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Research Article

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Use of chia seed on regular and low-fat crackers, their antioxidant properties, and *in-vitro* bioaccessibility

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Abstract

Although having functional properties, fat is known to be adversely effective in case of high consumption. High fat consumption causes health disorders such as obesity, cardiovascular diseases and high blood pressure, insulin balance disorders and cancer. For this reason, it is important to reduce fat consumption and create food formulations rich in bioactive components. In the scope of this study, *CS* (Chia seed) was replaced with wheat flour 10%, 20%, and 30% (w/w) and the fat amount was decreased in 25%, 50%, 75% (w/w) ratios for formulating low-fat crackers, and the antioxidative potential of the samples was evaluated. Extractable, hydrolysable, bioaccessible phenolic fractions of samples were analyzed in terms of $TEAC_{ABTS}$, $TEAC_{CUPRAC}$, $TEAC_{DPPH}$ and Total Phenolic Content (TPC) (Folin Ciocalteu's method). *CS* replacement was determined to be more effective than a fat reduction on AC and TPC results of samples. By 25, 50 and 100% fat reduction of extractable, hydrolysable and bioaccessible phenolic fractions, $TEAC_{ABTS}$ values increased respectively as 5.87%, 9.33% and 12.11%. 75% fat reduced-30% *CS* supplemented sample was 91.0% higher than 100% fat including-30% *CS* supplemented sample and 143.4% higher than the control sample in terms of $TEAC_{ABTS}$ for bioaccessible phenolic fractions. The dietary fiber, protein content and fatty acid composition are thought to be effective in the potential of *CS*. It is proved that *CS* could be expressed as a convenient pseudo-cereal for functional food formulations.

Keywords: Chia,Low-fat, Antioxidant activity, Total phenolic content, *in-vitro* bioaccessibility

Introduction

Fat is the most important component that affects the texture, mouthfeel, aroma, and all properties of foods (Marangoni et al., 2014). However, excessive energy intake resulting from the high fat intake is associated with obesity, cancer, high blood cholesterol, and coronary heart diseases (Chowdhury et al., 2014; Siri-Tarino et al., 2015). Reducing the amount of fat in the daily diet has become very important for public health (Borneo et al.,2010; EFSA, 2010). Consumers want to reduce the amount of fat without damaging the quality criteria of the food.

Crackers are popular snack foods in the human diet and often described as thin, hard-baked, crunchy wafers or biscuits (Sedej et al., 2011; Isik and Topkaya, 2016). In order to achieve the desired features in crackers, low moisture and high amount of shortening should be used in the dough (Lee and Inglett, 2006). For fat reduction or displacement in crackers; protein and carbohydrate-based fat substitutes (Laguna et al., 2014),

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the hydrogenated or saturated fat substitutes with vegetable oils, and recently stabilized shortenings which are consisting of newly developed water-in-oil emulsions are started to use (Tarancón et al., 2012).

Chia (*Salvia hispanica*) is an annual herbaceous plant native to Mexico and Central America, it has attracted the attention of consumers in Turkey and used various foods such as biscuits, pasta, bread and yoghurt in recent years (CC, 2009). *CS* is a good source of antioxidants, depending on the presence of polyphenols (Taga et al.,1984; Martı´nez-Cruz and Paredes-Lo´pez, 2014).

CS can be used as a fat substitute due to its fat and protein content, in addition to this they can be used as an enricher and functional properties developer in the cracker production. For this aim, *CS* was replaced with wheat flour at the levels of 10%, 20%, and 30% (w/w) and the fat amount was decreased in 25%, 50%, 75% (w/w) ratios for formulating low-fat crackers, and the antioxidative potential of the samples was evaluated.

Materials and Methods

Materials

The wheat flour obtained from Toru Flour Milling Co. Ltd. (Bandırma/Balıkesir/Turkey) and rest of the ingredients such as CS (Chia Seed), salt, baker's yeast, sodium bicarbonate ($NaHCO_3$), ammonium bicarbonate (NH_4HCO_3) and shortening were purchased from local stores in Bursa (Turkey) for production cracker production.

