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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Carob Flour Addition to Sourdough: Effect of Sourdough Fermentation, Dough Rheology and Bread Quality

Ekşi Hamura Keçiboynuzu Unu İlavesi: Ekşi Hamur Fermentasyonuna, Hamur Reolojisine ve Ekmeklik Kalitesine Etkisi

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Abstract

Carob flour (CF) has been widely used in bakery formulations since containing various bioactive compounds, high percentage of fibre, protein, vitamins and minerals. Sourdough fermentation is considered as a promising tool to improve sensorial, textural and nutritional features of baked goods. The aim of this study is to investigate the potential usage of Type I sourdough incorporated with carob flour in bread making. Empirical rheological measurements were also performed in the flour blended with carob flour (0%, 2%, 4%, 6%, 8%, and 12%). Water absorption capacity and dough development time increased significantly with the increase of carob flour ($P \leq 0.05$). Energy and extensibility value of the samples decreased according to control sample ($P \leq 0.05$). Yeast number of sourdough decreased with the increase of carob flour addition to sourdough ($P \leq 0.05$). The stimulation of Lactic acid bacteria growth were determined by the addition of carob flour when compared to the control ($P>0.05$) but no significant differences were observed among sourdoughs with the increasing level of carob flour. Usage of carob flour via sourdough fermentation increased the quality properties of bread with sourdough as compared to the commercial baker's yeast bread including same level of carob flour. Highest concentration of the CF (8% and 12%) in formulation caused the raise of hardness ($P \leq 0.05$), which in turn was associated with the decrease in the specific volume of the bread. CF addition of yeasted bread samples (CFYB) decreased the lightness (L*) of the bread crust ($P \leq 0.05$). Sourdough fermentation was also improved the sensory acceptance of carob flour incorporated breads. Low level of carob flour usage (from 2% to 6%) in sourdough making was not detrimental to any of the sensory parameters in this study.

Keywords: Carob flour, Sourdough, Type I, Rheology, Bread quality

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Öz

Keçiboynuzu unu, çeşitli biyoaktif bileşikler, yüksek oranda lif, protein, vitamin ve mineral içerdiğinden fırıncılık ürünleri formülasyonlarında yaygın olarak kullanılmaktadır. Ekşi hamur fermantasyonu, fırıncılık ürünlerinin duyusal, tekstürel ve besinsel özelliklerini geliştirmede iyi bir araç olarak kabul edilmektedir. Bu çalışmanın amacı, keçiboynuzu unu ilave edilmiş Tip I ekşi hamurunun, ekmek yapımında kullanım potansiyelinin araştırılmasıdır. Keçiboynuzu unu ile karıştırılmış unlarda (%0, %2, %4, %6, %8 ve %12) ampirik reolojik ölçümler de yapılmıştır. Keçiboynuzu ununun artmasıyla su absorbsiyonu ve hamur gelişme süresi önemli ölçüde artmıştır ($P \leq 0.05$). Numunelerin enerji ve uzayabilirlik değeri kontrol numunesine göre azalmıştır ($P \leq 0.05$). Ekşi hamura keçiboynuzu unu ilavesinin artmasıyla ekşi hamurun maya sayısı azalmıştır ($P \leq 0.05$). Keçiboynuzu unu ilavesi ile ekşi hamurda, kontrole (%0 keçiboynuzu) göre Laktik asit bakterilerinin gelişiminin teşvik edildiği belirlenmiştir ($P \leq 0.05$). Fakat, artan keçiboynuzu ilavesi ile ekşi hamurlar arasında istatistiksel olarak bir fark gözlemlenmemiştir ($P>0.05$). Ekşi hamur fermentasyonu yolu ile keçiboynuzu unu kullanımı, aynı oranda keçiboynuzu unu içeren ticari mayalı ekmeğe göre ekmeğin kalite özelliklerini artırmıştır. Formülasyondaki en yüksek keçiboynuzu unu konsantrasyonu (%8 ve %12) sertliğin artmasına ($P \leq 0.05$) neden olmuştur. Bu durum aynı zamanda ekmeğin spesifik hacmindeki azalma ile ilişkilendirilmiştir. Ticari mayalı ekmeğe, keçiboynuzu unu ilavesi, ekmeklerin parlaklık (L*) değerini azaltmıştır ($P \leq 0.05$). Ekşi hamur fermantasyonu, keçiboynuzu unu katkılı ekmeklerin duyusal özelliklerini de geliştirmiştir. Ekşi hamur yapımında düşük seviyede keçiboynuzu unu kullanımı (%2-%6), bu çalışmadaki duyusal parametrelerin hiçbirini olumsuz yönde etkilememiştir.

