MISS MAITE GAGNETEN (Orcid ID : 0000-0002-5539-1032)

DR. GRACIELA LEIVA (Orcid ID : 0000-0002-5154-0571)

DR. CAROLINA SCHEBOR (Orcid ID : 0000-0002-2417-4772)

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Gluten-free cookies added with fiber and bioactive compounds from blackcurrant residue

Running title: Cookies enriched with blackcurrant residue

Maite Gagneten[1,2,7], Diego A. Archaina[3,7], M. Paula Salas[4,5], Graciela E. Leiva[2], Daniela M. Salvatori[6,7], Carolina Schebor[1,2,7 *]

[1]CONICET – Universidad de Buenos Aires, Instituto de Tecnología de Alimentos y Procesos Químicos (ITAPROQ), Buenos Aires, Argentina

[2]Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina

[3]Laboratorio de Investigación y Servicios de Productos Apícolas (LISPA). Facultad de Bromatología. Universidad Nacional de Entre Ríos, Entre Ríos, Argentina

[4]Tecnología Alimentaria Rioplatense, Buenos Aires, Argentina.

[5]Asociación Argentina de Tecnólogos Alimentarios, Buenos Aires, Argentina.

[6]Instituto de Investigación y Desarrollo en Ingeniería de Procesos, Biotecnología y Energías Alternativas – PROBIEN (CONICET – Universidad Nacional del Comahue) Neuquén, Argentina

[7]CONICET, Buenos Aires, Argentina

*Corresponding author: cschebor@di.fcen.uba.ar

Intendente Güiraldes 2160 - Ciudad Universitaria - C1428EGA. Buenos Aires, Argentina. Tel. (++54 +11) 5285-7400

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Abstract

A blackcurrant by-product of juice production was dehydrated and added to a gluten-free chocolate cookies formulation as a source of dietary fiber and antioxidants. The blackcurrant ingredient showed high bioactive compounds content: total phenolics (TPC)= 37.5±0.9 mg GAE/g d.b., total monomeric anthocyanins= 18.0±0.4 mg cyanidin-3-glucoside/g d.b., antioxidant activity (AA)= 22.1±0.3 mg GAE/g d.b., and total dietary fiber (TDF)= 32.3±0.4% d.b. The cookie formulation was added with 3.75% blackcurrant powder without causing severe organoleptic changes. The cookies presented 62% and 70% increase of TPC and AA, respectively. The *in vitro* digestion showed that a relevant level of AA could reach the large intestine. Also, the TDF increased 2.5 times, reaching a final content that would enable the use of the claim "source of dietary fiber" in the labeling according to the Codex Alimentarius. The obtained cookies showed a high level of acceptance among celiac and non-celiac consumers in the sensory analysis.

Key words: Gluten free, cookies, blackcurrant, dietary fiber, antioxidant activity, polyphenols.

1. Introduction

Celiac disease is an autoimmune syndrome that causes inflammation of the intestine of patients with this pathology when they ingest gluten. It leads to acute symptoms, and also to malabsorption of essential nutrients such as minerals and vitamins, among others (Gujral et al., 2012). This pathology is being increasingly diagnosed all around the world due to the wheat-based diets, as well as to a better understanding of the disease, and improved analysis methods (Gallagher et al., 2004). It affects around 1% of the world's population and the only treatment is to follow a strict gluten-free diet for life (Singh et al., 2018).

There is nowadays a great variety of gluten-free products in the market. However, the refined flours and starches used as a replacement of wheat (typically rice flour and corn or potato starch) have small quantities of fiber and thus result in food with a very poor nutritional profile in terms of this nutrient (Šarić et al., 2019). The beneficial effects of a diet rich in fibers include prevention of constipation, the control of serum cholesterol levels, the reduction of the risk of diabetes and intestinal cancer, and the stimulation of beneficial microorganisms (Perry & Ying, 2016).