Methods

Cracker preparation

For the production of crackers, method described by Lee and Inglett (2006) applied with slight modifications. The control sample was prepared with 100% wheat flour and 100% fat (shortening). In rest of the cracker samples, *CS* was replaced with wheat flour at levels of 10%, 20%, and 30% (w/w) and the fat amount was decreased in 25%, 50%, 75% (w/w) ratios for evaluating low-fat crackers (Table 1.). For the production, firstly, dry ingredients were mixed for 30 secs in a mixing bowl, homogeneously. First part of the water and shortening were mixed in a separate container then added into the mixture and kneaded (5SS model 5SS, Kitchen-Aid, USA) for 120 sec. For the baker's yeast activation, the rest of the water was used. The cracker dough with all ingredients was kneaded for more 4 min. Then, the dough was proofed for 2 h at 35 °C. Lamination machine (TMM Inc., Turkey) was utilized for reducing the thickness of the proofed dough to 1.5 mm. The spread dough was docked and cut into 5×5 cm cracker size by the cutter-docker device. Afterwards, crackers were baked at 180 °C for 7 min in a convection oven (FKE 006 model, Inoksan, Turkey). Then, baked crackers were cooled down to room temperature (~30 min). The cracker samples were grounded and stored at -18 °C hermetically, till sample preparation of antioxidant capacity (AC) and total phenolic content (TPC) analysis.

Table 1. Formulation of crackers incorporated with chia seed

Fat Ratio	CS* Ratio	Sample Code	Wheat Flour (g)	CS* (g)	Shortening (g)	Water (g)	$NaHCO_3$ (g)	NH_4HCO_3 (g)	Salt (g)
100%	%0	C_{Aa}	100.00	0.00	13.00	40.00	0.50	2.00	1.60
	%10	C_{Ab}	90.00	10.00	13.00	40.00	0.50	2.00	1.60
	%20	C_{Ac}	80.00	20.00	13.00	40.00	0.50	2.00	1.60
	%30	C_{Ad}	70.00	30.00	13.00	40.00	0.50	2.00	1.60
75 %	%0	C_{Ba}	100.00	0.00	9.75	40.00	0.50	2.00	1.60
	%10	C_{Bb}	90.00	10.00	9.75	40.00	0.50	2.00	1.60
	%20	C_{Bc}	80.00	20.00	9.75	40.00	0.50	2.00	1.60
	%30	C_{Bd}	70.00	30.00	9.75	40.00	0.50	2.00	1.60
50%	%0	C_{Ca}	100.00	0.00	6.50	40.00	0.50	2.00	1.60
	%10	C_{Cb}	90.00	10.00	6.50	40.00	0.50	2.00	1.60
	%20	C_{Cc}	80.00	20.00	6.50	40.00	0.50	2.00	1.60
	%30	C_{Cd}	70.00	30.00	6.50	40.00	0.50	2.00	1.60
25%	%0	C_{Da}	100.00	0.00	3.25	40.00	0.50	2.00	1.60
	%10	C_{Db}	90.00	10.00	3.25	40.00	0.50	2.00	1.60
	%20	C_{Dc}	80.00	20.00	3.25	40.00	0.50	2.00	1.60
	%30	C_{Dd}	70.00	30.00	3.25	40.00	0.50	2.00	1.60

***CS:**Chia Seed; **C:**Cracker Sample; **A-D** for fat reduction; **a-d** for CS replacement*

Sample preparation

The AC and TPC of cracker samples were evaluated in terms of extractable, hydrolysable, bioaccessible fractions of phenolics (Vitali et al.,2009; Bouayed et al.,2012). For the extraction procedure, 2 grams of sample was mixed with HCl$_{conc}$/metanol/water (1:80:10, v/v) for 20 °C in shaking water-

bath (250 rpm, 2 h; Thermo Fisher Scientific Inc., USA) and centrifuged (3500 rpm, 4 °C, 10 min; 3 K 30 model, Sigma, Germany). The supernatant was taken as extractable fraction, the residue was subjected with methanol/H_2SO_4 (10:1) and shaken in shaking water-bath (250 rpm, 85 °C, 20 h) and centrifuged (3500 rpm, 4 °C, 10 min). The supernatant was taken as hydrolysable fraction. For bioaccessible fraction, the in-vitro digestion procedure that include the enzymatic extraction was mimic for the cracker samples (Bouayed et al.,2012) was applied to cracker samples. 2 grams of cracker sample was treated with the pepsin enzyme solution (40 mg/mL in 0.1 M HCl; Merck, USA) in shaking water-bath (250 rpm, 37 °C, 2 h). Then the extraction was continued with the intestinal digestion procedure with using a porcine pancreatic enzyme solution (2 mg/mL; Sigma-Aldrich, Germany) and porcine bile solution (12 mg/mL; Sigma-Aldrich) at 37 °C (250 rpm, 2 h), at the end of the time centrifuged (3500 rpm, 15 °C, 10 min). The extracts were kept at -18 °C and utilized in AC and TPC assays.

