to be statistically significant -($p < 0.05$).

Fairly high grades were awarded to all samples with APF and BPF incorporated (Table 5), but because P-APF and P-BPF, besides texture, proved to be the best rated in terms of taste (from 4.88 for P-APF and 4.50 for P-BPF shortly after production, to 4.44 for P-APF and 3.84 for P-BPF after 9 months of storage) these samples were selected for further analysis. Overall, all samples were sensory acceptable and stable during storage, thus representing a novel, functional food product, with potential interest for the food industry.

Texture analysis

Texture profile analysis of gelatin, agar and pectin-based jelly confections incorporated with APF and BPF is shown in Table 6. For hardness, all factors and the interactions PFxGA and GAxT had a statistically significant effect ($p < 0.05$), while the interactions PFxT and PFxGAxT did not show a significant effect ($p > 0.05$). For springiness, factors PF and GA had a statistically significant effect ($p < 0.05$), while T and all interactions (PFxGA, PFxT, GAxT, and PFxGAxT) did not have a significant effect ($p > 0.05$). Regarding cohesiveness, all factors and their interactions had a statistically significant effect ($p < 0.05$).

The incorporation of APF and BPF as rich source of whole range of various molecules originating form pulp, skin, stone, pips and stalks into a system such as jelly candies, which should be structured and arranged, significantly contributes to heterogeneity and complexity of its texture.

Effect of APF and BPF introduction on postprandial glucose and glycemic index

Anti-obesity and anti-diabetic potential of jelly candies has been further tested and validated through glucose response assessment and lowering of GI.

In the preliminary in vivo experiment, the respondents consumed the same amount (25 g) of jelly candies based on pectin which had superior sensorial properties and thus had been selected for both postprandial glucose surveying and GI determination. Glucose concentrations in capillary blood were measured at regular intervals: 0', 15', 30', 45', 60', 75', 90', 105' and 120'. These glucose concentration values were then plotted against time. Blood glucose appeared to rise 15 min after consumption of jelly candies but the increase was much lower and steadier after enriched candies consumption. The peak blood glucose was the highest at 30 min after the intake of control samples and much more prominent than upon ingestion of P-APF and P-BPF.

The glycemic curves and areas under them (IAUC) demonstrated a significantly improved glucose tolerance of both jellies in comparison to the control. The incorporation of anti-grain flour into the jelly matrix led to a reduction in IAUC by approx. 50% in comparison to the control sample (Fig. 3). The glycemic index of the jelly candies with pectin has also been determined to confirm that low GI can be achieved as a result of ABF and BPF incorporation. Based on calculated GI, both jellies were classified as low-GI types of food, with GI significantly below 55. It was also found that

Table 4 | The total content of betacyanins (Bc) and betaxanthins (Bx) determined spectrophotometrically in fresh and nine months stored jelly candies digested in vitro according to the INFOGEST procedure

Sample/ Month	BetacyaninsBc μg/g		Betaxanthins Bx μg/g	
	0	9	0	9
G-BPF	$99.7 \pm 2.9^{a,A}$	$99.5 \pm 3.6^{a,A}$	$76.7 \pm 3.2^{a,A}$	$72.8 \pm 3.0^{a,A}$
A-BPF	$101.9 \pm 4.7^{a,A}$	$91.3 \pm 3.1^{b,B}$	$61.3 \pm 2.5^{b,A}$	$55.6 \pm 2.1^{b,A}$
P-BPF	$102.7 \pm 3.5^{a,A}$	$89.2 \pm 2.9^{b,B}$	$69.1 \pm 2.7^{ab,A}$	$64.1 \pm 2.3^{b,A}$

The values are represented as mean ± SD ($n = 3$); different lowercase superscripts within the same row, and different uppercase superscripts within the same column and same parameter indicate a significant difference of means, according to Tukey's HSD test ($p < 0.05$).

