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Optimization of hemp seed oil extraction and functional testing of its by-product in gluten-free baked goods

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Abstract

Hemp is a non-psychoactive variety of cannabis. Its seed is rich in high quality protein, dietary fiber, and polyunsaturated fatty acids (PUFA). Oil extraction can be performed by cold pressing, and the by-product, known as press cake, is an interesting matrix to formulate functional foods. Response surface methodology was used for the optimization of hemp seed oil extraction by means of cold pressing. Experiments were carried out according to a face-centered central composite design with two independent variables (press and roasting temperature) at three different levels (60 °C, 65 °C, 70 °C; 120 °C, 140 °C, 160 °C). Optimized conditions were determined by a press temperature (PT) of 68.6 °C and a roasting temperature (RT) of 159.5 °C, with a predicted oil yield of 40.77% and an experimental one of 45.59%. With a circular economy approach, the by-product of the extraction process was used in the development of functional gluten free cookies, a source of protein and fiber, as well as antioxidant compounds. The cookie with higher press cake substitution showed the best results with a total phenolic content (TPC) after *in vitro* digestion of 3.97 ± 0.13 mg GAE/g and an antioxidant capacity measured by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and Ferric Reducing Antioxidant Power (FRAP) methods of 103 ± 4 μmol TEQ/g and 29 ± 1 μmol TEQ/g.

Keywords: Hemp oil extraction; Circular economy; Hemp seed press cake; Functional cookies; Bioaccessible antioxidants; Functional foods.

Highlights

- Optimization of hemp seed oil extraction achieved a 45.59% yield under optimal pressing (68.6 °C) and roasting (159.5 °C) conditions
- Hemp seed press cake was effectively reused in the formulation of gluten-free cookies, enriching them with protein, fiber, and antioxidant compounds
- Cookies with higher press cake substitution showed increased polyphenol content and antioxidant capacity compared to the control

1 Introduction

Hemp (*Cannabis sativa* L.) is the industrial, non-psychoactive variant of Cannabis, with levels of tetrahydrocannabinol (THC), which are below 1% (Lachenmeier et al., 2004). According to the FAO's FAOSTAT database, the most recent available data (2021) reported a global hemp seed production of 34.074 tons, with Canada, Australia, Chile, Russia and the United States of America (USA) being the main producers (Food and Agriculture Organization of the United Nations, 2023).

Hemp seeds are composed, on a dry weight basis, of 25%–35% oil, 20%–25% protein, 20%–30% carbohydrates, 10%–15% insoluble dietary fiber, 5%.4% soluble dietary fiber, and a smaller amount of minerals and vitamins (Callaway, 2004; Irakli et al., 2019). In addition, it contains all the essential amino acids, a wide range of polyunsaturated fatty acids (PUFA), including essential fatty acids (linoleic and linolenic acids), as well as antioxidant compounds (Vodolazska & Lauridsen, 2020).

Linolenic (ω-3) and linoleic acid (ω-6) are critical for human health as they serve as precursors for longer chain PUFA. There are significant global disparities in ω-3 levels and ω-6:ω-3 ratios. Besides, suboptimal ω-3 levels and imbalanced ω-6:ω-3 ratios are prevalent worldwide (Torrissen et al., 2025). In this sense, hemp seed oil represents an interesting alternative, as it has the most complete and balanced fatty acid profile in the plant kingdom (Callaway, 2004). Particularly, it exhibits an ω-3/ω-6 ratio close to 1:3, with approximately 17.4% ω-3 and 56% ω-6, which differentiates it from most available vegetable oils (Vodolazska & Lauridsen, 2020; Simopoulos, 2002).

Considering these characteristics, cold pressing is regarded as the most suitable extraction method (Crimaldi et al., 2017; Da Porto et al., 2011). This technique consists of a mechanical process without direct heat application and without refining steps (Çakaloğlu et al., 2018). It is a healthier and less polluting technique than solvent extraction, as it does not require the use of organic solvents such as hexane (Yusuf et al., 2014). Some authors (Callaway, 2004; Rabadán et al., 2018) indicate that, in certain cases, it may be necessary to apply low-temperature heat conditions to the pressing equipment; however, this should not lead to a significant increase in oil temperature due to the short residence time in the press barrel. Additionally, the roasting of seeds before pressing constitutes a key factor to improve oil release and should be considered in the optimization of the extraction process (Babiker et al., 2020).