Determination of total phenolic contents (TPC)

TPC of cracker extracts was evaluated by the Folin-Ciocalteu method with Apak et al. (2007a) procedures. The absorbance of the extracts was determined spectrophotometrically, gallic acid (Sigma-Aldrich, Germany) was utilized as standard and the results were expressed as mg gallic acid equivalents (GAE) per 100 g sample. The bioaccessibilities (%) of AC and TPC results were calculated according to Anson et al. (2009).

Antioxidant capacity

Different methods are used to determine the antioxidant capacity. Three methods commonly used to assess antioxidant capacity in vitro are the 2,2-azinobis (3-ethylbenzothiazoline6-sulfonic acid) (ABTS) test, DPPH, and the copper ion-reducing antioxidant capacity (CUPRAC) test.

The AC analysis determined according to methods of ABTS (Apak et al.,2007a), CUPRAC (Apak et al.,2007b) and DPPH (Brand-Williams et al., 1995) assays. The absorbance of the extracts was determined spectrophotometrically (Jenway, 6405 UV/Vis). Trolox equivalent (TE) calibration curve obtained for assays in between range of 0.02-0.08 µmol Trolox (Sigma-Aldrich, Germany) and the results were expressed as µmol Trolox equivalent (TE)/g sample.

For ABTS radical solution, 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12–16 h before use to obtain an absorbance at 734 nm. ABTS radical solution of blue-green color was diluted with ethanol (96%) at a ratio of 1:10. The stage was used adding 1 mL of the ABTS solution to (x) mL of extract and (4.0–x) mL of ethanol, and the absorbance measured at 734 nm after 6 min by using UV/Vis spectrometer (Optizen 3220 UV, Mecasys).

For CUPRAC assay briefly, One mL of 1 9 10^{-2} M CuCl2, 1 mL 7.5 9 10^{-3} M neocuproine, 1 mL ammonium acetate buffer solutions, x mL extract of samples and (4–x) mL of water were added and mixed. The final mixture at 4.0 mL total volume was let to stand at room temperature and after 30 min, the absorbance at 450 nm (Optizen 3220 UV, Mecasys) was recorded against a reagent blank.

The initial absorbance of the DPPH in methanol was measured at 515 nm and did not change throughout the period analysis. Briefly, all diluted extracts (0.1 mL) were added to the test tube and 3.9 mL of 6 x 10^{-5} M methenolic solution of DPPH was added and mixed. This mixture was left in the dark for 30 minutes and then the measurement was carried out.

Statistical evaluation

The obtained data was evaluated statistically by variance analysis with JMP IN 7.0.0 (Statistical Discovery from SAS 2005. Institute Inc.). The LSD (Least Significant Differences) test was used for determination of the statistical difference between the obtained mean values in terms of CS addition and fat reduction in cracker samples.

Results and Discussion

Phenolic compounds are present in three different forms such as free, extractable-conjugated and, non-extractable-bound forms in food. The bound forms of phenolic compounds can only be physically trapped in the structure of macro components without binding to the food matrix or various cellular structures (Gökmen et al.,2009; Nayak et al., 2015; Karabulut and Yemiş, 2019).

Bioaccessiblity of phenolic compounds in foods depends on components release from the food matrix, its absorption and its passage to the blood circulation system during digestion or intestinal fermentation. Especially, polyphenols in some foods bind to macromolecules such as proteins, carbohydrates and lipids in the cell wall structure and greatly affect the bioaccessibility in the gastrointestinal system. Because of being difficult to digest, bound phenolic compounds can reach the colon without alteration in the gastrointestinal tract (Arranz et al., 2010; Pastoriza et al., 2011; Karabulut and Yemiş, 2019).

The AC and TPC of cracker samples were evaluated in terms of extractable, hydrolysable, bioaccessible fractions of phenolics and given in Figure 1-4. Hydrolysable fractions of crackers were the highest results comparing to extractable and bioaccessible fractions. According to the extraction procedure, it is thought that the values of the hydrolysable fractions were increased due to the higher temperature and longer extraction duration of the cracker samples. Also, the bioaccessible fractions were shown higher results than the extractable fractions. The amount of released bioactive components were probably increased with the enzymatic treatment of *in-vitro* gastric and intestinal digestion with relatively higher extraction temperature (37 °C).

Evaluating the hydrolysable fractions for $TEAC_{ABTS}$, by the fat reduction, there were 7.70, 14.82 and 23.62% increase observed in antioxidative potential of crackers. For the C_D group of samples with 75% fat reduction, by the 10, 20, 30% *CS*-supplementation, there were 24.22, 54.32 and 82.36% increase for $TEAC_{ABTS}$ in C_{Db}, C_{Dc}, C_{Dd} samples. For the extractable fractions, a higher increase of antioxidative potential was observed as 48.24 , 91.45, 180.40% for $TEAC_{ABTS}$ in the same samples.