Table 5 | Sensory properties of fresh and nine months stored jelly candies

Sample	Period (month)	Sensory properties				Score
		Appearance	Texture	Odor	Taste	
G	0	4.25 ± 0.23^{a}	4.03 ± 0.21^{abcd}	4.75 ± 0.19^{a}	4.53 ± 0.25^{abcd}	4.39 ± 0.12^{abc}
G	9	4.19 ± 0.26^{a}	3.66 ± 0.13^{def}	4.50 ± 0.40^{a}	3.75 ± 0.27^{g}	4.02 ± 0.15^{efg}
G-APF	0	4.00 ± 0.13^{ab}	4.13 ± 0.19^{abc}	4.75 ± 0.35^{a}	4.25 ± 0.30^{cdef}	4.28 ± 0.08^{abcd}
G-APF	9	3.41 ± 0.30^{d}	3.56 ± 0.26^{f}	4.50 ± 0.40^{a}	4.00 ± 0.19^{efg}	3.87 ± 0.12^{gh}
G-BPF	0	4.13 ± 0.27^{a}	3.97 ± 0.16^{abcde}	4.75 ± 0.23^{a}	4.28 ± 0.36^{bcdef}	4.28 ± 0.15^{abcd}
G-BPF	9	3.44 ± 0.32^{d}	3.75 ± 0.33^{cdef}	4.50 ± 0.23^{a}	3.22 ± 0.25^{h}	3.73 ± 0.10^{h}
P	0	4.16 ± 0.23^{a}	4.22 ± 0.25^{a}	4.75 ± 0.00^{a}	4.75 ± 0.19^{ab}	4.47 ± 0.11^{a}
P	9	3.94 ± 0.22^{abc}	4.03 ± 0.16^{abcd}	4.50 ± 0.13^{a}	4.31 ± 0.37^{bcdef}	4.20 ± 0.12^{cde}
P-APF	0	4.03 ± 0.16^{ab}	4.09 ± 0.23^{abc}	4.75 ± 0.19^{a}	4.88 ± 0.19^{a}	4.44 ± 0.09^{ab}
P-APF	9	3.53 ± 0.21^{cd}	4.13 ± 0.19^{abc}	4.50 ± 0.23^{a}	4.44 ± 0.26^{abcde}	4.15 ± 0.11^{def}
P-BPF	0	3.63 ± 0.19^{bcd}	4.03 ± 0.21^{abcd}	4.75 ± 0.23^{a}	4.50 ± 0.19^{abcd}	4.23 ± 0.07^{bcde}
P-BPF	9	3.38 ± 0.19^{d}	3.66 ± 0.33^{def}	4.50 ± 0.00^{a}	3.84 ± 0.23^{fg}	3.84 ± 0.15^{gh}
A	0	4.13 ± 0.19^{a}	4.19 ± 0.18^{ab}	4.75 ± 0.33^{a}	4.38 ± 0.30^{bcde}	4.36 ± 0.13^{abcd}
A	9	3.50 ± 0.23^{d}	4.00 ± 0.19^{abcde}	4.50 ± 0.13^{a}	3.88 ± 0.13^{fg}	3.97 ± 0.09^{fg}
A-APF	0	4.13 ± 0.23^{a}	4.09 ± 0.13^{abc}	4.75 ± 0.35^{a}	4.63 ± 0.33^{abc}	4.40 ± 0.17^{abc}
A-APF	9	3.34 ± 0.27^{d}	3.81 ± 0.22^{bcdef}	4.50 ± 0.23^{a}	3.97 ± 0.31^{efg}	3.91 ± 0.15^{gh}
A-BPF	0	3.69 ± 0.26^{bcd}	4.00 ± 0.13^{abcde}	4.75 ± 0.27^{a}	4.50 ± 0.19^{abcd}	4.23 ± 0.09^{bcde}
A-BPF	9	3.31 ± 0.22^{d}	3.63 ± 0.23^{ef}	4.50 ± 0.33^{a}	4.06 ± 0.32^{defg}	3.88 ± 0.09^{gh}

Values are presented as mean ± SD ($n = 8$). Data were subjected to three-way ANOVA (between-subjects factors: Pomace Flour (PF) - three levels: control, APF, BPF; Gelling Agent (GA) - three levels: G, P, A; within-subject factor: Time (T) - two levels: 0 and 9 months); different lowercase letters within the same column indicate a significant difference of means, according to Tukey's HSD test ($p < 0.05$).

they belong to a low glycemic load (GL) food. Such data allowed for a much higher intake allowance of these jellies than commercial ones.

Discussion

The findings from this study offer insights into potential value-added applications for fruit and vegetable pomace, positioning them as a rich source of fiber and bioavailable molecules with significant health benefits. To our knowledge, this study is the first to use whole pomace flour, particularly APF and BPF, in jelly candy development. Previously, a dietetic jelly dessert containing biomolecules present in apple pomace was theoretically designed to be suitable for people dealing with diabetes and obesity[15] based on using pectin as a gelling agent, along with phloridzin, quercetin, fructose, L-arabinose, a colorant, citric acid and flavor compounds such as ethyl acetate and ethyl butyrate extracted from apples[15]. These ingredients were also used to create a functional gelled dessert at a laboratory scale level[16].The beetroot pulp was combined with strawberry, guava, etc to design functional jelly candies[17] while beetroot pomace extract was involved the development of candies with ginger (*Zingiber officinale*)[18]. Thus, the development of new formulations with APF and BPF introduces innovation in jelly candy production, particularly by utilizing minimally processed pomace as a raw material within entirely natural matrices, free from artificial additives.

Previous pomace preservation by removing water using dehydration and subsequent stabilization and grinding is a prerequisite for the production of thermally stable pomace flour with an extended shelf life[6]. The direct use of pomace flour in jelly candy production facilitates scaling up and maximizes the retention of bioactive compounds in the whole pomace. Consequently, the innovative functional products developed are free of synthetic aromas and colors and offer enhanced DF and bioactive molecules content, providing greater bioavailability and nutritional benefits. Unlike pomace extraction processes, which often result in the partial loss of bioactive compounds, the direct incorporation of pomace flour ensures that these nutrients are fully retained.

Previous studies have focused on enriching jelly candies with antioxidants and health-related biomolecules extracted from various natural sources, such as fruits, medicinal plants, and propolis[19,20]. However, these processes were mostly limited to lab-scale experiments, with minimal attempts to scale up production. The content of DF incorporated in developed jelly candies within the scope of this study exceeded the values reported in the literature, meeting the criteria for labeling them as a "source of DF" (3 g per 100 g of final product). The possibility of obtaining fruit jellies enriched with DF from apple, bamboo, psyllium, and wheat, in the concentration required to declare jellies as a "source of DF" was shown. However, the viscoelastic and mechanical properties, color, and syneresis followed during a month of cold storage, indicated that only jelly enriched with psyllium was without syneresis[21] while surveying of changes of bioactive molecules was not considered during the storage.

Simple metrics, such as the recommended carbohydrate-to-fiber ratio (10:1), can highlight the potential of pomace flour to enrich foods with DF, resulting in products with better nutritional quality[14]. In addition, this ratio could guide consumers and legislators and support producers to create healthier food through the incorporation of ingredients like pomace flour. The proximate composition (Table 2) showed the possibility of obtaining fruit jellies enriched with DF at the concentration that warrants being labeled as a "source of fiber"[21] and almost reaching carb:fiber ratio 10:1 recommended for processed food[22].

Total carb to fiber ratio enables identification of carb-rich products and surveying of the development of healthier carb-rich foods with better nutritional quality. According to our knowledge it has not been used until now to follow the transformation of energy burden confectionary products and their functionality improvement.