This extraction method is aligned with the concept of green extraction proposed by Chemat et al. (2012). Moreover, the fact that hemp seed oil preserves its antioxidant properties is not only beneficial to human health but also improves oil preservation, since, due to its high PUFA content, it is otherwise prone to rapid lipid oxidation (Faugno et al., 2019).

During the oil extraction process, hemp seed cake is generated. This by-product is rich in protein and dietary fiber and is also gluten-free, which is particularly relevant for the production of foods suitable for people with celiac disease or gluten sensitivity. Despite being defatted, the cake still contains 13.6% fat, of which 14.55% corresponds to ω-3 fatty acids (Siano et al., 2019). It also contains approximately 30.7% protein and 41% carbohydrates, as well as antioxidant compounds such as tocopherols, phenolic compounds, and phytosterols (Irakli et al., 2019). Hemp seed press cake represents a versatile ingredient with growing applications in the food industry. It has been used in the production of gluten-free breads and cookies, improving protein, dietary fiber, and bioactive compound content, while also contributing to the sensory acceptance of products (Korus et al., 2017; Mikulec et al., 2019). It has also shown potential in the formulation of extruded snacks enriched with plant proteins, where its incorporation enhances the mineral and nutritional profile (Ruszkowska et al., 2025). Furthermore, recent studies highlight its use in ice cream fortification as a partial substitute for dairy solids, providing functional and sustainable benefits (Lučan Čolić et al., 2024).

It should also be noted that hemp seeds may present allergenic potential. Recent findings identified storage proteins such as vicilins and edestins as possible allergens, with indications of cross-reactivity with hazelnuts (Beriziky et al., 2023). Although not the main focus of the present work, this aspect deserves consideration for future applications as hemp continues to gain popularity in human nutrition.

The valorization of this oil extraction by-product, by recovering and adding value to it, together with the fact that polluting organic solvents are not employed, allows the development of an integral process that takes advantage of the entire matrix without generating waste. This is in line with the concept of circular economy defined by the Ellen MacArthur Foundation (2017), understood as an economic activity that enables the elimination of waste generated by the system from its design.

In this context, the present study aims to optimize the cold pressing extraction of hemp seed oil and to explore the potential use of the resulting by-product (hemp seed press cake) as a functional ingredient in preliminary gluten-free cookie formulations.

2 Materials and methods

2.1 Hemp seed origin and pretreatment

Hemp seed "Futura 75" variety cultivated in France was provided by the hemp consulting company "Puerto Rural" (Montevideo, Uruguay).

The seeds were dried in a convection oven at 70 °C for 4 hours to avoid lipid oxidation and then stored at -18 °C until further use. These conditions were determined in preliminary assays.

2.2 Oil extraction and experimental design

An oil press (Mianyang Guangxin Import and Export Co., Ltd., Model YZYX40) was used to obtain hemp seed oil and hemp seed cake, operating at a screw speed of 35 rpm. Roasting of the seeds was carried out in a convection oven for 14 minutes at different temperatures, following the approach described by Babiker et al. (2021). A fixed roasting time of 14 minutes was adopted based on previous evidence showing that short-time convective roasting enhances oil release while minimizing thermal degradation of lipids and phenolic compounds. This strategy also ensured a controlled thermal load during cold pressing, in line with prior studies on hemp seed roasting for oil extraction (Babiker et al., 2021).

Within this constant time, a response surface methodology was established to maximize oil yield (Oy). A faced centred design, shown in Table 1, was established. Roasting temperature (120 °C to 160 °C) and press temperature (60 °C to 70 °C) were the independent variables.

A second-order polynomial model was set to predict the Oy (Equation 1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \tag{1}$$

Where Y is the Oy, X_1 and X_2 are the independent variables PT and RT, respectively, β_0 the intercept coefficient, β_1 and β_2 the linear coefficients, β_{11} and β_{22} the quadratic coefficients and β_{12} the cross-product coefficient. Additionally, the coefficient of determination (R^2) and lack of fit were determined to estimate the adequacy of the fit model obtained.

In order to calculate the Oy in each experiment, the remaining fat in press cake was determined following the ISO-6492-1999 procedure (International Organization for Standardization, 1999). Oil yield was determined using the fat percentages of whole seed ($F\%_S$) and press cake ($F\%_C$) as follows (Equation 2):

$$Oy = \left(\frac{F\%_S - F\%_C}{F\%_S} \right) \times 100 \tag{2}$$

Table 1. Effect of preheating temperature and press temperature on the oil yield.