It is an important parameter to determine the *in-vitro* bioaccessibility of the food bioactive components, for revealing their health potential and improving existing food formulations and processes. Evaluation of the effect of *CS*

on bakery product as a nutritional perspective, Pigni et al. (2020) produced supplemented wheat pasta with obtained by-product of the *CS* oil extraction and evaluated their *in-vitro* bioaccessibility of polyphenols. They did not determine the significant increase in oral or gastrointestinal digestion steps but by the intestinal digestion markable increase was observed in terms of TEAC (Trolox Equivalent Antioxidant Capacity). It can be expressed as the evidence of the bioactive compounds release in intestinal digestion step.

In terms of *CS* replacement, evaluating the C_A group of samples according to *CS* supplementation in 100% fat production, there are 9.1%, 20.0%, and 38.9% increases in TPC of bioaccessible phenolic fractions of C_{Ab}, C_{Ac}, and C_{Ad} respectively for 10%, 20% and 30% *CS* replacement. Withal, AC results were reflected higher increment in the same way of evaluation. There are 30.3%, 105.0% 121.5% increase for C_{Ad} comparing to C_{Aa} in terms of $TEAC_{DPPH}$, $TEAC_{CUPRAC}$, $TEAC_{ABTS}$. In terms of fat reduction, the highest increase was determined in AC content determined according to ABTS and CUPRAC methods. By 25, 50 and 100% fat reduction (Comparing to C_B, C_C and C_D with C_A group of samples) in extractable, hydrolysable and bioaccessible phenolic fractions, the AC results were increased respectively as 5.87%, 9.33%, 12.11% according to $TEAC_{ABTS}$ and respectively 5.87%, 9.33%, 12.11% increase were determined in $TEAC_{CUPRAC}$. *CS* replacement was determined to be more effective than fat reduction on AC and TPC results of cracker samples. 75% fat reduced-30% *CS* supplemented C_{Dd} sample is 91.0% higher than C_{Da} and 143.4% higher than C_{Aa} in terms of $TEAC_{ABTS}$ for bioaccessible phenolic fractions.

CS is a good source for bakery products with high protein (21.78%) and dietary fibre (38.70%) contents, including fatty acid varieties (Dundar et al., 2020) together with its bioactive potential. As a part of this study, by *CS* replacement; dietary fiber, protein, and ash contents were increased for cracker samples; and the fatty acid content was were enriched in terms of linoleic, oleic, α-linolenic acids. C_{Dd} was also expressed as the healthiest sample according to $C_{18:1}/C_{18:0}$ ratio (Dundar et al., 2020). In this study, C_{Dd} is also determined as the richest sample in terms of bioactive potential (Fig. 1-4) as a result of AC and TPC analysis.

Enes et al. (2020) published a systematic review according to electronic databases by following Prisma recommendations for understanding *CS* potential on health. Oxidative stress decreasing effect of *CS* associated with increasing the AC and providing antioxidant enzymes; decreasing the lipid peroxidation and amount of reactive oxygen species (ROS). Its antioxidant properties and bioactive potential comes form including the carotenoids, phospholipids, tocopherols, and phenolic acids such as chlorogenic acid, caffeic acid, kaempferol, quercetin, together with fatty acid content (especially α-linolenic acid: $C_{18:3}$) (Enes et al., 2020; Da Silva et al., 2016). Chlorogenic acid and caffeic acid are known with stronger inhibition effect on lipid peroxidation than the well-known antioxidative compounds such as vitamin E and C (Valdivia-López and Tecante, 2015).

Demin et al. (2020) evaluated the nutritional value of cracker samples based on *CS*, rye and buckwheat flours with extra virgin olive oil. *CS* amount provided a significant increase (p<0.05) in crude fiber, total ash and mineral (Cu, Fe, Mg, and Zn) contents of cracker samples. Especially with fatty acid content, *CS* was expressed as a convenient pseudo-cereal for functional food formulations by low amount of saturated fatty acids (<11%) and high amount of polyunsaturated fatty acids (> 89%). In the present study, *CS* supplemented cracker samples, comparing C_{Ad} with C_{Aa}; $C_{18:3}$ content increased from 0.05 to 5.52 g/100g (Also proportional increase of *CS* in content by decreasing fat in total dough amount). Evaluating the *CS* and fat effect together, $C_{18:3}$ content was determined 7.21 g/100g in C_{Dd} sample (Dundar et al., 2020). Therefore, the fatty acid content was found as one of the reasons of higher bioactive potential.