In commercially available jelly candies in the Serbian market, the ratios varied significantly, ranging from 42 to 470 and 912 for the candies based on pectin, agar, and gelatin. It should be noted that confectionery jellies, which are widely consumed by consumers with low to average income, often contain high levels of sugar (above 80%), as well as artificial flavors and additives, while content of DF is negligible and thus not declared at all. Their energy value is rarely below 1400–1500 kJ/100 g. The energy value of jelly candies enriched with APF and BPF was up to 20% lower than commercially available products. Apart from higher DF content samples with APF and BPF have lower content of dry matter and consequently lower carbohydrate content. Both APF and BPF have high water binding capacity and thus their addition increases the moisture of products that contain them.

Table 6 | Textural properties of fresh and nine months stored jelly candies

Sample	Period (month)	Textural properties		
		Hardness (g)	Springiness	Cohesiveness
G	0	503 ± 50^{e}	1.30 ± 0.10^{ab}	0.95 ± 0.01^{a}
G	9	529 ± 45^{e}	1.17 ± 0.10^{abcd}	0.97 ± 0.01^{a}
G-APF	0	520 ± 51^{e}	1.37 ± 0.35^{a}	0.93 ± 0.01^{a}
G-APF	9	511 ± 33^{e}	1.35 ± 0.40^{a}	0.91 ± 0.01^{a}
G-BPF	0	514 ± 16^{e}	1.20 ± 0.16^{abc}	0.92 ± 0.01^{a}
G-BPF	9	502 ± 48^{e}	1.08 ± 0.09^{abcde}	0.90 ± 0.02^{a}
P	0	1660 ± 80^{a}	0.86 ± 0.04^{bcde}	0.64 ± 0.03^{b}
P	9	1485 ± 276^{ab}	0.84 ± 0.03^{bcde}	0.58 ± 0.06^{bcd}
P-APF	0	1767 ± 224^{a}	0.78 ± 0.04^{cde}	0.61 ± 0.02^{bc}
P-APF	9	1625 ± 246^{a}	0.76 ± 0.08^{cde}	0.51 ± 0.09^{cde}
P-BPF	0	632 ± 81^{de}	0.68 ± 0.03^{e}	0.49 ± 0.06^{cde}
P-BPF	9	424 ± 60^{e}	0.69 ± 0.05^{e}	0.34 ± 0.03^{f}
A	0	1486 ± 141^{ab}	0.86 ± 0.12^{bcde}	0.59 ± 0.02^{bcd}
A	9	1021 ± 73^{cd}	0.77 ± 0.08^{cde}	0.33 ± 0.04^{f}
A-APF	0	1633 ± 204^{a}	0.88 ± 0.19^{bcde}	0.48 ± 0.04^{de}
A-APF	9	1605 ± 245^{a}	0.73 ± 0.02^{de}	0.44 ± 0.04^{ef}
A-BPF	0	1110 ± 109^{bc}	0.73 ± 0.03^{de}	0.56 ± 0.04^{bcde}
A-BPF	9	834 ± 31^{cde}	0.67 ± 0.07^{e}	0.32 ± 0.06^{f}

Values are presented as mean ± SD ($n = 3$). Data were subjected to three-way ANOVA (between-subjects factors: Pomace Flour (PF) - three levels: control, APF, BPF; Gelling Agent (GA) - three levels: G, P, A; within-subject factor: Time (T) - two levels: 0 and 9 months); different lowercase letters within the same column indicate a significant difference of means, according to Tukey's HSD test ($p < 0.05$).

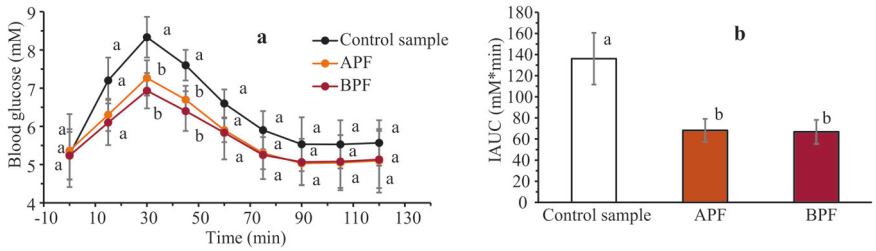

Fig. 3 | Postprandial glucose response. a Glycemic curve and **b** IAUC after consumption of 25 g of jelly candies without pomace flour (control sample) and with APF and BPF flour addition. Different lowercase letters at the same time indicate a significant difference in means, according to the t-test ($p < 0.05$) ($n = 10$).

Previous studies in both animals and humans have confirmed the health-related benefits of DF associated with DPs in different foods. An essential function of DF is the transportation of DP through the gastrointestinal tract[4]. Around 50% of DPs traverse the small intestine linked to DF. The release of DPs by the action of the bacterial microbiota contributes to the anti-obesity effect ascribed to DF. Changes in gut microbiota and the production of metabolites such as short-chain fatty acids, and the creation of an antioxidant environment in the colon represent emerging mechanism that explain the protective role of high DF content in obesity prevention and treatment. Thus, both DF and DP are approached in this study, along with Bx and Bc as additional potential contributors to food efficiency and GI reduction.

It is worth to note, that the importance of diversifying functional products to facilitate bioactive molecules delivery is supported by animal studies that show DPs' effects on obesity parameters, such as reducing body weight, fat mass, and triglycerides by enhancing energy expenditure and glucose homeostasis[23]. The link between obesity and the amount and bioavailability of consumed DPs[24] highlights importance of easy DP delivery from fruit and vegetables pomace flour. DPs' potential to reduce inflammation, decrease adipocyte viability, inhibit triglyceride accumulation, and stimulate lipolysis and fatty acid β-oxidation highlights the promising role of jelly candies enriched with APF and BPF as a delivery system.