Design point	Coded level		Uncoded Level		Response Variable
	X_1	X_2	Roasting temperature (°C)	Press temperature (°C)	Oil yield (Oy) (%)
1	1	-1	160	60	28.79 ± 2.94
2	1	0	160	65	37.82 ± 2.32
3	1	1	160	70	43.52 ± 3.89
4	0	-1	140	60	37.21 ± 4.08
5	0	0	140	65	33.32 ± 4.52
6	0	1	140	70	29.97 ± 0.67
7	-1	-1	120	60	32.2 ± 1.76
8	-1	o	120	65	38.61 ± 4.07
9	-1	1	120	70	27.52 ± 2.47
10	0	0	140	65	20.53 ± 1.73
11	0	0	140	65	35.29 ± 0.40

2.3 Proximate analyses

Proximate analyses were performed on the seed and press cake. Protein and Total Dietary Fiber were determined using the Association of Official Analytical Chemists (AOAC) methods 984.13 and 985.29, respectively (Association of Official Analytical Chemists, 2012). Fat was estimated following the ISO-6492-1999 (International Organization for Standardization, 1999) procedure. Moisture content was determined by gravimetric analysis in a convection oven at 105 °C until constant weight. Ash was determined in a muffle furnace following ISO 5984-2002 (International Organization for Standardization, 2002). Total carbohydrate content was obtained by difference.

2.4 Cookie formulation

Hemp seed press cake cookies were prepared with the cake resulting from the optimized pressing conditions. Two cookies were formulated (as shown in Table 2) which comply with the "source of protein" and "high protein" claims from the C1 Regulation (EC) No 1924/2006 of the European Parliament and of the Council (European Union, 2006). Also, both cookies comply with the "high in fiber" claim according to the same regulation. Control cookies were formulated substituting the press cake with the gluten free flour mix. Basic ingredients were obtained from the local market, and the gluten free flour mix used was Exent's "Gluten free cook mix" made in Uruguay. It consists of a mix of rice flour, corn starch, and cassava starch and contains 3,4% of protein and 0% of dietary fiber.

All ingredients were mixed and kneaded for the preparation of the dough, which was then rolled out to a height of 0.3 mm and cut into 4.7 cm diameter discs. The cookies were then baked in a convection oven at 180 °C for 15 minutes. Cookie A complied with the "source of protein" and "high in fiber" claims, whereas cookie B complied with the "high in protein" and "high in fiber" claims. Control cookie is represented by the letter C.

Note: The formulated cookies were evaluated in a non-structured preliminary test (n=6) by members of the research team. While this allowed for basic assessment of feasibility and acceptability, it does not substitute a statistically robust sensory evaluation. Further studies are required to evaluate consumer acceptance through formal sensory analysis.

Table 2. Cookie formulations.

Ingredients	Control Cookie	Cookie A	Cookie B
Hemp seed flour (press cake)	0%	23.2%	50.8%
Gluten free flour mix	59.4%	36.2%	8.6%
Egg	14.3%	14.3%	14.3%
Water	12.2%	12.2%	12.2%
Vegetable oil	12.2%	12.2%	12.2%
Baking powder	1%	1%	1%
Salt	0.9%	0.9%	0.9%
Nutrition Facts			
Protein (g per 28g portion)	1.4	3.7	6.5
Dietary Fiber (g per 28g portion)	0	3.6	7.8
Fats (g per 28g portion)	5	6.2	7.6
Carbohydrates (g per 28g portion)	18	15	10
Energetic Value (kcal per 28g portion)	118	123	129
EU Nutritional Claim for Dietary Fiber	No claim obtained	Source of dietary fiber	High in dietary fiber

A = Cookie formulated to comply with "source of protein" and "high in fiber" claims; B = Cookie formulated to comply with "high in protein" and "high in fiber" claims. Control cookie prepared with gluten-free flour mix.

2.5 Phenolic compounds extraction

Phenolic compounds were extracted from hemp seeds, press cake, and all three cookie formulations following the methodology of Pérez-Jiménez et al. (2008). Extractable polyphenols (EPC) were obtained through sequential aqueous-organic extraction using methanol/water and acetone/water solutions, and quantified directly in the resulting supernatants. The non-extractable polyphenols (NEPC), corresponding to bound phenolics, were determined from the solid residue after EPC extraction. In this study, NEPC were released by acid hydrolysis of the residue, omitting the HCl/butanol/$FeCl_3$ step described in the original protocol. Both EPC and NEPC were analyzed separately, and total polyphenol content (TPC) was expressed as the sum of EPC and NEPC.

2.6 Total polyphenol content (TPC)

Total phenolic content (TPC) of the extracts was determined by the Folin-Ciocalteu method, first proposed by Singleton & Rossi Junior (1965), modified by Georgé et al. (2005). Results were expressed in mg of Gallic acid equivalents per gram of wet sample.