Jethwani et al. (2020) prepared bars with mango, apple and guava supplemented with chia seeds. When the antioxidant activity and total phenolic components of these bars were examined; while the antioxidant (DPPH) values of the control bars were 76.38%, mango, apple and guava supplemented with chia seeds were found 80.09%, 78.43%, 78.14%, respectively. The antioxidant activity ABTS (mmolTE / 55g) values of the control bars were found 38.17 mmolTE / 55g, while the bars with chia seed mango, apple and gouava were 45.49 mmolTE / 55g, 42.30 mmolTE / 55g, 39.6 mmolTE / 55g, respectively. The results for total phenols reveals the significant increase ((p <0.01) in the selected antioxidant rich bars as compared to the control bar. The control bar was seen to have a total phenols content of 64.50 mgGAE / 55g. A highest total phenol among the selected bars was found to be in guava chiaseed bar (97.01 mgGAE / 55g) while lowest was observed in apple chiaseed bar (69.93 mgGAE / 55g).

Antonini et al. (2019) made beef burgers that contain chia seeds and goji puree (2.5 and/or 5%), and total phenolics and antioxidant capacity were determined. The ORAC and DPPH methods revealed a higher antioxidant capacity when goji puree and chia seeds where added, respectively, thus highlighting the different ability of polyphenols to scavenge free radicals.

Sharma et al. (2020) prepared six different formulations of muffins. One formulation was prepared with wheat flour (control), and in other five formulations, wheat flour was replaced with chia seeds at different levels (5%,10%,15%,20%,25%). Total antioxidant activity was found between 0.39 (mg TE/100g) and 0.78 (mg TE/100g). It was determined the lowest total antioxidant activity in control.

Mesías et al. (2016) determined significant and progressive increase in the AC ($TEAC_{ABTS}$, $TEAC_{DPPH}$, $TEAC_{ORAC}$ and $TEAC_{FRAP}$) by means of *CS* increase in formulations. In other respects, increasing amounts of *CS,* in chips (Coorey et al., 2012) and bread (Costantini et al., 2014) formulations, higher AC potentials were observed according to analyze results.

Together with the recent studies, using chia (*Salvia hispanica*) in the functional food formulations could be expressed to supportive. *In-vitro* studies have shown the health potential of *CS* with different properties, and further *in-vivo* studies could be recommended to conduct for understanding the health properties with the mechanism.