Here, the substantial potential of APF and BPF to enrich jelly candies with DP was demonstrated by analyzing samples upon in vitro digestion. Content of TPC and TFC in fresh jelly candies with APF and BPF was much higher than in control samples produced with apple juice that have only moderate TPC, as well as AO activity. For instance, a jelly made primarily with apple juice (900 mL), sucrose (80 g), and gelling agents (20 g) also showed moderate TPC levels[25]. However, the difference in experimental approach makes comparison with previously publsihed data difficult. Commonly TPC and AO activity were determined upon extraction of jelly candies, not upon in vitro digestion. The exceptionally high TPC and AO activity was determined in extract of jelly candies with pomegranate juice (TPC of 1.59 mg GAE/g and a DPPH value of 14.8 mg TE/100 g)[19], rosemary extract (TPC increase from 190–197 to 283–411 mg GAE/100 g, AO activity determined by the ABTS from 1.4/1.5 to 3.0/5.1 mM TE)[26] and propolis[20]. Data on bioaccessibility of DP from enriched jelly candies remained limited, both in fresh samples or stored ones. Values of TPC, TPF and AO activity determined upon in vitro digestion of jelly candies with APF and BPF were found lower than in jellies enriched with propolis, rosemary extract or pomegranate juice, as expected, but upon storage of nine months both TPC and TPF showed significant retention while significant part of AO activity was maintained. The results obtained upon digestion of fresh and nine months stored jelly candies confirmed the findings of Gan et al.[27], indicating that candies can effectively serve as carriers for DPs. Obviously, jelly candies enriched with APF and BPF can transport and release polyphenolic compounds with AO activity in the intestines efficiently during the whole period of storage.

Along with TPC and TFC content of betaxanthins (Bx) and betacyanins (Bc) was surveyed. They are approved as colorants by the European Union and the Food and Drug Administration and recognized as a valuable antioxidant resource, potentially enhancing protection against free radicals. Since betalains exhibit greater stability within a pH range of 3 to 7, they are particularly suitable for low-acid foods, such as jelly candies. The stability of red beet Bx and Bc from fresh or processed food was assessed through simulated oral, gastric, and small intestinal digestion and compared with the digestive stability of purified pigments. Bx were found to be fully soluble in the aqueous (bioaccessible) fraction after ultracentrifugation of the post-intestinal digest, whereas the release of Bc from the matrix was incomplete. This indicates that digestive stability significantly influences the bioaccessibility of Bx, while additional factors related to the food matrix and processing methods impact Bc bioaccessibility[28]. In this study, content of Bx and Bc in various jelling matrix digested in simulated oral, gastric and intestinal fluid was followed during nine month storage. All jelly matrixes enabled good retention of Bx and Bc whose changes were not significant. Obviosly, betalains stability in the tested matrixes can facilitate BPF's promising application in various types of confectionery popular among children. Storability of jelly candies with beetroot pulp[17] or beetroot pomace extracts[18] remained unexplored. Also, the stability of jellies colored with betalains was not surveyed in long term manner. Betalains (0.1 or 0.3% of red beet extract) used instead of synthetic colorants in jellies based on gellan gum (0.5 or 1.5%) as the gelling co-agent, flavored with Salix aegyptiaca distillate, contributed to the antioxidant properties, redness, and chroma of the prepared candies[29]. However, the color stability during storage was not tested[29]. In another study, gummy candies were selected as a model food system to incorporate betalain-rich capsules obtained by ionic gelation with calcium alginate from a betalain-rich extract. No significant variation in total color parameters was observed during storage at 4 °C for only 30 days[30].

The whole idea is to offer consumers more health-related bioactive molecules with good retention during storage as well as significant amount of DF while maintaining the same indulgent experience. Since consumer acceptance is pivotal in food development sensorial analysis by trained panelist was included. It was found that the incorporation of APF and BPF into the jelly candies affected the texture, making it less firm and slightly grainy, though the gel structure was maintained in all samples. Enrichment with pomace flours led to a change in the color of the jelly candies, in terms of darkening. Also, a loss of a bright fracture and the appearance of a cloudy section was noticed upon flour addition. The samples with BPF were appreciated for their rich color, imparted by betalains. Having in mind that the taste has been cited as the major reason guiding food choices[31] and that for jelly candies a gel-like texture is compulsory, significant attention needs to be devoted to the overall sensory quality of developed products. The best scores for texture attributes were assigned to samples prepared with pectin, while those for samples with agar, although similar in the first period of the study, were affected by the occurrence of more spreadable consistency and sporadic syneresis after 9 months (Table 5). The use of gelatin brought the expected distinct springiness and gumminess to the samples, which were less noticeable with the addition of APF and BPF. The most pronounced evaluated parameter was taste. The panelists considered that APF and BPF enriched the flavor of the samples to which they were added, making them fuller and more natural compared to the control samples. Similar findings were reported by Tsurumi et al.[32] who showed that the flavor obtained from apple pomace was almost equal to the flavor of an apple. It should be emphasized that the taste of jelly candies with BPF was acceptable even to those evaluators who normally do not appreciate the taste of red beets, due to the absence of the "earthy" taste note typical of beets.

In general, some decline in grades was visible after 9 months of storage, which is caused by common changes during aging, such as loss of aroma, discoloration, or syneresis. Syneresis was not pronounced even after 9 months of storage, and it occurred more in samples with BPF than with APF, confirming the ability of the fibers from apple to reduce syneresis[21].

Having in mind that texture is a crucial attribute for jelly candies, as consumers expect a specific texture and are less likely to accept significant alterations, even if they enhance the product's functionality, choses parameters were surveyed using texture analyzer. In terms of hardness, it is known that agar often gives a firmer gel than pectin and, especially, gelatin, but in the present research, jelly candies with pectin showed slightly higher hardness values (Table 6). This is probably due to the increased effective amount of hydrocolloid, because the added pomace flours already contain pectin. Due to the high WHC, the increase in pectin concentration may increase the hardness of the finished products[33]. Thus, the higher hardness values for P-APF samples are somewhat expected. On the other hand, the pectin from red beet roots exhibits the properties of low methylated pectin[34] and could diminish gelling power and hardness of a system with a lot of sugar. Gelatin as a gelling agent showed a lower hardness, especially at the first test point (0 months), since it, unlike agar, and especially pectin, displays slow-setting properties[35].

