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Effect of sourdough prepared with the combination of chickpea and carob on bread properties

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ABSTRACT

This study investigated the quality attributes of wheat bread made from chickpea-carob sourdough (CCS). Four types of dough formulations were prepared by adding 0 (2% yeast added) (control), 10, 20, and 30% CCS (based on the weight of wheat flour). The bread samples' physical properties, proximate composition, antioxidant properties, mold count, and sensory attributes were investigated. An increase in the CCS amount caused an increase in the ash content, protein content, total antioxidant capacity, and total acidity. The loaf-specific volumes were measured as 2.85, 3.45, 3.49, and 3.29 cm^3/g for 10%, 20%, 30% CCS added, and control samples (yeast-leavened bread), respectively. Furthermore, CCS addition decreased the mold count from 0.5 to 1.5 log cfu/g compared to the control sample after 3, 6, and 9 days of storage time. CCS addition to bread formulations did not affect the sensory attributes significantly ($p < 0.05$). The results suggest that the CCS can be used to improve bread's nutritional and functional properties.

1. Introduction

In recent years, consumer demand has urged the bakery industry to produce products with positive effects on health and improved nutritional value (Gobbetti et al., 2014). Using sourdough from non-wheat sources became an alternative way of production to increase the nutritional value of bread while improving its functional and technological properties (Cakir et al., 2021). Sourdough, which can be produced traditionally or industrially, is a source of various metabolites, including enzymes, organic acids, antioxidants, bioactive peptides, amino acid derivatives, nutritive substances, and complex carbohydrates (i.e., exopolysaccharides) (Principato et al., 2019; Turfani et al., 2017). Microbial metabolism during sourdough fermentation may increase bioactivity and bioavailability and decrease the glycemic response of baked goods (Gobbetti et al., 2014). In addition, it has been reported that sourdough exhibited antifungal activities, improving the final product's shelf-life (Meziani et al., 2015). It was also reported that sourdough helps to prolong bread staling (Caglar et al., 2021).

Although the legumes are rich in nutritive substances, the consumption level is below the recommended dose (McCrory et al., 2010). It has been reported that refined wheat flour lacks some bioactive substances such as polyphenols, dietary fiber, and some essential amino acids (i.e., threonine and lysine) (Boye et al., 2010). Therefore, fortification with other cereals or legumes would be an effective means to improve its nutritional quality. Legume flours are raw materials with high dietary fiber and phenolic compounds, and sourdough fermentation is a helpful process to exploit their potential to fortify baked goods (Gobbetti et al., 2020). Chickpea (*Cicer arietinum* L.) is rich in high-quality protein containing well-balanced amino acid composition, histidine, Ca, P, Mg, K, and vitamin A (Gaur et al., 2015). Similarly, carob (*Ceratonia silique* L.) is rich in minerals (Fe, Ca, K, P, Na, S), vitamins (E, D, C, B6, folic acid, Niacin), hydrocolloids, gums, carbohydrates (sucrose, glucose, and fructose), dietary fiber (around 35%), and polyphenols (Dülger Altıner and Hallac, 2020; Durazzo et al., 2014). They help reduce the risk of degenerative and neurodegenerative diseases (Scalbert et al., 2005).

Chickpeas and carob have a mild taste, high nutritional value, several health benefits, and low antinutritional factors (Yatmaz and Turhan, 2018). There needs to be more information about using carob and chickpea together as a sourdough starter to elucidate their additive potential for improving the bread quality. Therefore, this study aimed to produce sourdough from a mixture of chickpea and carob flours to be used as a starter to produce sourdough wheat bread. For this purpose, chickpea-carob sourdough was produced and added into dough formulations at three different levels. To determine the effect of chickpea-carob sourdough, bread samples' textural, physicochemical,

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microbial, and sensory properties were compared with yeast-leavened bread produced using baker's yeast.

2. Materials and methods

2.1. Materials

Baker's wheat flour, chickpea flour, and dried carob were purchased from a local market. The chemical compositions are given in Table 1 (based on wet basis). The whole dried carob (including pod and seed) was grounded using a bench-size centrifugal mill (Retsch ZM200, Germany) and sieved by a laboratory-type sieve (Retsch AS200, Germany) to obtain a powder having a particle size range of 100–250 μm. The powders were kept in sealed plastic bags and stored at +4 °C for further use. Analytical grade reagents, chemicals, growth media, and standards were procured from a local distributor of Sigma–Aldrich Company (Sigma-Aldrich Chemie GmbH, Germany).