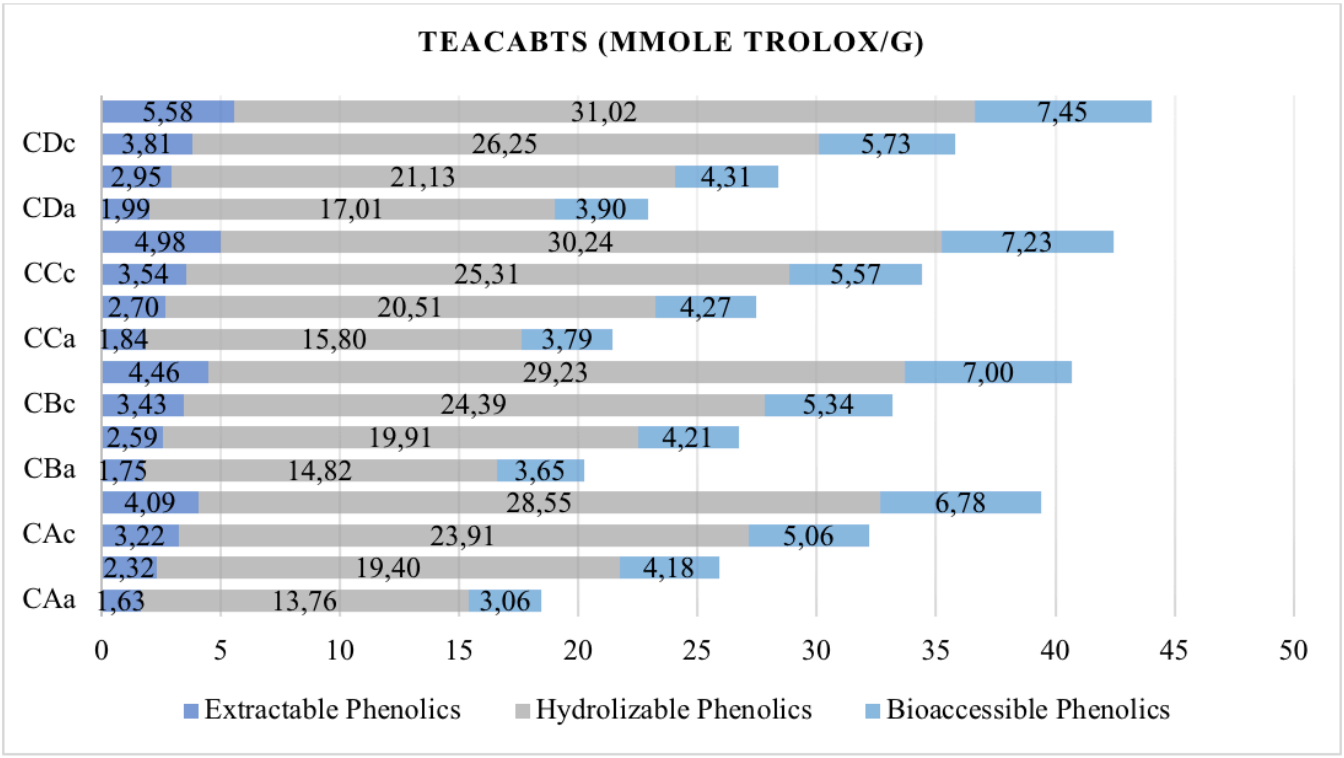

Figure 1. $TEAC_{ABTS}$ results of cracker samples
*CS:Chia Seed; A:100% fat; B:%75 fat; C:%50 fat; D:%25 fat, a:0% CS; b:10% CS; c:20% CS; d:30% CS in cracker samples

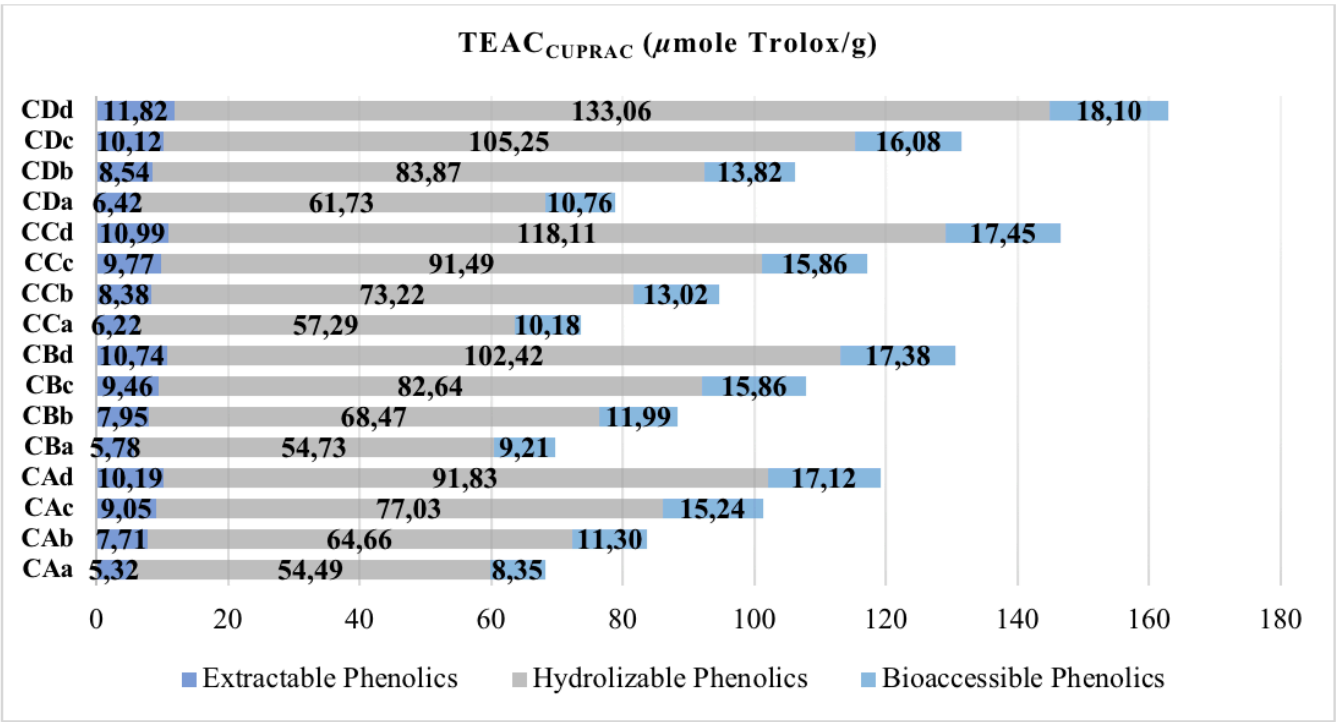

Figure 2. $TEAC_{CUPRAC}$ results of cracker samples
*CS:Chia Seed; A:100% fat; B:%75 fat; C:%50 fat; D:%25 fat, a:0% CS; b:10% CS; c:20% CS; d:30% CS in cracker samples