In the literature, the effect of ingredients rich in DF on both decreasing[36] and increasing[37,38] of hardness values of various food has been

reported. Fruit and vegetable powders, such as grape skin powder[38] and watermelon exocarp powder[37], had the effect of hardening the jelly candy texture. Kapoor et al.[39] suggest that DF from pomaces possibly can act as filler in the gel matrix. However, a reduction in the hardness of jelly candies with the addition of APF and BPF, observed in the present study, corresponds to the moisture content (Table 2). Also, it is in agreement with tasting observations. The addition of red pitaya fruit puree to gummy candies also reduced their hardness. This can be attributed to decrease of flexible cross-links in jelly candies, leading to an increase in the heterogeneity of the structure, along with the plasticizing effect of water promoting formation of hydrogen bonds with hydrocolloids and thus softening of a texture[40].

Gelatin, as the most suitable agent in the production of gummy candies, brought pronounced springiness to the samples that contained it[41]. The same samples also had the highest values for cohesiveness, as well as the smallest influence of the addition of pomace flours on this texture attribute. Lower cohesiveness was measured in jellies with pectin, and the lowest in those with agar. This result suggests that samples with agar may be more prone to syneresis during storage, which is in compliance with scores obtained by sensory analysis, as well as with findings of other researchers[41].

In this study, the effect of the APF and BPF incorporation on postprandial glucose and glycemic index was taken as crucial. The association between the introduction of pomace flour and the strategy of obesity prevention was indicated by its high content of DF, TPC, TFC, and betalains, along with the lowered carb:fiber ratio of jelly candies. The prediction of glycemic index (GI) based on macronutrients is recommended for developing foods with lower glucose response[42]. In addition to macronutrients such as DF, DPs play a crucial role in lowering GI due to their anti-obesity and anti-diabetic properties. It is widely known that beyond their antioxidant properties DP demonstrate therapeutic functions, such as enhancing insulin sensitivity, reducing hepatic glucose output, inhibiting key carbohydrate-digestive enzymes, and modulating glucose absorption. These activities collectively contribute to improved post-prandial glycemic control[43].

Decline in postprandial glucose and reduction of glycemic index and load associated with APF and BPF incorporation into pectin matrix unequivocally confirmed pomace flour effect. GI significantly below 55 and a low glycemic load (GL) of pectin jelly candies with APF and BPF permitted for a much higher intake allowance of these jellies than commercial ones. Values of GI for commercially available jellies varied but were commonly very high. The effect of pomace flour addition on GI has been found similar to sucrose replacement reported in the literature. The glycemic index of jelly candies with sucrose partially or completely replaced with maltitol and erythritol, determined through a prospective crossover study, dropped from 81.9 to 54.1 and 49.9 respectively[27]. The mechanism behind GI reduction includes the mutual effect of fiber and bioactive polyphenolics and betalains.

Fiber swells in the stomach, increasing the viscosity of its contents and delaying sugar absorption[44]. The higher percentage of fiber corresponds with slower digestion of food and a lower increase in glucose since fiber causes glucose retardation and markedly reduces the access of glucose to the epithelium. In addition to AO properties, phenolic bioactives improve insulin sensitivity, reduce hepatic glucose output, inhibit the activity of enzymes involved in carbohydrate digestion, and modulate glucose absorption in the bloodstream. Studies also confirmed the antidiabetic effect of betalains, which reduced glycemia by 40%[45]. Developed candies can provide healthier options for individuals who strive to have better control over glucose homeostasis and obesity contrasting the general perception that jelly candies cause a high increase in blood sugar levels. These compositionally optimized products containing apple and beetroot flour contribute better control of glycemic response reaching the criteria to fit into dietary guidelines for diabetes and obesity prevention[46].

Since an increasing body of evidence suggests that a low-glycemic-index (GI) diet has a preventive potential in relation to the insulin resistance syndrome, diabetes and obesity, GI lowering power of APF and BPF should be employed in various food formulations. It is especially important to develop alternatives to energy burden products popular among children and young adults. Such approach can contribute obesity and diabetes prevention[47]. Future work will focus on product diversification, including replacing sucrose to fully satisfy these criteria.

Methods

Flour preparation

The starting material was apple and beetroot pomace (AP and BP) obtained from the fruit processing plant "Fruvita" (Smederevo, Serbia) and Zdravo Organik (Selenča, Serbia), where it was classified as a waste. Apple and beetroot pomace remained after squeezing whole, healthy, carefully selected and washed apples and beetroots. Wet pomace was dried at an industrial scale level. The dehydrator chamber's internal temperature never exceeded 55 °C. The dried AP and BP were ground to the usual particle size of standard flour (below 300 µm) allowing a very wide usage. The final APF and BPF moisture levels were 4–8%, while water activity (aw) was 0.2–0.4.

Experiment design

Jelly candies enriched with pomace flour were developed at a small scale level. A randomized design was performed with 3 gelling agents, 2 pomace flour and sucrose as a sweetener. Nine manufacturing batches were sampled (agar, pectin and gelatin as thickening agents) ×3 levels of enrichment (with APF or BPF and without flour addition). Proximate composition, content of insoluble and soluble fiber, cellulose and fructans were determined in developed samples while TPC, TFC, betalains and AO activity upon in vitro digestion in fresh and nine months stored jelly candies. Control samples are marked only with the initial letter of the gelling agent (A-agar, P-pectin, G-gelatin). Samples are marked with the starting letter of the respective gelling agents accompanied by abbreviations for pomace flour (APF and BPF): A-APF, A-BPF, P-APF, P-BPF and G-APF, G-BPF.