The TPC was determined as the sum of EPC and NEPC.

2.7 Antioxidant capacity

The antioxidant capacity of hemp seed, press cake, and gluten-free cookies was determined using two assays: the ABTS radical cation discoloration assay (Re et al., 1999, with modifications) and the FRAP assay (Benzie & Strain, 1996, with modifications).

For the ABTS assay, the stock solution was prepared by diluting the reagent in distilled water to a concentration of 2.5 mM. Then, 2.5 mL of this solution were mixed with 44 µL of potassium persulfate and kept in the dark for 16 h to allow complete formation of the ABTS radical. A Trolox standard curve was prepared using ethanol:water (50:50, v/v) dilutions in concentrations ranging from 0 to 2.0 mM. After activation, the radical solution was diluted with ethanol:water to reach an absorbance of 0.70 ± 0.02 at 734 nm. Subsequently, 3 mL of this solution were mixed with 30 µL of either Trolox standard or sample extract and incubated for 30 min in the dark. Absorbance was measured at 734 nm using a Shimadzu 1800 Ultraviolet–Visible (UV-Vis) spectrophotometer. Results were expressed as µmol Trolox equivalents per gram of wet sample.

For the FRAP assay, the working reagent was prepared by mixing equal volumes of $FeCl_3 \cdot 6H_2O$ (20 mM), TPTZ (10 mM in 40 mM HCl), and sodium acetate buffer (0.3 M, pH 3.6, adjusted with glacial acetic acid). A Trolox standard curve was prepared with aqueous dilutions ranging from 25 µM to 750 µM. Samples or standards were mixed with distilled water and FRAP reagent in a ratio of 1:1:30 (v/v/v), then incubated for 30 min at 37 °C in a water bath. Absorbance was recorded at 595 nm using a Shimadzu 1800 UV–Visible spectrophotometer. Results were expressed as µmol Trolox equivalents per gram of wet sample.

2.8 *In vitro* digestion

To determine the bioaccessibility of phenolic compounds in the formulated cookie, an *in vitro* digestion was performed according to the methodology described by Hollebeeck et al. (2013).

The polyphenol content and antioxidant capacity of the supernatants were determined as described in 2.6 and 2.7, respectively. In each case, the bioaccessibility percentage of TPC was calculated (Equation 3), as described by Tomé-Sánchez et al. (2021).

$$Bioaccessibility\left(\%\right) = \frac{TPC\ after\ digestion}{TPC\ \ before\ digestion} X\,100 \tag{3}$$

2.9 Statistical analysis

Analyses were carried out in triplicate, and all data were reported as mean ± SD. Design Expert Software (version 11.0) was used for response surface methodology analysis. One-way analysis of variance (ANOVA) was performed on each assay using InfoStat (Di Rienzo et al., 2020), and differences between samples were determined by Tukey's test ($\alpha \leq 0.05$).

3 Results and discussion

3.1 Oil extraction optimization

Results obtained after conducting the response surface analysis are shown in Table 3. The lack of fit was non-significant ($p > 0.05$), which means that the mathematical model correctly fits the oil yield (Oy) data. In addition, the R^2 of 0.959 shows that almost 96% of the variations could be explained by the current model.

Looking at the coefficients of the linear regression, the linear term of press temperature (PT) was not significant ($p > 0.05$), while all the other coefficients were significant. Taking this into consideration, the second order equation for the model is presented below in terms of actual factors (Equation 4).

$$\%Oil\ yield\ Y = -394.95135 - 4.20596X_1 + 22.01230X_2 + 0.005035X_1^2 - 0.218111X_2^2 + 0.045227X_1X_2 \tag{4}$$

Regarding the rest of the coefficients and considering their corresponding p-values (Table 3) the most significant terms were the linear term for preheating temperature (β_1), the quadratic term for press temperature (β_{22}) and the interaction (β_{12}) ($p < 0.0001$) followed by the quadratic term for preheating temperature (β_{11}) ($p = 0.0008$).

The effects of press and roasting temperature on oil yield can be seen in Figure 1. Oil yield is higher when both roasting and press temperature increase. Roasting affects the seed's structural stability, particularly its shell, which can be taken apart easily after it, and due to this, it is a proven and commonly used method to enhance oil yield (Babiker et al., 2021). Therefore, it makes sense that a higher roasting temperature implies a higher oil yield. In this line of thought, the higher press temperature may have had a positive effect on the Oy because of its repercussions on the seed's structure. Moreover, higher temperatures imply a decrease in the oil viscosity, which is favorable to its flow and contributes to the increase in oil yield (Savoire et al., 2012).