Table 1
Chemical compositions of refined wheat flour, chickpea flour and carob flour (Turkomp, 2023).

Content (per 100 g of sample)	Unit	Refined wheat flour	Chickpea flour	Carob flour
Moisture	g	12.02	8.76	12.22
Ash	g	0.46	2.97	2.47
Protein	g	10.96	18.56	4.18
Yağ, toplam	g	1.44	5.33	0.69
Carbohydrate	g	71.36	41.35	54.61
Fiber, total dietary	g	3.76	23.03	25.83
Fiber, water-soluble	g	–	–	2.68
Fiber, water-insoluble	g	–	–	23.14
Starch	g	58.32	30.98	–
Saccharose	g	0.23	3.16	16.4
Glucose	g	0	0	8.96
Fructose	g	0	0.37	11.47
Maltose	g	1.66	0	0.06
Fe	mg	1.49	5.92	2.2
P	mg	98	397	211
Ca	mg	69	99	311
Mg	mg	28	139	119
K	mg	159	1171	992
Na	mg	0	19	5
Zn	mg	5.93	3.16	1.45
Se	μg	0	31.1	2.5
Thiamin	mg	0.245	0.572	–
Riboflavin	mg	0.039	0.164	–
Niacin equivalents, total	NE	3.788	6.11	–
Niacin preformed	mg	1.606	3.146	–
Vitamin B-6, total	mg	0.047	0.535	–
Folate, food	μg	36	46	–
Vitamin E	α-TE	–	2.92	–
Vitamin E, IU	IU	–	4.35	–
Alpha-tocopherol	mg	–	2.92	–
Tryptophan	mg	131	178	–
Threonine	mg	192	748	–
Isoleucine	mg	555	937	–
Leucine	mg	972	1517	–
Lysine	mg	310	2438	–
Methionine	mg	212	358	–
Cystine	mg	139	292	–
Phenylalanine	mg	758	1103	–
Tyrosine	mg	480	700	–
Valine	mg	637	1001	–
Arginine	mg	180	975	–
Histidine	mg	145	634	–
Alanine	mg	395	773	–
Aspartic acid	mg	254	1008	–
Glutamic acid	mg	2176	2710	–
Glycine	mg	405	928	–
Proline	mg	1443	805	–
Serine	mg	328	1000	–

3. Methods

3.1. Sourdough preparation

The back-slopping method reported by Rizzello et al. (2014) was used to produce Type I sourdough with minor modifications. For this purpose, 500 g chickpea and 500 g carob flours (1 kg in total) were mixed with 1 L drinking water in a 2 L glass jar. The mixture was stirred vigorously and then incubated at 30 °C for 15 days to obtain chickpea-carob sourdough (CCS). Chickpea-carob flour (25 + 25 = 50 g) and water (50 mL) were added to propagate sourdough after every five days. The final sourdough was stored in a glass jar at +4 °C for further use.

3.1.1. Technological properties of the dough samples

The technological properties, including water absorption of the wheat flour, were determined using a Farinograph (Brabender, Germany) according to AACC method 54–21 (AACC, 2000). The viscoelastic properties of the dough samples were determined using an Extensograph (Brabender, Germany) according to AACC method 54–10 (AACC, 2000). Extensograms were recorded at room temperature for 45, 90, and 135 min proofing times.

3.2. Bread production

This study prepared three different doughs by adding CCS at three different amounts (10, 20, and 30%, w/w, based on wheat flour weight), as shown in Table 2. In addition, the control dough sample was prepared using 2% baker's yeast. In the breadmaking process, the water amount is crucial since it directly impacts gluten development and bread quality. The same happens with the kneading time and the fermentation time. Therefore, the total water amount was set at the same amount (650 mL) for all the bread formulations based on the water content of the CCS added. After kneading, the dough was rested at room temperature for 40 min. Then, the dough was cut into 200 g pieces, rolled by hand, and placed on baking pans. Fermentation was done at 30 °C for 4 h at 80% relative humidity. The doughs were baked at 180 ± 5 °C for 50 ± 5 min. The loaves were rested 1 h to cool at room temperature.