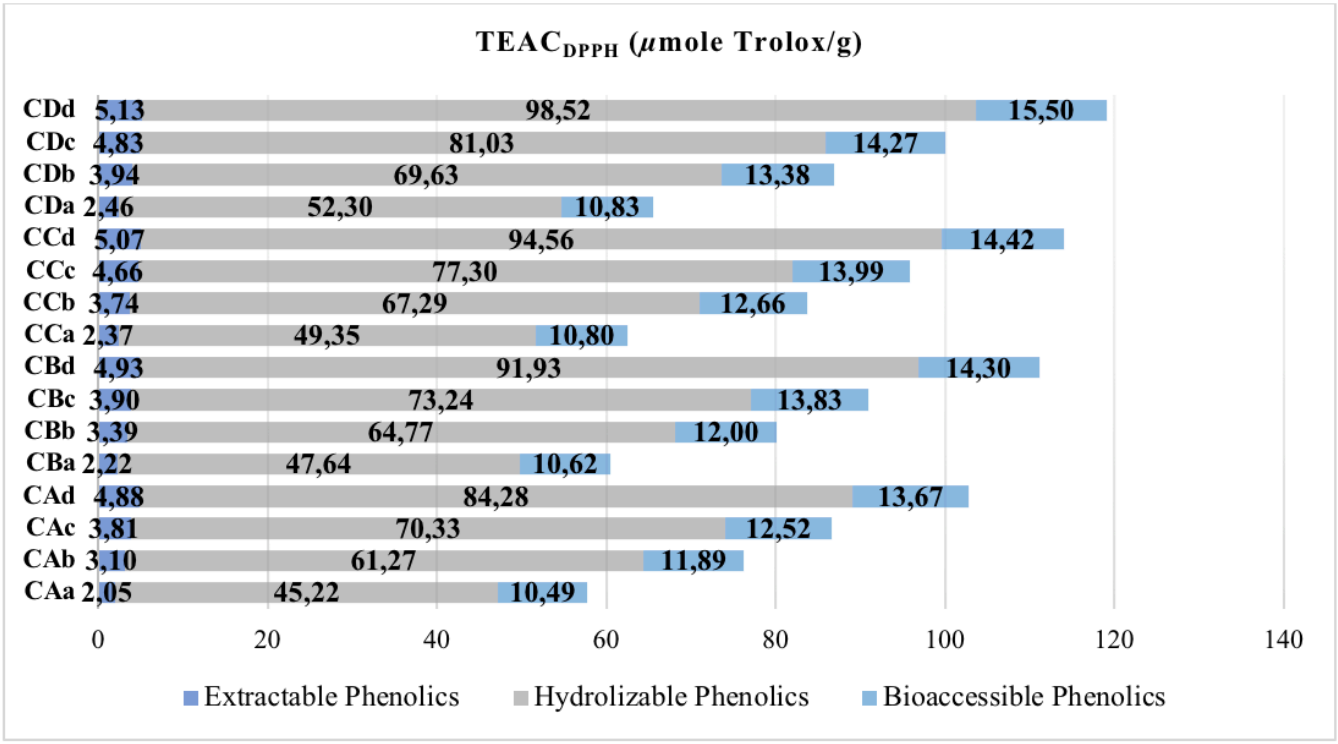

Figure 3. TEAC$_{DPPH}$ results of cracker samples
*CS:Chia Seed; A:100% fat; B:%75 fat; C:%50 fat; D:%25 fat, a:0% CS; b:10% CS; c:20% CS; d:30% CS in cracker samples