As seen in Table 1, Oy varied from 20.53% to 43.52%, the highest oil yield from the design points was 43.52% and was obtained at the most extreme pressing conditions. This result is concordant with the one obtained by Crimaldi et al. (2017), who obtained the highest Oy of their design at the most extreme temperature conditions.

The optimal point selected from the model to use as working conditions to obtain the maximum oil extraction yield (Oy) was the one determined by a press temperature of 68.6 °C and a roasting temperature of 159.5 °C. As can be seen in Figure 1, there are several points that maximize extraction, but this was the chosen one because it was easily replicable in the conditions of the press. For these conditions, the model predicts an oil yield of 40.77%, and the experimental result was 45.59%. Further studies should be conducted regarding the oxidation of free fatty acids to determine the oil quality in the selected conditions.

Table 3. Estimated regression coefficients (β) in terms of coded factors, determination coefficient (R^2), and lack-of-fit of the predicted second-order polynomial model for the oil yield in terms of coded factors. β_0 corresponds to the intercept coefficient, β_1 and β_2 the linear coefficients for PT and RT, respectively, β_{11} and β_{22} the quadratic coefficients for PT and RT, respectively and β_{12} the cross product coefficient.

Item	Coefficients	*p*-value*
β_o	35.74	
Linear		
β_1	2.87	< 0.0001
β_2	-0.0519	0.8804
Quadratic		
β_{11}	2.01	0.0008
β_{22}	-5.45	< 0.0001
Interaction		
β_{12}	4.52	< 0.0001
	Fit statistics	
R^2	0.9590	
Lack-of-fit	2.92	0.2233

*Significance level $\alpha \leq 0.05$.

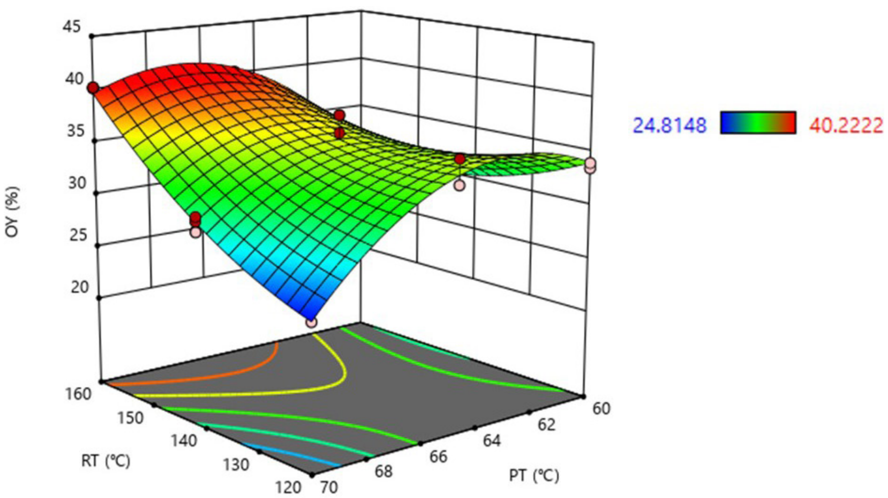

Figure 1. Response surface for oil yield. OY means oil yield. PT means press temperature. RT means roasting temperature.

3.2 Proximate composition of hemp seed and press cake

Results for the proximate composition of hemp seed and press cake are presented in Table 4. The values obtained for all the parameters analyzed for hemp seed and its press cake are consistent with the expected ranges (Siano et al., 2019). When the hemp seeds went through the pressing process, an ingredient significantly higher in fiber and protein was obtained, that is, the press cake. For this reason, the pressing process is not only of value because of the hemp seed oil extraction but because it results in a good option for the manufacturing of products, a source of fiber and protein.

Table 4. Proximate composition of hemp seed and press cake.

	Hemp seed (g/100 g)	Press cake (g/100 g)
Protein	23.97 ± 0.83^{a}	30.23 ± 0.14^{b}
Lipids	26.95 ± 0.07^{b}	14.69 ± 0.24^{a}
Ash	6.1 ± 0.2^{a}	6.98 ± 0.14^{b}
Total dietary fiber	35.76 ± 0.3^{a}	45.78 ± 1.1^{b}
Carbohydrates[1]	3.29	1.44
Moisture	3.73 ± 0.09^{b}	0.873 ± 0.003^{a}

[1]Carbohydrates content obtained by difference. Values within a row that do not share a letter differ significantly ($p < 0.05$).