3.2.1. Chemical and physicochemical analyses

Moisture and ash contents were analyzed using AOAC methods (AOAC, 2000). Using a UV–vis spectrophotometer, total protein content was determined following the Bradford assay (Bradford, 1976) based on the reading of absorbance values at 595 nm. pH was determined using a pH meter (Hanna HI2211, Germany). To determine total titratable acidity (TTA), 10 g of loaves were mixed with 90 mL of distilled water in a Stomacher (VWR Star Blender-LB400) for 2 min. This mixture was titrated with 0.1 N NaOH solution to a final pH of 8.5 (Katina et al., 2006b). TTA was given as mL of 0.1 mol L^{-1} NaOH needed to reach the final pH. For both pH and TTA determination, all samples were analyzed in triplicate.

3.2.2. DPPH (2,2-diphenyl-1-picrylhydrazyl) method

The 80% methanolic extracts of bread samples were prepared as described by Ermiş et al. (2020). 0.1 mL of extracts were added to 3.9 mL of a methanol DPPH solution ($6E10^{-5}$ M). The mixture of 0.1 mL of 80% methanol and 3.9 mL of DPPH solution was used as a control. The mixtures were rested in the dark for 30 min at room temperature before measuring the absorbance values at 517 nm using a UV-VIS spectrophotometer (Schimadzu, Japan). % inhibition (DPPH scavenging) was calculated using the following equation (Karamać et al., 2002).

$$\% \text{ inhibition} = [1 - (A_s/A_c)] \times 100 \quad (1)$$

Where A_s and A_c are the absorbance readings for bread sample and control sample, respectively.

Table 2
Ingredients used to produce bread samples.

Sample code	CCS (g)	Wheat sourdough	Baker's yeast(g)	Wheat Flour (g)	Water (mL)	Salt (g)	Total (g)
Control	–	–	20	1000	650	15	1685
A (CCS100)	100	–	–	1000	580	15	1695
B(CCS200)	200	–	–	1000	520	15	1735
C(CCS300)	300	–	–	1000	450	15	1765

CCS: chickpea-carob sourdough.

3.3. *Technological properties of bread samples*

The rapeseed displacement method (AACC method 10–05) (AACC, 2000) was used to determine the volume of bread loaves. The specific volume was calculated by dividing the volume captured by the loaf sample by its weight. Texture Profile Analysis (TPA) was carried out with a Texture Analyser TA. HD plus (Stable Micro Systems) (Godalming, UK). The measurement was applied on loaf slices having rectangular prism shape with a thickness of 1.25 cm and a length of 2.5–3.0 cm. The crust was not removed. Hardness, firmness, springiness, resilience, and chewiness for the crumb, and crispiness for the crust (Demirkesen-Bicak et al., 2021; Katina et al., 2006a; Limbad et al., 2020) were determined by Stable Micro System software program (Yildirim and Arici, 2019).

3.3.1. *Total LAB, yeast, and mold count analysis*

The total number of LAB and yeast counts in CCS samples were determined using MRS (de Man, Rogosa, and Sharpe) agar (Merck, Germany) and malt extract agar (Merck, Germany), respectively. DRBC (Dichloran Rose Bengal Chloramphenicol) Agar (Merck, Germany) was used for mold count in bread samples. The LAB and yeast colonies were counted after incubation at 30 ± 1 °C for 24 h under anaerobic conditions. For mold growth, the incubation was done at 25 ± 1 °C for four days (Sabillón et al., 2021). The colony counts were expressed as log CFU (colony forming unit) per gram of sample.

3.4. *Sensory evaluation*

For the sensory evaluation of the loaf samples, 15 semi-trained panelists (Food Engineering Program 4th year students) were employed. A slice of each samples were placed on a plastic dish separately and labeled with random three-digit codes containing numbers and letters before given to the panelists to evaluate the loaf samples using a rating scale between 1 and 5 (1-poor, 2-non-satisfactory, 3-acceptable, 4-good, 5-very good) (ISO, 2016; Zebib et al., 2020). The attributes asked panelists to evaluate are color, aroma, crumb structure, taste, texture, and overall acceptability. The panelists were asked to wash their mouth with spring water served in a plastic glass for the taste neutralization between tastings of different bread samples. Sensory evaluation was conducted roughly 6 h after baking.