Blackcurrant (*Ribes nigrum* L.) is a berry known for its particularly high concentration of bioactive compounds with antioxidant capacity (Gagneten et al., 2019). This fruit is particularly rich in phenolic compounds, especially phenolic acids, flavonoids and tannins. Among these compounds, anthocyanins account for approximately 80% of the total phenolic content (Paunović et al., 2017). The numerous biological activities that blackcurrant intake might provide to human health have been shown in different *in vitro* and *in vivo* experiments. These activities include anti-inflammatory, vaso-modulatory and immuno-modulatory responses, antimicrobial, and anticarcinogenic activities and improvement of visual function (Gopalan et al., 2012). However, unlike other berries, blackcurrant is scarcely consumed as fresh fruit due to its strong astringency, acid taste, and very high perishability. Therefore, this berry is mainly commercialized in the form of juices, jams, or frozen fruits (Diaconeasa et al., 2019; Rohm et al., 2015). The processing operations involved in the production of many of these products often leave large amounts of by-products. In particular, during juice production from berries, around 20% of the fruits are discarded in the form of pomace (Alba et al., 2018; Rohm et al., 2015). Consequently, the aim of this work was to develop gluten-free cookies with added fiber content and bioactive compounds, by incorporating a blackcurrant powder ingredient obtained from a waste of juice production.

2. Materials and methods

2.1. Materials

Individually quick-frozen blackcurrants (*Ribes nigrum*, Titania cultivar) were purchased at a local market (Desde La Tierra, Buenos Aires, Argentina). A commercial powder to prepare gluten-free chocolate cookies (Feinkö™), and the ingredients of the same commercial product were provided by Tecnología Alimentaria Rioplatense (Buenos Aires, Argentina). The label and nutritional information are shown in **Figure SM1**. Butter and eggs were purchased at a local market. The reagents were analytical grade.

2.2. Lyophilized blackcurrant ingredient

The frozen fruit was thawed in a water bath at 40–45 °C for 25 min, until it reached 10 °C. Then, it was processed with a commercial slow juicer (Peabody by Hurom PE-HSJO2, China): power=150 W, velocity=70 rpm, pressing time=1.5 min. The obtained residue, similar to that generated in the berry juice industries, was lyophilized for 48 h in an Alpha 1–4LD/2–4LD-2 equipment (Martin Christ Gefriertrocknungsanlagen GMB, Osterode, Germany) operating at -50°C and 0.04 mbar. The dry product was manually milled, seeds were separated with a 18 mesh sieve and a fine powder was obtained by milling with an electric grinder Moulinex (China).

2.3. Gluten-free chocolate cookies

The dry ingredients (corn starch, rice flour, sugar, cocoa powder, sodium bicarbonate, xantic gum, chocolate flavor, vanilla flavor, and salt) were mixed and the blackcurrant ingredient was added. For the cookies preparation, egg and butter were added (according to the commercial product´s recipe) and the dough was kneaded, rolled, cut and cooked in an electric oven at 150 °C for 9 min. The cookies containing the blackcurrant ingredient were named B-cookies. A control set of samples without the addition of the blackcurrant powder were prepared with the same procedure (C-cookies).

2.4. Blackcurrant powder and cookies characterization

2.4.1. ***Composition***

Water content: it was determined by Karl Fisher titration with a Karl Fisher TIM 980 titration manager (Radiometer Analytical, France), applying the one component technique with Hydranal Titrant Composite 5 from Riedel-de Haën (Seelze, Germany). Pure methanol was used as solvent.
Water activity: it was measured using an electronic dew point water activity meter Aqualab series 3 (Decagon Devices, Pullman, Washington, USA).
Crude protein content: it was determined following the Kjeldahl-Arnold-Gunning method (AOAC, 928.08, 2005), using the correction factor=6.25.
Fat content: it was determined following the direct method with solvent extraction (AOAC, 960.39, 2005). Cookies were dried in vacuum oven at 70 °C before fat content determination.
Ash content: it was determined following the direct method (AOAC, 923.03, 2005).
Total dietary fiber (TDF) and Insoluble dietary fiber (IDF): they were determined following AOAC methods (AOAC, 991.43 and 985.29, 2005).

2.4.2. ***Color***

Superficial color was determined using a handheld colorimeter (Minolta Co, model CR400, Japan). Color functions of the CIELAB uniform color space were calculated for illuminant C at 2° standard observer. The global color variation (ΔE^*_{00}) was calculated according to Luo et al. (2001).