Production of jelly candies

The preparation of the pre-cooking mixtures differed depending on the used gelling agent: agar powder obtained from *Gelidium* algae (E 406), beef gelatin and HM pectin (E440). The appearance of the mixture after the gelling and cutting processes is shown in Fig. 4.

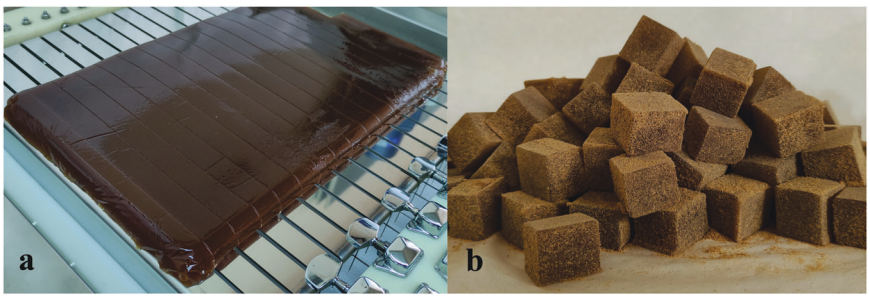

Fig. 4 | Jelly candies production. a Cutting of jelly candies with APF based on pectin matrix (P-APF); **b** Appearance of finished P-APF coated with APF instead of crystal sucrose.

Preparation of a mixture with pectin

APF or BPF were added into apple juice and well combined in a solution brought to a low boiling. Half of the intended quantity of sucrose was added, with thorough stirring. Then, a dry mixture of pectin and remaining sucrose was conjoined with other ingredients. The mixture thus obtained was subjected to further cooking.

Preparation of a mixture with agar

The agar was dissolved directly in the apple juice, APF or BPF was added and intensively stirred. When the boiling temperature was reached, sucrose was added and cooking was continued.

Preparation of a mixture with gelatin

The mixtures with gelatin were prepared in the same way as with agar, with the only difference being that after the addition of gelatin to apple juice with APF and BPF, the resulting solution was left until swollen.

Preparation of jelly candies

From the pre-cooking mixtures obtained in the described manner, jelly candies were made using the same procedure. Cooking was performed until the desired dry matter content of about 75% was reached. Then the mixture was separated from the heat source and citric acid solution (50% w/w) was added in the range of 0.57–0.65%, depending on the gelling agent and the ratio of other ingredients. Shortly stirred mass was poured into rectangular molds covered with cellophane. Upon cooling at room temperature, the jelly mass was refrigerated (4 °C) for at least 24 h to allow the gel to structure, after which it was removed from the mold and cut into equal pieces with a guitar cutter. To protect the surface without jeopardizing the composition and increasing energy value, prepared candies were rolled in APF instead of crystal sugar which represents an innovative way of finishing the treatment of candies that has not been applied or published by other researchers so far. This manner of coating represents the final part of a specific production procedure for which a patent application[48] to the Serbian intellectual property office was submitted. Subsequently, prepared jellies were packed in glass jars for further analysis and monitoring of changes during storage.

Determination of the proximate composition of APF, BPF and jelly candies

Nutritional components were determined by procedures described by the Association of Official Analytical Chemists. Total carbohydrate content was calculated as: 100 – (fat (g) + ash (g) + moisture (g) + protein (g)). Water activity (aw) of APF, BPF and jelly candies determined by the aw meter NovasinaLabSwift Bench-model Water Activity Meter (Neutec Group Inc 1 Lenox Avenue Farmingdale, NY, USA) at (25 ± 2) °C.

Determination of sucrose, D-glucose and D-fructose

Sucrose, D-glucose, and D-fructose were determined using an enzymatic assay kit from R-biopharm (R-BIOPHARM AG, Darmstadt, Germany). A homogenized sample was incubated (70 °C, 15 min), treated with carezz I ($K4[Fe(CN)_6]$) and cerezz II ($ZnSO_4$) solutions, (because of precipitation of proteins, turbidity elimination or emulsions breaking) and filtered. An aliquot was mixed with buffer pH 7.6, NADP + plus ATP solution, and hexokinase plus glucose-6-phosphate dehydrogenase suspension. D-fructose was determined after isomerisation by phosphor glucose isomerase and the NADPH produced was measured at 340 nm. Sucrose content was calculated from the difference in D-glucose concentrations before and after hydrolysis by β-fructosidase. For determination of D-glucose after hydrolysis, filtrate aliquot was mixed with β-fructosidase solution, buffer pH 7.6, NADP + plus ATP solution, and hexokinase plus glucose-6-phosphate dehydrogenase suspension. NADH produced was measured at 340 nm. Total sugars were expressed as the sum of glucose, fructose, and sucrose.

Determination of cellulose

The cellulose content was determined using the standard method SRPS ISO 6541:1997[49]. A sample was boiled with a mixture of acetic, nitric, and trichloro acetic acid (after necessary grinding and defatting). The residual raw cellulose was filtered using a filter crucible, then dried and weighed.

Determination of fructan

The content of fructan was determined according to the instruction manual Megazyme, Bray, Ireland, K-FRUCHK (AOAC 999.03-2005)[50]. Homogenized sample was dissolved in distilled water, heated in a boiling water bath for 10 minutes, and then centrifuged at 13,000 rpm for 5 min. An aliquot of obtained supernatant was incubated with sucrase/amylase solution at 30 °C for 30 min. Then, alkaline borohydride solution (10 mg/mL sodium borohydride in 50 mM sodium hydroxide) was added and incubated at 40 °C for 30 min. Afterward, 200 mM acetic acid was added to adjust the pH to approximately 4.5. The aliquots of this solution were placed into 3 glass test tubes. In two of them fructanase solution was added, and to the third (sample blank), sodium acetate buffer pH 4.5, was added. The tubes were incubated at 40 °C for 30 min to hydrolyze fructan to D-fructose and D-glucose. These sugars were determined by measuring PAHBAH color complex formed after addition of the PAHBAH (p-hydroxybenzoic acid hydrazide), reducing sugar reagent, at 410 nm.