3.5. *Statistical analysis*

The data obtained were presented as means ± sd. Statistical analyses were done by ANOVA and Tukey's multiple comparison tests to determine the differences between the mean values ($p < 0.05$) using Minitab 17 software (Minitab Inc., Pennsylvania USA).

4. Results and discussion

4.1. *Farinograph properties of wheat flour*

A Brabender farinograph was employed to evaluate the technological properties of the flour sample in the kneading process. The initial moisture of wheat flour was measured as 14%. Based on the data obtained from farinograph, the development time, stability, degree of softening, farinograph quality number, and water absorption values were determined as 1.9 min, 15.7 min, 43 FU, 41, and 63%, respectively. Longer dough development time and stability values indicate higher flour strength leading to stronger doughs. High water absorption and a low degree of softening indicate good-quality flour (Shongwe et al., 2022).

4.2. *Viscoelastic properties of dough*

Extensibility and resistance to extension indirectly characterize the limit of the gas bubbles' expansion. The formed gas bubbles should expand without tearing during the baking of fermented dough to obtain a good bread volume. The influence of CCS on the viscoelastic properties of dough samples was evaluated using an extensograph, and the results are exhibited in Table 3. Based on the data obtained, adding CCS into dough formulations at different amounts affected the resistance and extensibility properties. However, the energy value was not significantly affected by different levels of CCS added ($P < 0.05$). The extensibility values of CCS-added dough samples decreased from around 120 to 85 mm at each three proofing times of 45, 90, and 135 min as the amount of added CCS increased from 100 to 300 g. The extensibility of the control dough sample was more remarkable than CCS-added dough samples. A decrease in extension by adding more CCS might be attributed to the weakening of the gluten network and its elasticity by adding CCS (Novotni et al., 2020). Another possible explanation could be the dilution of the gluten network by chickpea proteins (Arendt et al., 2007). In addition, the hydroxyl groups and the soluble fiber content of carob and chickpea might form more hydrogen bonding with water than starch and gluten, or they directly interact with gluten proteins, thus delaying gluten hydration (Turfani et al., 2017). Another possible reason might be attributed to the activity of proteolytic enzymes of lactic acid bacteria during the fermentation step (Spicher and Nierle, 1988) after adding CCS into the dough formulation.

The resistance to extension (Rmax) of dough samples containing CCS was higher than that of the control. It is expected that the proteolytic

Table 3
Extensograph data of dough samples at different proofing times ($n = 1$).

	R_{max} (BU)	Extensibility (mm)	Energy (cm^2)
	45 min		
Control	377 ± 14 ^{b}	119 ± 7 ^{a}	61 ± 4 ^{ab}
A	457 ± 23 ^{ab}	101 ± 9 ^{b}	65 ± 2 ^{a}
B	472 ± 28 ^{ab}	92 ± 7 ^{bc}	74 ± 6 ^{a}
C	512 ± 32 ^{a}	85 ± 5 ^{bd}	77 ± 3 ^{a}
	90 min		
Control	550 ± 31 ^{b}	120 ± 10 ^{a}	74 ± 2 ^{ab}
A	556 ± 21 ^{b}	103 ± 12 ^{ab}	79 ± 2 ^{a}
B	593 ± 18 ^{ab}	95 ± 8 ^{b}	79 ± 3 ^{a}
C	618 ± 31 ^{a}	88 ± 6 ^{bc}	82 ± 5 ^{a}
	135 min		
Control	579 ± 16 ^{b}	124 ± 11 ^{a}	71 ± 6 ^{ab}
A	627 ± 22 ^{ac}	105 ± 10 ^{ab}	76 ± 4 ^{a}
B	649 ± 27 ^{ab}	94 ± 9 ^{ac}	81 ± 3 ^{a}
C	685 ± 30 ^{a}	90 ± 4 ^{bc}	82 ± 4 ^{a}