2.4.3. ***Bioactive Compounds Content and Antioxidant Activity***

A spectrophotometer ultraviolet-visible Jasco V-630 (Tokyo, Japan) was used in all cases. Ethanolic extracts were prepared by adding 7.5 mL of acidified ethanol to 0.5 g of sample, shaking for 15 min using a magnetic stirrer and filtering under vacuum with a Büchner funnel. The pellet was extracted two more times with 7.5 mL of acidified ethanol. The extracts were combined, and acidified ethanol was added to constitute a total volume of 25 mL.
Total Phenolic Content (TPC): it was determined using the Folin–Ciocalteu reagent following Archaina et al. (2018) procedure. A calibration curve was constructed with gallic acid (R^2 =0.990) and the results were expressed as mg gallic acid equivalents per g of dry sample (mg GAE/g d.b.).
Antioxidant Activity (AA): it was measured using the bleaching method of 2.20-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid] radical cation ($ABTS^{+\bullet}$), according to Archaina et al. (2018). A calibration curve was done with gallic acid (R^2=0.988) and the results were expressed as mg GAE/g d.b.

Total Monomeric Anthocyanin Content (TMA): it was determined following the pH differential method according to Archaina et al. (2018).

2.5. Simulated *in vitro* digestion

The oral, gastric and intestinal phases were simulated according to the method proposed by Minekus et al. (2014). Cookie samples were incubated under constant stirring at 100 rpm with an orbital shaker Vicking M-23 (Vicking SRL, Argentina) at 37 °C in a Function Line 7000 drying stove (Heraeus, Germany).

After *in vitro* digestion, the samples were centrifuged at 13,000 g, at 4 ºC for 10 min to separate the soluble and insoluble fractions.

Antioxidant activity of the digested fractions: for the soluble fraction it was determined using the procedure previously described (item 2.4.3). For the insoluble fraction, the quencher procedure (Gökmen et al., 2009) was used. The same calibration curve was used for the soluble and insoluble fractions and the results were expressed as mg GAE/g d.b.

2.6. Sensory analysis

Three analyses were done: An Attributes' intensity level test, an Attributes' satisfaction level test, and a Global satisfaction test (Lawless & Heymann, 2010). The studies were carried out in specially designed cabins (Meilgaard et al., 2006) at the Sensory Analysis Laboratory of the Facultad de Bromatología, UNER (Argentina). 95 consumers, comprised of students, staff of the university, and members of a celiac association (ACELA) were surveyed. For the satisfaction tests a 7-point hedonic scale that ranged from "like extremely" to "dislike extremely" was used, while a 5-point scale that ranged from "very low" to "very high" was employed for the intensity level test.

2.7. Statistical analysis

It was performed using Graph Pad Prism 6 (CA, USA, 2014). An ANOVA analysis and the Tukey's test were carried out to detect differences ($p<0.05$) between samples. All physicochemical measurements were made in triplicate and the average values were informed.

3. Results and discussion

3.1. Lyophilized blackcurrant ingredient characterization

The blackcurrant powder ingredient was characterized (**Table 1**), showing low water activity and water content values, which suggests microbiological and physical stability of this ingredient. Reißner et al. (2019) reported higher fat and protein contents in a blackcurrant pomace containing stems, seeds and skins, and around the same ash content. Regarding dietary fiber content, more than 60% corresponded to the insoluble fraction (20 ± 2% d.b.). Reißner et al. (2019) also reported values of IDF of 55.1% (d.b.) for blackcurrant pomace. Alba et al. (2018) informed values of 46.9 ± 4.6 and 47.4 ± 5.4 % (d.b.) for IDF and 30.0 ± 1.5 and 25.1 ± 1.0 % (d.b.) for soluble dietary fiber in two commercial blackcurrant pomaces, which results in a percentage of around 60% of IDF respect to TDF.