3.3 Functional characterization of hemp seed and press cake

The EPC and NEPC of hemp seed and press cake, as well as antioxidant capacity, are shown in Table 5.

According to Siano et al. (2019), the TPC (TPC=EPC+NEPC) as determined by Folin-Ciocalteu method after following the extraction process established by Vonapartis et al. (2015) of the seed was 0.767 mg GAE/g and 0.744 mg GAE/g for the press cake. They also semi-quantified polyphenols by Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) and determined a total of 1.540 mg GAE/g for the seed and 1.078 mg GAE/g for the press cake. In the case of this study, TPC for the seed was 14.14 mg GAE/g and 19.2 mg GAE/g for the press cake. This difference could be explained by the difference in the extraction method. As stated by Pérez-Jiménez et al. (2008), differences in sample preparation, extraction of antioxidants and expression of results may vary between methods, leading to different results. Irakli et al. (2019) obtained for the Futura 75 hemp seed flour variety a TPC of 7.798 mg GAE/g; this value more closely resembles the one obtained in the present study for the same variety, and its difference could also be explained by the polyphenol extraction method.

Even though the TPC quantified is not comparable to the available studies, EPC can be compared, and the results obtained were in the same range. Current research does not usually take into consideration the NEPC, which is not significantly released during the salivary, gastric, or duodenal steps of digestion, nor does it get released during conventional extraction methods that are carried out with the use of organic solvents like methanol, ethanol, and acetone in aqueous solutions. To extract these polyphenols, protocols consisting of acid hydrolysis have to be applied. Whereas, in the case of human consumption, they do not get released and absorbed in the small intestine because of their bond with dietary fiber, and instead are carried out to the colon where they can be metabolized by the microbiota and some enzymes present there. This has been reported to result in positive health outcomes (Pérez-Jiménez et al., 2013).

Apart from that, EPC in the press cake was higher ($p < 0.05$) than in the hemp seed; this could be explained by the effect that the pressing process has on the structure of the food matrix, making the polyphenols initially present easier to extract when it comes to the press cake.

Furthermore, antioxidant capacity was significantly higher for the press cake in every case except for the non-extractable fraction as measured by the ABTS assay. The first result stated is concordant with the higher TPC. The exception could be explained firstly by the difference that exists between the two assays used to determine antioxidant capacity. They are both single electron transfer (SET) reactions and are usually used to determine hydrophilic compounds' antioxidant capacity, but ABTS can also detect some lipophilic antioxidants. Since the seed has a higher lipid content, this could explain why the ABTS assay detects a higher antioxidant capacity for the seed, but the FRAP assay does not (Pérez-Jiménez et al., 2008). Secondly, the fact that this was only observed for the non-extractable fraction could be explained because usually lipophilic antioxidants remain mostly bound to the fiber that remains in this fraction.

Table 5. Extractable and non-extractable polyphenol content and antioxidant capacity of hemp seed and press cake.

	EPC (mg GAE/g)	TEAC (mmol TEQ/g) *ABTS assay*	TEAC (mmol TEQ/g) *FRAP assay*
Hemp seed	1.24 ± 0.05[a]	0.0126 ± 0.0004[a]	0.0105 ± 0.0004[a]
Press cake	6.8 ± 0.2[b]	0.0130 ± 0.0004[b]	0.051 ± 0.001[b]
	NEPC (mg GAE/g)	**TEAC (mmol TEQ/g) *ABTS assay***	**TEAC (mmol TEQ/g) *FRAP assay***
Hemp seed	12.9 ± 0.4[a]	0.0288 ± 0.0005[a]	0.049 ± 0.001[b]
Press cake	12.4 ± 0.4[a]	0.017 ± 0.001[b]	0.064 ± 0.002[a]

Values within a column that do not share a letter differ significantly ($p < 0.05$). EPC: extractable polyphenolic content; NEPC: non-extractable polyphenolic content; TEAC: Total equivalent antioxidant capacity.