A, B, C: sourdough bread samples containing 10, 20, and 30% CCS.
BU: Brabender unit, R_{max}: maximum resistance.
For a given row, the values with different letters are significantly different ($P < 0.05$).

activity of bacteria and yeast during sourdough fermentation leads to gluten degradation and, therefore, a loss of resistance to extension. However, a decrease in pH values due to sourdough fermentation and the presence of chickpea and carob proteins might lead to dough strengthening and hence increase the Rmax value. Previous researchers reported similar results (Arendt et al., 2007; Clarke et al., 2004; Schober et al., 2003; Voinea et al., 2020). They concluded that an increase in acidity results in the gluten protein network's disentanglement, leading to increased resistance and decreased extensibility. Contrarily, Gocmen et al. (2007) concluded that increasing the sourdough level decreased the resistance to extension.

4.3. Chemical and physicochemical properties of bread

Chickpea flour (10.34% moisture, 18.56% protein, and 2.97% ash) and carob flour (12.72% moisture, 4.18% protein, and 1.94% ash) were used to prepare CCS. As shown in Table 4, the addition of CCS increased the content of proteins, minerals, total acidity, and antioxidant power of the bread samples. The moisture content of bread samples are varied from 39.63 to 43.45% as only sample B showed a statistically significant difference in moisture compared to the control. The protein content of bread samples B and C (12.79 and 14.87%, respectively) was found to be significantly higher than A and the control sample (11.18 and 7.96%, respectively) ($P < 0.05$). The increase in protein content with the increased amount of % CCS added might be linked to the higher protein content of chickpea flour (Gómez et al., 2008).

Based on the data presented in Table 4, adding CCS affected the pH and TTA of the bread. Increasing the amount of CCS added to the dough caused a significant decrease in the pH value of the bread samples (varied from 4.35 to 5.83) and an increase in TTA values from 3.95 to 5.98 mL (0.1 M NaOH per 10 g of bread sample). Similar pH values (around 4.0) were reported by Curiel et al. (2015), who studied the fermentation of different legume flours with LAB strains. An increase in TTA as increasing the amount of CCS added could be attributed to the number of initial LAB and yeast cell populations responsible for the acid production during the dough fermentation. These findings agree with previously reported data (Curiel et al., 2015; Karrar et al., 2019; Novotni et al., 2020).

Based on the data outlined in Table 4, a significant increase in % inhibition (DPPH) can be noticed as a result of increasing the amount of added CCS ($P < 0.05$). The inhibition values of sourdough bread samples ranged from 22.62 to 31.28%, depending on the amount of CCS added. The inhibition level for sample C was almost two-fold higher than the control sample. The increase in antioxidant power would be attributed to the inclusion of carob flour containing both the pulp and seed, which increases the amount of polyphenols in bread, thus providing higher levels of antioxidant capacity (Turfani et al., 2017). Another reason might be that the level of phenolic compounds increased after sourdough formation (Slavin, 2000). Xiao et al. (2016) reported that the addition of fermented chickpea flour into dough formulation improved the antioxidant capacity of the bread. Similarly, Curiel et al. (2015) and Novotni et al. (2020) demonstrated a significant increase in the total phenolic compounds and antioxidant capacity after sourdough fermentation of different legumes. Improved antioxidant capacity of the bread would be beneficial for human health and help delay bread staling. In addition, an increase in organic acid production may lead to an increase in the activity of enzymes (i.e., proteases and amylases) in the dough, thus causing a reduction in bread staling and, therefore, an extension of shelf-life (Arendt et al., 2007).

Table 4
Physico-chemical characteristics of bread samples.

Characteristics	A	B	C	Control
Moisture (%)	40.52 ± 0.67 ^{ab}	39.63 ± 1.27 ^{b}	42.03 ± 2.2 ^{ab}	43.45 ± 0.76 ^{a}
Protein (%)	11.18 ± 1.60 ^{ab}	12.79 ± 1.43 ^{a}	14.87 ± 2.73 ^{a}	7.96 ± 0.44 ^{b}
Ash (%)	2.02 ± 0.14 ^{a}	2.09 ± 0.11 ^{a}	2.15 ± 0.08 ^{a}	1.50 ± 0.09 ^{b}
pH	5.17 ± 0.22 ^{b}	4.65 ± 0.11 ^{c}	4.35 ± 0.09 ^{d}	5.83 ± 0.10 ^{a}
TTA (mL)	4.78 ± 0.18 ^{c}	5.58 ± 0.22 ^{b}	5.98 ± 0.27 ^{a}	3.95 ± 0.30 ^{d}
Inhibition (DPPH) (%)	22.62 ± 2.57 ^{bc}	26.59 ± 4.29 ^{ab}	31.28 ± 4.16 ^{a}	14.94 ± 2.77 ^{c}