TPC, TMA and AA values were almost two to three times higher than the values obtained in previous works for the fresh fruit (Archaina et al., 2018; Gagneten et al., 2019). This suggests that the residue remaining after juice extraction holds a large fraction of the fruit's bioactive compounds which are retained in the press cake. From these results, it becomes of great interest the use of this residue for the production of functional ingredients and to add value to the production of blackcurrant.

The powder color parameters represented the deep red color of the fruit, in accordance with its high anthocyanins content (Paunović et al., 2017).

3.2. Gluten-free cookies formulation and characterization

An internal sensory panel (7 persons) performed preliminary studies testing cookies prepared with different levels of blackcurrant powder addition. It was decided to use 3.75% of powder, replacing 8.3% of the rice flour and 10.4% of the corn starch. The powder concentration addition was limited by severe organoleptic changes in the cookies, mainly related to an unpleasant aroma and acidic flavor.

The water content was 9.5 ± 0.1 for C-cookies and 11.3 ± 0.2 g/100g for B-cookies, showing a significant difference. The water activity was 0.733 ± 0.004 and 0.740 ± 0.001 for C-cookies and B-cookies, respectively, with no significant differences. The higher water content but same water activity of B-cookie may be due to the fiber contribution to the water binding capacity and the presence of low molecular weight compounds given by the fruit ingredient, which could provide water interaction sites and could also decrease the water activity (Šarić et al., 2019; Reißner et al., 2019).

Regarding color, the L* and b* parameters were significantly lower in B-cookie compared to C-cookie, while a* did not show significant changes. These results indicate a darkening of the sample as well as a slight decrease in the yellowish color, as it can be clearly seen in **Figure SM2**. The total color variation (ΔE^*_{00}) was 10.3 ± 0.5. According to Keraitė et al. (2017) classification, ΔE^*_{00} values between 2 and 10 are perceptible at a glance, indicating that the two cookies showed appreciable different colors for the human eye.

Table 2 shows the proximate composition of the cookies. Ash, fat and protein contents did not show significant differences between the two samples. Regarding the fiber content, Šarić et al. (2019) enriched gluten-free cookies with raspberry concentrates (by replacing 30% of the gluten-free flour) and reached a TDF content of 6.92 ± 0.018% d.b. In this work, a lower addition of blackcurrant ingredient significantly enhanced the TDF content by 2.5 times as well as the IDF by 2.7 times. This shows an important improvement in the nutritional profile of the cookies and, moreover, it would enable the use of the claim "source of dietary fiber" in the labeling of such products according to the Codex Alimentarius (CXG 23-1997, FAO/WHO) (TDF=4.2 ± 0.7% w.b.).

B-cookies presented a TPC value of 2.5 ± 0.03 mg GAE/g d.b., which corresponded to a 62% increase in comparison to C-cookie. Filipčev et al. (2016) enriched gluten-free cookies with sugar beet molasses in a 10-40% solids proportion and reached TPC values that ranged between 9.23 and 23.55 mg GAE/g d.b. Žilić et al. (2016) studied the TPC of cookies prepared with anthocyanins-rich corn flour and informed values between 1.8 and 3.3 GAE/g for TPC. These results show that the addition of the blackcurrant ingredient can represent, not only a valuable contribution to the fiber content of the gluten-free cookies, but also provide interesting amounts of compounds with potential bioactive activities.

In order to have a better idea of the bioaccesible AA, an *in vitro* digestion was conducted (**Table 3**). As expected, B-cookie presented higher AA than C-cookie in both soluble and insoluble fractions after *in vitro* digestion. On the other hand, the soluble fraction presented a slight increase in AA in comparison to the chemical extraction, indicating a probable release of compounds with antioxidant properties during the digestion process (Karaś et al., 2017; Pastoriza et al., 2011), although a direct comparison cannot be done because of the different experimental methodologies. Regarding the insoluble fraction, Karaś et al. (2017) explained that the bioaccessibility of phenolic compounds depends on their interaction with other components present in the food matrix. In the present work the AA in the insoluble fraction was one order higher than in the chemical extraction and in the soluble fraction. Moreover, after the *in vitro* digestion, more than 50% of the initial product belonged to the insoluble fraction. This suggests that an important amount of compounds with antioxidant activity could reach the large intestine were they could exert their antioxidant function until excretion. Paturi et al. (2018) explained that when blackcurrant polyphenols are consumed together with dietary fiber, the protective effect of this latter macronutrient helps the phenolic compounds reach the large intestine where they are metabolized by the resident microbiota, showing a synergetic effect between the two components. Other studies show that the gastrointestinal system is constantly exposed to reactive oxygen species (Halliwell et al., 2005). Therefore, the activity of antioxidant compounds that help maintaining the redox balance in these organs could help prevent diseases of the gastrointestinal tract related to the generation of these reactive oxygen species during digestion processes (Leopoldini et al., 2011).