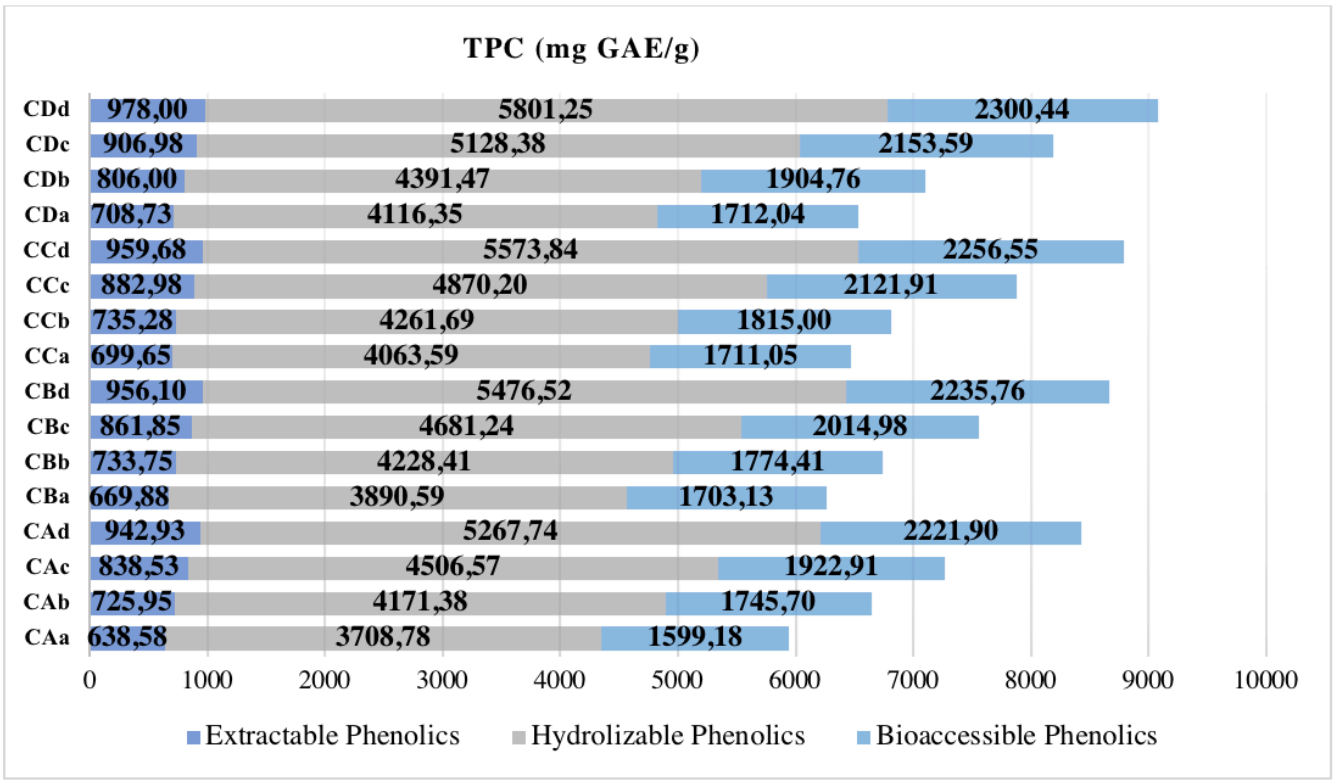

Figure 4. Total Phenolic Compound analysis results of cracker samples
*CS:Chia Seed; A:100% fat; B:%75 fat; C:%50 fat; D:%25 fat, a:0% CS; b:10% CS; c:20% CS; d:30% CS in cracker samples

Conclusion

In addition to providing energy, the fat has a nourishing function because of providing intake of essential fatty acids and fat-soluble vitamins. The effects of fat on the bakery products are important in regard of the mouthfeel, texture and taste properties. Although having functional properties, fat is known to be adversely effective on health in case of high consumption. Reducing the amount of fat in the daily diet, which has become a public health issue, is a problem and concern for many consumers. Cracker is one of the common bakery products because of being satisfying, nutritious, and delicious. For creating a functional and nutritious formulation, *CS* was replaced with wheat flour at the levels of 10%, 20%, and 30% (w/w) and the fat amount was decreased in 25%, 50%, 75% (w/w) ratios for production of low-fat crackers. For determination of the bioactive potential of the crackers, extractable, hydrolysable, and bioaccessible phenolic fractions of samples were analyzed in terms of $TEAC_{ABTS}$, $TEAC_{CUPRAC}$, $TEAC_{DPPH}$ and total phenolic content. Hydrolysable fractions of crackers were the highest results comparing to extractable and bioaccessible fractions. *CS* supplementation is remarkably more effective on bioactive compounds of cracker samples comparing with the fat reduction. In terms of *CS* replacement, evaluating the C_A samples according to *CS* supplementation in 100% flour production, there are 9.1%, 20.0%, and 38.9% increases in TPC of bioaccessible phenolic fractions of C_{Ab}, C_{Ac}, and C_{Ad} respectively for 10%, 20% and 30% *CS* replacement. *CS* could be expressed as a convenient pseudo-cereal for functional food formulations by increasing the bioactive potential and also providing the quality parameters. The results of our study showed that the addition of chia seeds plays an important role, especially in increasing antioxidant capacity and phenolic compounds. Thanks to this feature, it is recommended as a guide for future studies in order to increase the functionality of commercial products.

Compliance with Ethical Standards

Conflict of interest

The authors report no declarations of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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