A, B, C: sourdough bread samples containing 10, 20, and 30% CCS.
TTA: Total titratable acidity (mL 0.1 N NaOH per 10 g sample).
For a given row, the values with different letters are significantly different ($P < 0.05$).

4.4. Physical quality characteristics of bread

The loaf volume is a quality criterion that is vital in attracting consumers (Costantini et al., 2014). Loaf-specific volume, baking loss, and other textural attributes are presented in Table 5. CCS significantly affected bread's textural properties and baking loss compared to the control bread sample. The loaf-specific volume ranged from 3.45 to 3.49 cm^3g^{-1} after adding CCS as only sample A showed a difference in loaf volume compared to the control sample (3.29 cm^3g^{-1}) ($p < 0.05$). The lower volume of sample A might be attributed to the lower amount of CCS added to the dough. The initial number of microorganisms affects the fermentation process and CO_2 gas production, one of the main factors for a volume increase in bread. There was no statistically significant difference between the B and C sample compared to the control. Similar data reported by Turfani et al. (2017) and Novotni et al. (2020) reveal an increase in bread volume after adding carob flour to wheat flour. Mohd Roby et al. (2020) studied the effect of fortification of wheat bread with encapsulated kombucha sourdough and reported an increase in the loaf volume compared to yeast bread. The addition of carob flour improved dough stability. It could be linked to water absorption capacity and gelling ability, empowering the expanding cells within the dough structure and improving gas retention and bread volume. An increase in the volume of CCS-added bread samples could also result from using carob flour together and its seeds, which are rich in hydrocolloids (mainly carob gum or locust bean gum) (Dakia et al., 2007). Hydrocolloids may prevent bigger cell formation by disrupting the coalescence of small cells in the dough (Das et al., 2013). The increased number of small cells would improve CO_2 retention and result in a uniform breadcrumb structure with increased volume. However, these findings do not agree with the findings of some previous studies. Salinas et al. (2015) reported that adding a mixture of carob pulp and seed flour at

Table 5
Physical quality parameters of bread samples.

Properties	A	B	C	Control
Loaf specific volume (cm^3/g)	2.85 ± 0.15 ^{b}	3.45 ± 0.07 ^{a}	3.49 ± 0.07 ^{a}	3.29 ± 0.10 ^{a}
Baking loss (%)	11.38 ± 1.60 ^{b}	9.95 ± 0.81 ^{c}	12.81 ± 1.03 ^{b}	16.46 ± 0.64 ^{a}
Hardness (N)	3.21 ± 0.32 ^{a}	3.02 ± 0.34 ^{b}	1.91 ± 0.33 ^{c}	0.49 ± 0.14 ^{d}
Crispiness	0.76 ± 0.09 ^{b}	0.73 ± 0.21 ^{b}	0.79 ± 0.11 ^{b}	0.93 ± 0.04 ^{a}
Firmness	0.75 ± 0.05 ^{b}	0.76 ± 0.03 ^{b}	0.76 ± 0.05 ^{b}	0.81 ± 0.04 ^{a}
Springiness	2.37 ± 0.92 ^{a}	2.29 ± 1.24 ^{ab}	1.43 ± 0.17 ^{b}	0.40 ± 0.18 ^{c}
Chewiness (N)	1.31 ± 0.62 ^{a}	1.70 ± 0.47 ^{a}	1.12 ± 0.18 ^{ab}	0.37 ± 0.19 ^{b}
Resilience	0.45 ± 0.05 ^{a}	0.45 ± 0.05 ^{a}	0.46 ± 0.05 ^{a}	0.48 ± 0.06 ^{a}

A, B, C: sourdough bread samples containing 10, 20, and 30% CCS.
For a given row, the values with different letters are significantly different ($P < 0.05$).