3.3. Sensory studies

The results of the Attributes' intensity test are shown in **Figure 1**. Regarding aroma and color, the majority of consumers perceived them as ideal for both formulations. However, 46% of consumers considered the color of B-cookie as higher than ideal. The chocolate flavor perception of B-cookie showed the largest deviations from the ideal point, being sensed as higher for some consumers and lower for others.

In the Attributes' satisfaction test, high satisfaction levels prevailed for the three studied attributes for both cookies (**Figure 2**). However, the addition of blackcurrant powder caused a slight decrease in the liking categories, especially for the aroma.

For the Global satisfaction test, both samples were overall accepted by the majority of the consumers. However, the addition of the blackcurrant powder caused a decrease in the percentage of responses in the liking categories from 95% to 79%.

Additionally, the surveyed population was divided in two groups conditioned to whether they followed a gluten-free diet (Group II) or not (Group I). The results of the Global satisfaction test for B-cookie between both groups showed that the liking categories were larger for Group II, reaching 85%, compared to 79% for Group I. Moreover, in this group 25% of consumers chose the "like extremely" category (**Figure SM3**). This could be related to the organoleptic properties of gluten-free bakery products, which are usually different from those of regular products, and may not be so well accepted by people who are not used to these kind of foods (Pagliarini et al., 2010).

4. Conclusions

The blackcurrant waste from juice production presented a relevant content of dietary fiber and phenolic compounds. When added to a gluten-free cookie formulation, it proved to have a very good potential to improve the product´s quality, contributing to a 62% and 70% increase of TPC and AA, respectively, and leading to a product with more than twice the dietary fiber content. Moreover, this work showed that a moderate addition of the fruit waste powder was sufficient to achieve a fiber content high enough to enable the product to carry the claim "source of dietary fiber" according to the Codex Alimentarius guidelines.

The *in vitro* digestion showed that more than 50% of the digested sample belonged to the precipitate and that this fraction presented a high antioxidant activity. These findings suggest that a relevant antioxidant activity could be delivered to the large intestine were it could play a fundamental role in the preservation of the health in the gastrointestinal tract.

The cookies enriched with the blackcurrant powder showed good organoleptic properties in the sensory test. A consumer's panel expressed a high acceptance of the product, both from people used to consume gluten-free food, and from those usually consuming conventional bakery products; with more than 79% people choosing liking categories. These results show that the obtained product could be targeted not only to people that follow gluten-free diets, but also to a wider market of consumers that may be interested in the bioactive contribution.

5. Acknowledgements, Conflict of interest and Ethical guidelines

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Table 1. Physicochemical and functional properties of blackcurrant powder.

Property		
Water activity (at 25 °C)		0.25 ± 0.01
Water content (%)		3.50 ± 0.03
pH		3.25 ± 0.01
Total acidity (mg citric acid/100 g)		6.02 ± 0.04
Total dietaryfiber (% d.b.)		32.3 ± 0.4
Proteincontent (% d.b.)		4.2 ± 0.1
Fat content (g/100 g d.b.)		1.98 ± 0.05
Ash content (g/100 g d.b.)		2.9 ± 0.1
TPC (mg GAE/g d.b.)		37.5 ± 0.9
TMA (mg cyanidin-3-glucoside /g d.b.)		18.0 ± 0.4
AA (mg GAE/g d.b.)		22.1 ± 0.3
Color coordinates	L*	29 ± 2
	a*	31 ± 3
	b*	7.5 ± 0.7

d.b. = dry basis

Table 2. Proximate composition of B-cookies and C-cookies.