Determination of total, soluble and insoluble fiber

Soluble and insoluble dietary fiber (SDF and IDF) were determined using the enzymatic–gravimetric method (AOAC 991.43-1994(2000))[51]. Approximately 1 g of sample was weighed in duplicate and mixed with 40 mL of MES/TRIS buffer (MES: 2(N-morpholino) ethanesulfonic acid: TRIS: tris(hydroxymethyl) aminomethane), pH 8.2). The mixture was incubated with 50 µL of heat-stable amylase in a boiling water bath for 30 min. After cooling, protease solution was added and incubated at 60 °C for 30 min. Following another cooling, the pH was adjusted to 4.1–4.8 with 0.561 M HCl, 200 µL of amyloglucosidase was added, and the solution was incubated at 60 °C for 30 min. After enzyme treatment, the solution was filtered, and the residue was washed with warm distilled water to obtain the IDF. The combined filtrate and washings were precipitated with 4 volumes of 95% ethanol to determine the SDF. The precipitate was then filtered and dried. Both SDF and IDF residues were corrected for protein, ash, and blank values. Total dietary fiber calculated as the sum of SDF and IDF does not include all fractions, as the fructan fraction dissolves during ethanol treatment. Therefore, the fructan content should be added.

In vitro digestion of jelly candies in simulated gastrointestinal fluids

The Infogest network recently published a consensus method for the evaluation of in vitro food digestion that simulates the oral, gastric and intestinal phases of human digestion[52]. The protocol was applied in triplicate on 9 samples of fresh and nine months of stored jelly candies, APF and BPF. The blank solution was prepared with all reagents but without the sample, to correct possible contamination.

Mouth simulation

Briefly, jelly candies (10 g) or flour (2 g) were placed in a 50 mL conical tube and mixed with oral fluid (2.5 mL), 0.5 mL of 1500 U/mL α-amylase in $NaHCO_3$ buffer pH 6.8, 25 mL of 0.3 M $CaCl_2$ and 7.475 mL of water. pH was adjusted to 7.0 and the mixture was incubated at 37 °C for 2 min.

Stomach simulation

The addition of 7.5 mL of gastric electrolyte solution prepared from the stock solutions of the following salts KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2$ and $(NH_4)_2CO_3$ according to Infogest procedure to 10 mL from oral phase was followed by the addition of 1.6 mL of 25,000 U/mL pepsin and 5 µL of 0.3 M $CaCl_2$. The 1 M HCl was used to adjust the pH to 3, water was added to final volume of 20 mL and the mixture incubated for 2 h at 37 °C under agitation of 200 rpm.

Small intestine simulation

For the final stage of digestion, the intestinal stage, 11.0 mL of intestinal solution made according to Infogest procedure using the stock solutions of the following salts KCl, KH_2PO_4, $NaHCO_3$, NaCl and $MgCl_2$, 5.0 mL of 800 U/mL pancreatine, 2.5 mL 160 mM bile salts in $NaHCO_3$ buffer pH 7.4 and 40.0 μL of 0.3 M $CaCl_2$ were added to 20.0 mL from gastric phase. The 1 M NaOH solution was used to adjust the pH to 7.0. After incubation for 2 h at 37 °C under agitation of 200 rpm, the tubes were kept in an ice bath for 15 min to stop intestinal enzymatic activity and centrifuged at 13,400 rpm. The supernatant (bio accessible fraction) was collected and subjected to further analysis.

Determination of bioactive compounds and antioxidant activity

Supernatants of digested jellies were subjected to determination of TPC, TFC, Bc, Bx and total AO activity using spectrophotometric assays DPPH and FRAP, immediately upon production and after nine months of storage.

Determination of total phenolic content

Folin-Ciocalteu (FC) assay was applied for the determination of total phenolic content[53]. Properly dilute samples (0.5 mL) were mixed with FC reagent diluted 11 times (2.5 mL), allowed to react for 5 min in the dark and 2 mL of 7.5% sodium carbonate solution was added, shaken and left to react for two hours in the dark. The absorbance at 760 nmwas measured. Distilled water was used as blank. Measurements were done in triplicate. Results were expressed in gallic acid equivalent (GAE) per g of sample (mg GAE/g).

Determination of total flavonoids

Total flavonoid content was determined by a colorimetric method as described previously[54]. Briefly, 150 μL of digested jelly candies with flour and 0.8 mL of the digested jelly candies without flour addition were filled up to 1.5 mL with distilled water, 75 μL of 5 g/100 g $NaNO_2$ solution was added and after 6 min 150 μL of 10 g/100 g $AlCl_3x6H_2O$ solution was added to the mixture. The mixture was allowed to stand for 5 min, 0.5 mL of 1 M NaOH and distilled water to 2.5 mL were added. The solution was mixed well and the absorbance was measured immediately against the blank at 510 nm. The results were expressed as mg of catechin equivalents per g of samples.

Determination of betacyanins and betaxanthins content

Digested samples of jelly candies with beetroot pomace (100 μL) were diluted with 5 mL of water and absorbance was measured at 538 and 480 nm. Betacyanins (Bc) and betaxanthins (Bx) content were determined as absorbance units of 1% relative to the dry matter of the digested samples at 538 and 480 nm for Bc and Bx, respectively. The betalains content (BLC) was calculated as: BLC [mg L^{-1}] = (A × Df C × MW × 1000)/(ϵ × 1), where A is the absorption value, Df the dilution factor and 1 the path length (1 cm) of the cuvette. For the quantification of Bc and Bx, the molecular weights (MW) and molar extinction coefficients (ϵ) were respectively, 550 g mol^{-1} and 60,000 L $mol^{-1}cm^{-1}$ in H_2O: λ = 538 nm for betanin, 339 g mol^{-1} and 48,000 L $mol^{-1}cm^{-1}$ in H_2O: λ = 480 nm for vulgaxanthin[55]. Results are expressed as micrograms of Bc or Bx per g of digested jelly candies (μg/g).