10% decreases the specific volume of wheat bread by about 10%. Similarly, Šoronja-Simović et al. (2016) concluded that 20% of added carob flour caused a 25% lower specific volume than the control wheat bread. Furthermore, Xing et al. (2021) found that fortifying wheat bread with protein-enriched chickpea sourdough caused a decrease in specific volume and denser crumb structure.

CCS-added bread samples exhibited a little darker brown color than control bread samples, possibly due to the Maillard reaction that occurred during baking and the color of the CCS. Compared to the control, a decrease in crispiness and firmness was observed with the addition of CCS, while an increase in springiness and chewiness was noted (Table 5). However, no significant difference in crispiness and firmness attributes were observed with increasing concentration of CCS. The hardness was reduced with increasing concentration of CCS but remained substantially higher than that of the control sample. Karrar et al. (2019) reported that adding sorghum sourdough and nabag pulp flour improved dough elasticity, gas retention, water absorption, and loaf volume. According to Mohd Roby et al. (2020), encapsulated kombucha sourdough reduced the crumb firmness. In this study, adding CCS adversely affected the hardness, firmness, springiness, crispiness, and chewiness compared to the control sample. No significant differences existed between the loaf-specific volume and resilience properties of all samples ($P < 0.05$).

Bourré et al. (2019) report that adding red lentil flour to wheat bread decreased the specific volume and increased the crumb's hardness. Similarly, Turfani et al. (2017) stated that adding lentil flour to wheat bread decreased the dough's stability by reducing the gluten network. They noted that their findings might be due to the alterations in the development and hydration of the gluten network due to the disturbance of the wheat protein-starch interface following the inclusion of legume flours. They concluded that the increase in crumb's hardness might be linked to the fibers, proteins, resistant starch, and insoluble carbs found in legume flours. According to Boukid et al. (2019), fibers may prevent carbohydrates and proteins from absorbing water, resulting in a dense crumb structure. Olojede et al. (2020) revealed that adding chickpea and cowpea flour into sorghum sourdough bread formulation led to higher cohesiveness and lower springiness. In a study by Turkut et al. (2016), adding quinoa flour did not cause a significant difference in crumb springiness compared with the control sample. Rizzello et al. (2016) and Rouhi et al. (2023) found that fortifying wheat bread with quinoa sourdough increased crumb hardness.

4.5. *Microbiological assessment*

Based on the findings of previous studies, around 20 yeast species (mostly *Saccharomyces* and *Candida* species) and more than 50 LAB species (mostly *Lactobacillus, Pediococcus, Leuconostoc, Enterococcus, Streptococcus and Weissella* species) were identified in spontaneous sourdoughs (Boyaci Gunduz et al., 2020; Sáez et al., 2018). The interaction between yeasts and LAB is reported, and this symbiotic relationship is responsible for leavening the dough and generating a typical taste and aroma in the final product (Ertop and Şeker, 2018).

Generally, the number of LAB and yeasts of a typical sourdough ranges from 7 to 9, and 5 to 7 log CFU g^{-1}, respectively (Paramithiotis et al., 2006). In this study, total LAB and yeast counts of CCS after 15 days of incubation were determined as 8.50 and 3.84 log CFU g^{-1}, respectively. Similar numbers were reported by Ertop and Şeker (2018), Curiel et al. (2015), and Novotni et al. (2020), with slight differences. The differences in counts depict that sourdough microbial ecology and acidity rely on several endogenous and exogenous factors (Gänzle, 2014).

Table 6
Mold counts of sourdough bread samples.

	Mold count (log CFU/g)			
	1st day	3rd day	6th day	9th day
Control	0	3.70 ± 0.15 ^{a}	4.85 ± 0.12 ^{a}	6.23 ± 0.22 ^{a}
A	0	2.70 ± 0.17 ^{b}	4.00 ± 0.14 ^{b}	5.83 ± 0.18 ^{b}
B	0	2.22 ± 0.08 ^{c}	3.60 ± 0.11 ^{c}	5.24 ± 0.07 ^{c}
C	0	2.23 ± 0.10 ^{c}	3.35 ± 0.09 ^{d}	5.11 ± 0.08 ^{d}

A, B, C: sourdough bread samples containing 10, 20, and 30% CCS.
CFU: colony forming unit.
For a given coloum, the values with different letters are significantly different ($P < 0.05$).