Property		C-cookie	B-cookie
Dietary fiber (% d.b.)	TDF	1.9 ± 0.4 [a]	4.8± 0.7 [b]
	IDF	1.1 ± 0.1 [a]	3.0 ± 0.9 [b]
Protein content (g/100 g d.b.)		5.31± 0.08 [a]	5.36 ± 0.09 [a]
Fat content (g/100 g d.b.)		22 ± 3[a]	23 ± 2[a]
Ash content (g/100 g d.b.)		1.3 ± 0.1[a]	1.4 ± 0.1[a]

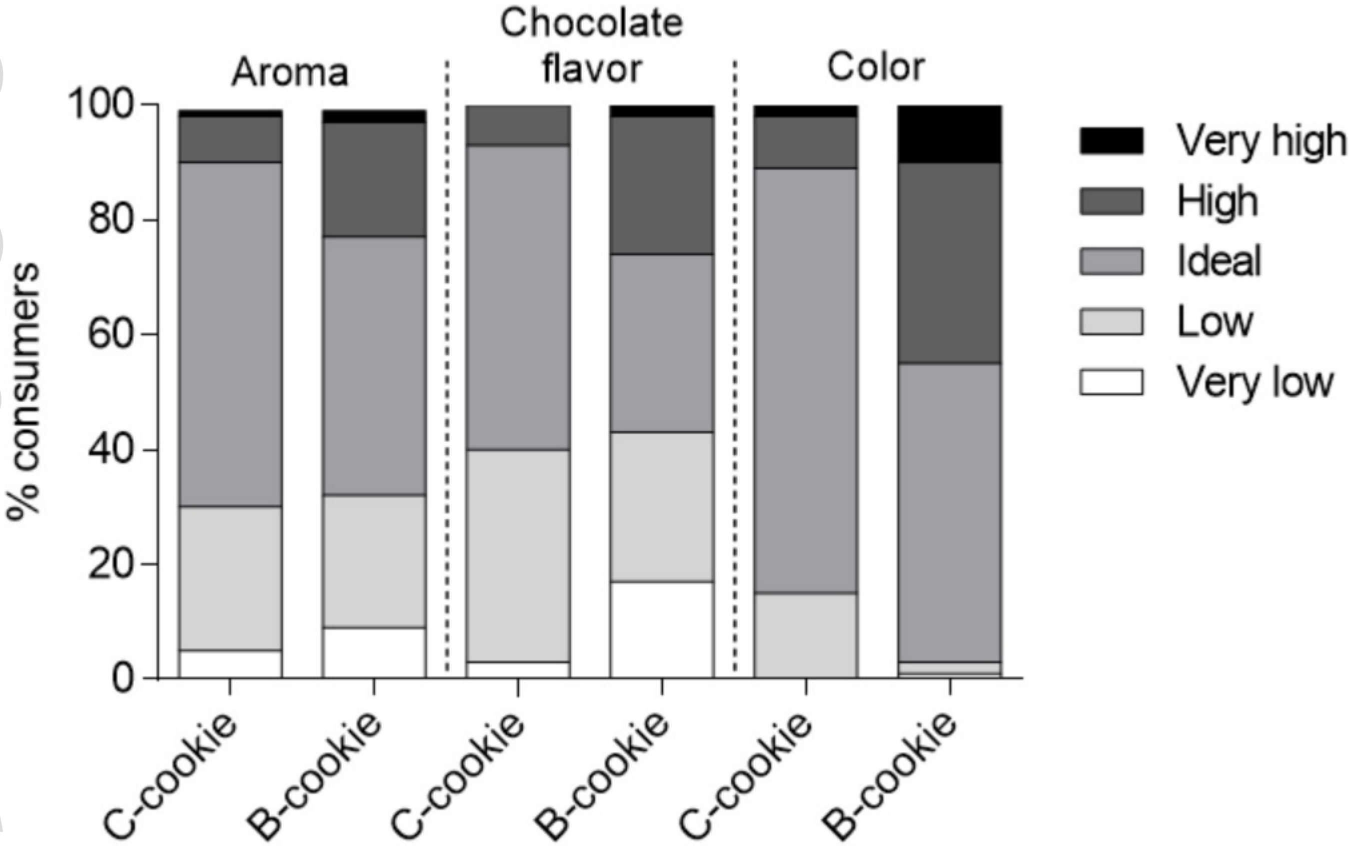
d.b. = dry basis.