Determination of antiradical activity using DPPH assay

The antiradical activity of samples against DPPH radical was measured by a modified method of Blois[56]. Three different concentrations of diluted samples (0.2 mL) were mixed with 2.8 mL of a DPPH solution (0.1 mM) in a 2:1 (v/v) mixture of EtOH and 0.1 M acetate buffer pH 4.3. The mixture was shaken and left for 1 h in the dark before the absorbance measurement at 515 nm. The results are expressed as mM of Trolox equivalents per g of sample (mM TE/g).

Determination of total reducing power by FRAP

The FRAP assay was performed according to the procedure previously described[57] with some modifications introduced. The FRAP reagent solution was made by mixing 0.3 M acetate buffer (pH 3.6), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) (10 mM TPTZ solution in 40 mM HCl) and 20 mM $FeCl_3$ in volume ratio 10:1:1, respectively. Aliquots of diluted extracts (0.1 mL) were added to distilled water (0.3 mL) and FRAP reagent (3 mL) to form mixtures. After 40 min incubation in the dark absorbance at 593nmwas measured. The results were expressed as mM Trolox equivalent per g of digested sample (mM TE/g).

Sensorial analysis

To provide an insight into samples sensorial properties and select the samples for further in vivo analysis all 9 samples were subjected to a sensory analysis conducted by a panel of 8 evaluators (5 female and 3 male) who were certified according to ISO 8586:2023[58]. Testing was performed in the laboratory in two testing periods: shortly after production, and after 9 months of storage. The following sensory properties were evaluated: appearance (surface appearance, coating, size, shape, color), texture (hardness, snap, springiness, chewiness, adhesiveness), and flavor (odor and taste). The scoring method (1–5) was applied, according to the procedure described by Zlatanović et al.[10].

Texture analysis

Texture analysis of fresh and nine months stored jelly candy was examined using TA.XT Plus Texture Analyzer (Stable MicroSystems Ltd., UK) with a cell capacity of 5 kg, by texture profile analysis (TPA) method. The sample preparation included cutting into pieces in the shape of a cylinder with a diameter of 12 mm and a height of 10 mm. A cylindrical probe (P/25 mm) was used. TPA was performed with two consecutive compressions without breaking the sample. The samples deformation was 50% (distance 5 mm according to the height of the sample). The compression speed of the probe was 1 mm/s. The following texture parameters were determined: hardness (g), springiness and cohesiveness[59]. The calculation of the values of the texture parameters was performed by the program EXPONENT (Micro Stable System, Great Britain) specially designed for the used texture analyzer.

In vivo determination of glycemic index

Determination of the glycemic index was carried out in accordance with the requirements of the ISO 26642:2010[60] standard. The selection of participants was defined in the standard. The main exclusion criteria were glycemic disorders, restrictive or specific diet, food allergies or hypersensitivities. The subjects included in the study were not dependent on diabetic drugs. Also, participants were not using steroids, protease inhibitors or antipsychotics. The study included 10 non-diabetic adults aged 22–59 years old, with body mass index (BMI) within the range of 19–22 kg/m^2 and fasting glucose level ≤6 mmol/L. Informed consent was obtained from all of them. The Ethics Committee of the Institute of Public Health of RS approved the study (No of ethics approval 5646/1 issued 07.10.2022). Two tests were carried out per week. The participants fasted for at least 12 h and avoided exercise, smoking, alcohol and drug consumption. Blood was taken 5 min before consumption of samples (0 min) and 15, 30, 45, 60, 75, 90, 105 and 120 min after sample consumption.

For determination of postprandial glucose, fasting glucose tests were performed with 25 g of jelly candies based on pectin (P, P-APF and P-BPF), taken with 250 mL of water within 5 min. Glucose concentration was plotted against time. Area under the curve (IAUC) was calculated geometrically as the sum of the areas of the trapezoids over 2 h excluding the area below the initial fasting glucose concentration. The percentage of IAUC decrease was calculated and related to APF and BPF incorporation.

For the determination of glycemic index, fasting glucose tests were performed with 25 g of glucose or the amount of pectin jellies with APF and BPF (P-APF and P-BPF) containing 25 grams of carbohydrates, consumed within 5 min with 250 mL of water. Subsequent blood glucose tests were carried out at 0', 15', 30', 45', 60', 75', 90', 105' and 120' after sample consumption. The glycemic index (GI) was calculated by averaging the IAUC for each jelly, dividing it by IAUC for glucose and multiplying by 100. The glycemic load was calculated by multiplying GI with the weight in grams of

the available carbohydrates in jellies and then dividing by 100(SRPS ISO 26642:2013)[61].

Statistical analysis

Statistical analysis of the data was conducted using XLSTAT (version 2014.5.03, Addinsoft, New York, USA), analysis and statistics add-in for MS Excel. The results were expressed as means ± standard deviation (SD). Significant differences between means were determined by t-test and ANOVA with post-hoc Tukey's HSD test ($p < 0.05$).

Data availability

Data available under request to the corresponding author: SnežanaZlatanović (snezana.zlatanovic@gmail.com).

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Author contributions

S.G.: Conceptualization, Methodology, Investigation, Writing - original draft; Supervision, S.Z.: Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision, J.L.P.: Methodology, Investigation, Writing - review & editing, M.D.: Investigation, Writing - review & editing, D.M.: Formal analysis, Visualization, M.S.: Formal analysis, F.P.: Methodology, Formal analysis, Writing - review & editing. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

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