Anahtar Kelimeler: Keçiboynuzu unu, Ekşi Hamur, Tip I, Reoloji, Ekmeklik Kalite

1. Introduction

Incorporation of nutritious foodstuffs into food formulations is considered as a tool to increase the consumption of healthy foods. Legumes (lentils, beans, carob etc.) and lupin are good examples of nutritious foodstuffs and provide potential beneficial health effects (Rizzello et al. 2014; Yaver and Bilgiçli, 2021). Bakery products, mainly breads since mostly consumed, are considered as a good carrier for functional compounds of nutritious foods. There are different types of manufacturing processes of bread making and sourdough bread making is one of them.

Sourdough is considered as a key element in traditional bread baking. The popularity of sourdough is increased gradually since contributing significantly to the flavour, texture and nutritional quality of breads (Arendt et al. 2007; Gobbetti et al. 2019). The use of sourdough fermentation is also one of the options to improve the sensory and functional quality of breads, containing nutritious foodstuffs. White bread enrichment is important for improving the nutritional density and fibre content since white bread is lack of the essential amino acid (lysine), minerals and vitamins. Many studies have been carried out to enhance the minerals, vitamins and other bioactive compounds of breads (Gawlik-Dziki et al. 2017; Hayta and İşçimen 2019; Ranjbar et al. 2019). Dietary fibre enrichment was also carried out such as inulin (Bojnanska et al. 2015; Rubel et al. 2015; Sirbu and Arghire 2017), sugar beet fibres (Filipovic et al. 2007), oat, and apple fibres (Kurek et al. 2018). Carob (*Ceratonia siliqua* L.) has also been used in order to enrich bakery products.

Carob is a kind of Mediterranean food, which contains low-molecular-weight carbohydrates, especially sucrose, high amounts of polyphenols and dietary fibre (Owen et al. 2003). Carob flour is milled from the pulp, seeds and germ of the carob fruit. The whole carob fruit contains 1–5 % w/w proteins, and 0.2–0.8 % w/w lipids, 9–13 % w/w crude fiber and 50–65 % w/w sugars. There are also significant amount of minerals (1–6 % w/w), mainly calcium, magnesium, sodium, phosphorus, and iron (Salinas et al. 2015). Different parts of the carob fruit can be used in various products such as beverages, confectionery and bakery goods. Salinas et al. (2015) conducted a study which includes carob flours from seed germ (G) and from fruit pulp (P) fractions in white bread production. Increasing levels of carob flour in breads showed lower specific volume and higher crumb firmness and chewiness although protein content for G breads was higher than wheat bread. Several studies have also been performed for incorporating the carob flour into cakes (Rosa et al. 2015), and biscuits (Vujić et al. 2014). Carob flour usage is very promising approach for gluten free formulations since having caroubin which is a specific water-insoluble protein isolated from carob bean embryo. Smith et al. (2010) reported that viscoelastic properties of caroubin are gluten-like and the formation of wheat-like dough is possible disulphide bonded high molecular weight proteins.

The adjustment of sensorial and technological parameters in bread formulation incorporated with nutritious foodstuffs is one of the first requirements to receive the consumer's acceptance. The manufacturing of the sourdough enriched with carob flour and incorporating this sourdough into white bread are the main objectives of this study. Three types of breads were produced as carob flour yeasted bread (CFYB), carob flour sourdough yeasted bread (CFSYB) and carob flour sourdough bread (CFSB). The comparisons with each other were performed in order to find optimal bread type in terms of sensorial and quality attributes. Chemical and microbiological characteristics of sourdough and the quality characterization of the breads were investigated.