3.4 Characterization of hemp seed cookies

3.4.1 Nutritional value of functional cookies

As seen in Table 2, cookie formulations were developed to reach different nutritional claims according to EU guidelines (Regulation (EU) No 1047/2012). This approach has already been used in previous research (Presa-Lombardi et al., 2023). Its particularly important to reach nutritional claims in ready-to-eat products such as cookies, since these claims have been shown to influence the purchase intent for all types of consumers (Pinto et al., 2017)

Similar strategies have been reported with other cereal by-products. For instance, whole grain rice flours from traditional landraces (Kalanamak and Chak-hao) have been successfully incorporated into gluten-free cookies, improving their content of bioactive compounds and antioxidant capacity while maintaining desirable sensory attributes (Itagi et al., 2023). Likewise, the use of fermented rice bran enriched the nutritional profile of gluten-free cookies with higher protein, fiber, and phenolic content, besides extending shelf life and consumer acceptance (Christ-Ribeiro et al., 2021). These findings support the present study, showing that hemp press cake, like other agro-industrial by-products, can be effectively valorized as a functional ingredient in gluten-free baked goods.

Other studies have evaluated the use of hemp seeds in pasta (Teterycz et al., 2021 bread (Mikulec et al., 2019), and its influence on nutritional value. According to Mikulec et al. (2019) and Presa-Lombardi et al. (2023), the incorporation of hemp flour into ready-to-eat products shows clear limitations in terms of formulation. Both studies identified a threshold of approximately 30% in bread and 50% in snack bars, beyond which product quality begins to be negatively affected. When these levels are exceeded, undesirable changes occur in key parameters such as color, rheological behavior, and sensory attributes. These alterations not only compromise the technological performance of the dough or mixture but also reduce consumer acceptance significantly, highlighting the importance of balancing nutritional enrichment with the maintenance of desirable organoleptic properties (Mikulec et al., 2019; Presa-Lombardi et al., 2023). As one of the main limitations of this study is the lack of a sensory analysis, this threshold may be of particular importance.

3.4.2 Functional characterization of hemp seed cookies

EPC and NEPC of hemp fortified cookies, as well as antioxidant capacity, are shown in Table 6.

Results in the case of control cookies were the lowest ($p < 0.05$), which suggests that the addition of hemp seed press cake to the cookies may have a positive effect in the polyphenol content and antioxidant capacity.

Results obtained for antioxidant capacity of the extractable and non-extractable polyphenols were significantly different ($p < 0.05$) for all cookies except in the case of the FRAP assay for the extractable fraction of cookie A and cookie B. This result could be explained by the difference, which was previously mentioned, between the ABTS and the FRAP assay: the former is able to detect some lipophilic antioxidants that the latter is not. This could explain the reason why only this method resulted in a significant difference in TEAC (Total equivalent antioxidant capacity) of the B cookie in comparison to the A cookie, as cookie B has the highest lipid content of the three cookies studied (Pérez-Jiménez et al., 2008).

For the polyphenol content, the B cookie presented the highest content of EPC and NEPC, and it has a TPC of 14.3 mgGAE/g. The results indicate that the addition of a higher amount of press cake leads to an increase in the amount of TPC in the cookie. The B cookie also has the highest antioxidant capacity for the ABTS assay and the NEPC of the FRAP assay. These results are consistent with the ones reported by Ertaş & Aslan (2020).

Table 6. Extractable content (EPC) and non-extractable polyphenol content (NEPC), and antioxidant capacity of hemp seed and control cookies.

	EPC (mg GAE/g)	TEAC (mmol TEQ/g) *ABTS assay*	TEAC (mmol TEQ/g) *FRAP assay*
Control Cookie	0.68 ± 0.01[a]	0.0049 ± 0.0002[a]	0.0067 ±0.0004[a]
Cookie A	2.03 ± 0.07[b]	0.016 ± 0.001[b]	0.019 ± 0.004[b]
Cookie B	2.5 ± 0.1[c]	0.026 ± 0.001[c]	0.022 ± 0.001[b]
	NEPC (mg GAE/g)	**TEAC (mmol TEQ/g) *ABTS assay***	**TEAC (mmol TEQ/g) *FRAP assay***
Control Cookie	3.06 ± 0.04[a]	0.0069 ± 0.0003[a]	0.0085 ± 0.0004[a]
Cookie A	5.4 ± 0.1[b]	0.015 ± 0.001[b]	0.015 ± 0.001[b]
Cookie B	11.8 ± 0.4[c]	0.0349 ± 0.0005[c]	0.061 ± 0.002[c]

Values within a column that do not share a letter differ significantly ($p < 0.05$). Control cookie had 0% of hemp press cake. Cookie A had 23.2% of hemp press cake. Cookie B had 50.8% of hemp press cake.

3.5 Bioaccessibility of bioactive compounds of cookies

The content of antioxidant compounds in the cookies does not guarantee that they will be able to be absorbed and possibly result in health benefits. The *in vitro* digestion method used in this study allows for simulating a gastrointestinal human digestion and estimating the bioaccessibility of bioactive compounds, that is to say the amount of antioxidants that could be potentially absorbed by the intestine. Results for TPC and polyphenol bioaccessibility are shown in Table 7.