Table 6 shows the mold counts of the bread samples after different storage times (1, 3, 6, and 9 days). An increase in the amount of CCS added affected fungal growth at all storage times. A significant decrease in the number of fungal colonies for bread samples A, B, and C was noted compared to the control. However, the difference in the number of colonies for B is not statistically significant compared to sample C ($P < 0.05$). These data agree with the findings reported by Principato et al. (2019). The antifungal property of sourdough bread might be attributed to the organic acids and other metabolites produced by dominant strains growing during the fermentation.

4.6. *Sensory attributes*

The consumer acceptability of bread produced with CCS was evaluated. For this purpose, the sensory attributes of bread samples were determined using color, aroma, crumb structure, taste, texture, and overall acceptability properties (Table 7). According to the scores given by panelists, adding CCS to bread formulations did not affect the sensory attributes significantly ($P < 0.05$). The scores ranged from 3.60 to 4.57, revealing that the sourdough bread was acceptable. Color is an essential sensory attribute for consumers (Hathorn et al., 2008). Even though the increase in CCS caused a decrease in the score of the color attribute, the differences were not statistically significant ($P < 0.05$). The texture and aroma properties of samples demonstrated very close scores. Similar findings were reported by Gül et al. (2018) and Novotni et al. (2020). Aguilar et al. (2016) reported that adding chestnut sourdough did not affect consumers' perceptions adversely regarding crumb hardness, aroma, and taste. Similarly, Herken & Aydin (2015) and Caglar et al. (2013) reported no significant effect on the scores of the overall acceptability of tarhana soup supplemented with carob flour (up to 15%) in comparison with the control.

5. Conclusion

In this research work, we investigated the potential use of CCS to improve the nutritional quality of wheat bread and its consumer acceptability. Based on the results obtained, CCS positively affected the nutritional, textural, and sensory properties despite differences in dough rheology. The addition of CCS has not affected the sensory attributes

Table 7
Sensory properties of bread samples.

Attributes	A	B	C	Control
Color	4.47 ± 1.47 a	4.00 ± 1.00 a	3.93 ± 1.00 a	4.57 ± 1.76 a
Aroma	4.27 ± 1.27 a	4.27 ± 1.27 a	4.00 ± 0.90 a	4.29 ± 1.07 a
Crumb structure	4.07 ± 1.07 a	4.47 ± 0.53 a	4.47 ± 1.47 a	4.12 ± 0.94 a
Taste	4.20 ± 1.20 a	4.20 ± 1.20 a	3.60 ± 1.60 a	4.26 ± 1.23 a
Texture	3.80 ± 1.80 a	3.93 ± 1.33 a	3.93 ± 0.90 a	3.96 ± 1.50 a
Overall acceptability	4.13 ± 1.13 a	4.27 ± 1.27 a	3.99 ± 0.90 a	4.23 ± 1.03 a

A, B, C: sourdough bread samples containing 10, 20, and 30% CCS.
For a given row, the values with different letters are significantly different ($P < 0.05$).

significantly ($P < 0.05$). Therefore, as a natural ingredient, CCS can improve wheat bread's nutritional and textural quality to satisfy consumers' natural and healthy product expectations without decreasing its technological quality. In addition, the CCS addition may help delay mold growth. Future studies should focus on evaluating the effect of CCS on the digestibility of bioactive compounds (i.e., starch digestibility, phytate degradation, and improvement of mineral bioavailability) and the glycemic index of the wheat bread. Furthermore, the potential use of CCS in gluten-free bakery products should also be investigated.

CRediT authorship contribution statement

Hatice Eraslan and Jana Wehbeh: Investigation, Formal analysis, Writing - Original Draft. Ertan Ermis: Conceptualization, Methodology, Writing - Review & Editing, Project administration.

Implications for gastronomy

The incorporation of sourdough affects the texture and rheology of dough and of bread. It also contribute to the taste and aroma of bread. The inclusion of sourdough directs the fermentation of dough and has a positive impact on the texture, smell and taste of the baked product. It increases the storage life of products and may make them more digestible.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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