2. Materials and Methods

2.1. Materials

Carob flour (CF) used in this study was obtained from local seller (Atışeri Ltd., Mersin). CF was obtained by the grinding of carob pulp and seed parts together. Wheat flour (Ova flour, Konya), bakery yeast (Saccharomyces cerevisiae), and salt used in the bread making were supplied commercially.

2.2. Physicochemical and empirical rheological analyses of the composite flours

American Association of Cereal Chemists (AACC) International Standard Methods were used for determining moisture (No: 44-15A), ash (No: 08-01), and sedimentation (No: 56-60A) values of the wheat flour and composite flours containing 0%, 2%, 4%, 6%, 8%, and 12% carob flour (w/w). Wet gluten contents and gluten index (No: 38-12) were also determined according to AACC International (2000).

Mixing properties of the wheat flour and the composite flours containing 0%, 2%, 4%, 6%, 8%, and 12% carob flour (w/w) were determined by Farinograf (Brabender, Farinograf AT, Germany) according to AACC Method

No: 54-21. Extensogram characteristics of the samples were determined by using AACC Method No: 54-10 (AACC International, 2000). All of the tests on the samples were performed at least in duplicate.

2.3. Sourdough Preparation

Type I sourdough was produced with the mixing of 187.5 g flour and 112.5 ml tap water (dough yield [dough weight X 100/flour weight], 160) and fermented at 25°C for 24 h. 30 g of fermented dough was subsequently added as an inoculum to start the fermentation of new mixture of flour (168.75 g) and water (101.25 ml). It is repeated five times to reach the constant technological properties (Ercolini et al. 2013). In addition, the composite flours using in sourdoughs were prepared by mixing carob and wheat flour at the ratio of 0%, 5%, 10%, 15%, 20%, and 30% (w/w) aiming at the production of the carob flour level in the final bread dough as 0%, 2%, 4%, 6%, 8%, and 12%, respectively.

2.4. Chemical and Microbiological Characterisations of Sourdough

The measurement of pH, Lactic acid bacteria (LAB) and yeasts enumeration was carried out to determine the technological properties of six different sourdoughs. 10 g sourdough was homogenized with 90 ml of sterile peptone water (1 g/L) for the enumeration of yeast and LAB. Yeast and LAB counts were determined by using Sabouraud dextrose agar (SDA, Oxoid) and De Man, Rogosa and Sharpe agar (MRS, Merck), respectively. The pH of the sourdough was measured by pH meter (HANNA instrument, Germany).

2.5. Bread Making

Carob flour yeasted bread (CFYB), carob flour sourdough yeasted bread (CFSYB) and carob flour sourdough bread (CFSB) were produced in this study. Carob flour sourdough yeasted bread (CFSYB) means that a bread containing both sourdough (Type I) and baker's yeast. Baker's yeast (*Saccharomyces cerevisiae*) was used in yeasted bread formulations. 100 g blended flour containing 0%, 2%, 4%, 6%, 8%, and 12% CF, 2 g bakery yeast, 1.5 g salt, and additional water determined by farinograph were mixed until the optimum dough development in the mixer (Öztiryakiler, OM10, Turkey) for the production of CFYB. 30% sourdough was added as dough basis in the formulation of CFYSB and CFSB. Both CFYB and CFYSB dough were fermented at 30°C and 65% humidity for 2 hours while CFSB fermented firstly at room temperature for 1 hour and then fermented at 30°C and 65% humidity for 22 hours. All bread samples were baked at 200°C for 30 min in stone based oven (Fimak, Turkey).

2.6. Quality Analysis of the Breads

Hardness (N) value of the breadcrumbs was obtained from texture profile analyser (SMS TA.XT2 Plus, UK). 5 kg load cell and 36 mm diameter cylindrical probe were used. Two slices of 25 mm thickness were cut from each loaf of bread and three measurements were performed for each bread sample. Bread volumes were measured according to rapeseed displacement method using a loaf volumeter (Simsek Laborteknik A.S., Turkey) after the cooling of the breads to room temperature. Colour values (L*, a*, b*) of the bread crumb and crust were determined by using the CIE L*a*b* colour system on Chromameter (CR-100 Konica Minolta, Japan). Duplicate measurements were carried out for each bread sample.