The B cookie was the one with higher TPC after the digestion, it has twice the TPC content of the C cookie. Therefore, cookie B is the one with the highest amount of bioaccessible polyphenols (Table 7).

However, cookie B was the one with the lowest bioaccessibility percentage. This could be explained by its high content of NEPC compared to cookies A and C (Table 6). Cookie B was formulated with the highest substitution of hemp seed press cake and therefore has the highest fiber content. Previous studies have proven that dietary fiber forms structures with polyphenols that are difficult to break during digestion, causing most of the polyphenols to remain in the pellet. This means that most of the polyphenols initially present in the cookie will not be available for absorption after digestion (Gutiérrez Barrutia et al., 2019; Quirós-Sauceda et al., 2014).

The polyphenol content that is not bioaccessible after going through the duodenum corresponds to the non-extractable fraction. It is bound to dietary fiber, and it is carried through the small intestine into the colon, where it can be metabolized by the gut microbiota, forming short chain free fatty acids (Pérez-Jiménez et al., 2013). Several studies have shown that these non-extractable compounds result in several health benefits, such as anti-inflammatory activity and a reduction of oxidative stress because of the effect of polyphenol-derived metabolites (Pérez-Jiménez et al., 2013).

In concordance with the TPC results, antioxidant capacity was significantly higher for the B cookie, making it the best option when it comes to the potential health benefits. Further investigation is needed regarding the bioavailability of the polyphenols, as well as sensory analysis to evaluate the acceptance of the cookie.

Table 7. Polyphenol content, antioxidant capacity and recovery index of digested cookies.

	TPC (mg GAE/g)	TEAC (mmol TEQ/g) *ABTS assay*	TEAC (mmol TEQ/g) *FRAP assay*	Bioaccessibility (%)
Control Cookie	2.03 ± 0.06[a]	0.046 ± 0.001[a]	0.017 ± 0.001[a]	54.2 ± 1.6[c]
Cookie A	3.09 ± 0.13[b]	0.055 ± 0.003[b]	0.023 ± 0.002[b]	41.9 ± 1.8[b]
Cookie B	3.97 ± 0.16[c]	0.103 ± 0.004[c]	0.029 ± 0.001[c]	28.6 ± 1.2[a]

Values within a column that do not share a letter differ significantly ($p < 0.05$). Control cookie had 0% of hemp press cake. Cookie A had 23.2% of hemp press cake. Cookie B had 50.8% of hemp press cake.

3 Limitations of the study

This study has certain limitations that should be acknowledged. First, although the functional properties of hemp press cake were evaluated in gluten-free cookies, no formal sensory analysis was conducted. This restricts the possibility of assessing consumer acceptance, which is essential for determining the potential of these formulations in real markets.

Second, oil recovery under the chosen cold-pressing conditions was lower than yields typically obtained with solvent-based methods. Nevertheless, the decision to apply a solvent-free extraction was deliberate, aiming to minimize the use of hexane and other organic solvents that present environmental and toxicological concerns. In this regard, Cravotto et al. (2024) highlighted the importance of developing alternative green extraction strategies that move away from hexane in the food industry. Future research should therefore complement these findings with comprehensive sensory evaluation and further exploration of environmentally friendly extraction techniques to improve oil yield while maintaining safety and sustainability (Cravotto et al., 2024).

4 Conclusion

This study demonstrated that hemp seed oil can be obtained under optimized cold-pressing conditions using a solvent-free methodology, reaching acceptable yields while preserving the nutritional and functional qualities of the by-product. The resulting press cake proved to be a valuable ingredient for the formulation of gluten-free cookies, enhancing their protein, fiber, and bioactive compound content, as well as their antioxidant capacity after digestion. These results provide evidence that hemp by-products can be effectively valorized within a circular economy framework, contributing to the development of sustainable functional foods.

At the same time, the work highlights trade-offs between environmental considerations and technological performance, since solvent-free extractions achieve lower oil recovery than conventional solvent-based methods. Nevertheless, adopting greener approaches aligns with current demands to reduce the use of hexane in food processing and to minimize toxicological and ecological impacts.

Future studies should address sensory analysis to evaluate consumer acceptance and investigate alternative eco-friendly extraction strategies that could further improve oil yield without compromising safety or sustainability.

Data Availability Statement

All data generated or analyzed in this study are included in this published article.

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