Rows with different lowercase letters are significantly different ($p<0.05$).

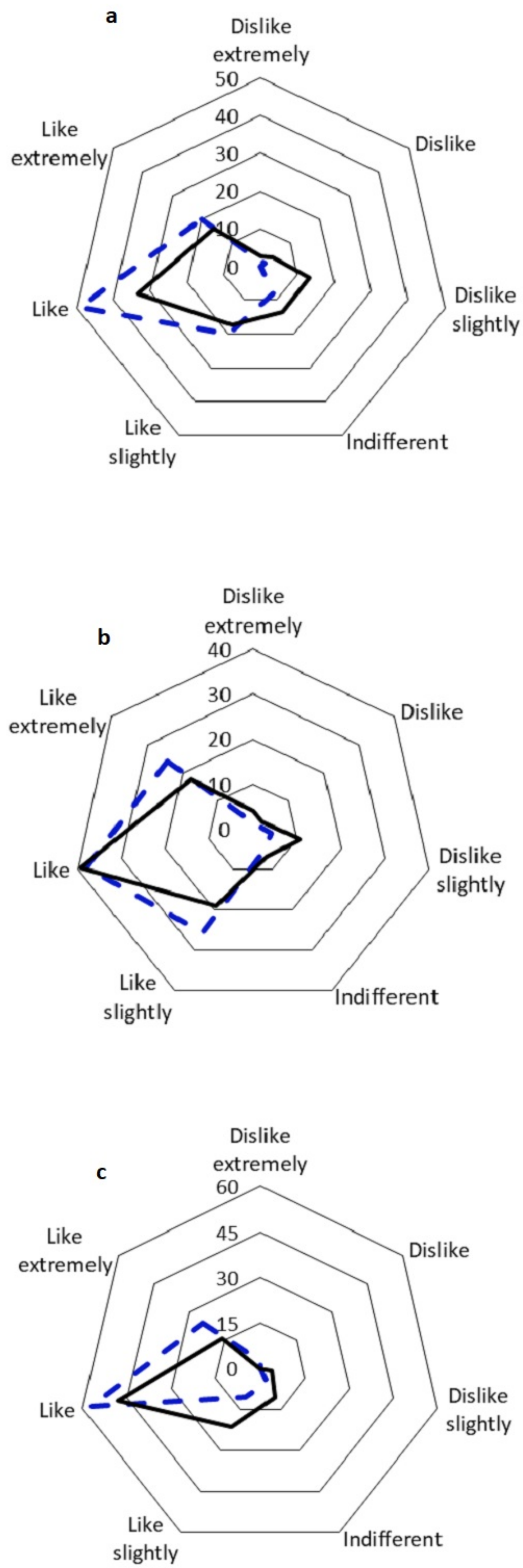
Table 3. Antioxidant activity (mg GAE/g d.b.) obtained by chemical extraction and after *in vitro* digestion (soluble and insoluble fractions).

	Chemical extraction	Soluble fraction	Insoluble fraction
C-cookie	0.71 ± 0.02[a]	0.83 ± 0.01 [b]	19 ± 1[e]
B-cookie	1.03 ± 0.01 [c]	1.27 ± 0.04 [d]	26 ± 2 [f]

Values with different lowercase letters are significantly different ($p<0.05$).



ijfs_14798_f1.jpg


a
Dislike extremely
50
40
30
20
10
0
Dislike
Dislike slightly
Indifferent
Like slightly
Like
Like extremely
b
Dislike extremely
40
30
20
10
0
Dislike
Dislike slightly
Indifferent
Like slightly
Like
Like extremely
c
Dislike extremely
60
45
30
15
0
Dislike
Dislike slightly
Indifferent
Like slightly
Like
Like extremely