2.7. Sensory Analysis

A total of 14 untrained panellists between 25 and 40 years old participated in the sensory analysis. Crumb and crust colour, smell, taste, and overall acceptability were the parameters to evaluate each sample for quality attributes by the panellists. Each parameter was rated with a score from 1 to 5 (1 = dislike extremely, 3 = neither like or dislike, 5 = like extremely). Breads were served in randomly to the panellists and the means of the data were evaluated.

2.8. Statistical analysis

Statistical differences in the mean values of the results were determined by using one-way ANOVA with the JMP ver.6 software (SAS Institute, Inc. 2005).

3. Results and Discussion

3.1. Physicochemical and empirical rheological properties of the blended flours

Table 1 shows the physicochemical and mixing properties of the flour samples. Wheat flour using in this study was characterized with the highest moisture content (14.35%) and sedimentation value (37.50 ml) in all blended flours sample. Ash content for the flour samples ranged from 0.537 to 0.833 %. The higher carob flour addition to blended flours, the higher the ash content was obtained as expected since the carob flour contains high amounts of elements such as calcium, potassium, magnesium, sodium and phosphorus (Ayaz et al. 2009; Musa Özcan et al. 2007). The significant reduction was observed in the blended flour containing 6% and 8% CF in terms of wet gluten content ($P \leq 0.05$). Diluting effect caused by decreasing the amount of gluten was not observed in the gluten index value of the samples with the exception of the blended flour containing 4% CF as compared to control flour (0% CF). This result can be supported by the study of Kaur et al. (2013) who reported that any significant relationship between flour protein content and gluten index value. Addition of carob flour decreased the sedimentation value of the composite flour as compared to control (0% CF). The sedimentation value of flour is related to the swelling of the gluten fraction of flour in lactic acid solution (Shewry and Tatham 2000). In this study, swelling of the protein (gluten from wheat flour and caroubin from carob flour) were higher in the blended flour containing 6%-12% CF than the other blended samples. This can be the effect of water holding capacity of caroubin described as also gluten-like protein and dietary fibre content of CF (Tsatsaragkou et al. 2014).

Increase in the level of CF additions provided an increase in water absorption (WA, %), as shown in *Table 1*, from 58.38 % (control flour, 0%CF) to 60.95 % (blended flour containing 12% CF), which is in agreement with the observations of Miś et al. (2012). Wang Jinshui et al. (2002) previously found that different fibres including carob fibre increased water absorption capacity of the wheat flour. This phenomena was caused by the hydroxyl groups in the fibre structure since allowing more water interactions through hydrogen bonding, as was previously reported by Rosell et al. (2001). Similar trend was also observed in stability value, which was increased from 5.04 min for 0% CF to 11.10 min for 12% CF. The dough development time initially increased significantly with addition of 2% and 4% CF and then decreased in the addition level of above 4% *(Table 1).* These results are in line with the study carried out on bread dough enriched with carob fibre and oat wholemeal, in which the dough development time and stability increased up to 2% for carob fibre addition (Miś et al., 2012). However, some studies demonstrated that carob fibres did not modify the dough development time or the stability value (Wang Jinshui et al. 2002).

Table 1. Physicochemical and mixing properties of blended flours

Carob Flour Level (%)	Moisture (%)	Ash (%)	Sedimentation value	Wet Gluten Content (%)	Gluten index (%)	WA (%)	DDT (min)	Stability (min)
0	14.35±0.07^{a}	0.537±0.004^{e}	37.50±0.71^{a}	26.35±0.07^{ab}	99.10±0.14^{a}	58.38±0.03^{c}	2.12±0.01^{c}	5.04±0.02^{e}
2	14.15±0.07^{ab}	0.572±0.002^{d}	29.50±0.71^{c}	27.40±0.71^{a}	97.45±1.63^{ab}	58.60±0.42^{c}	8.24±0.01^{a}	8.20±0.14^{d}
4	14.15±0.07^{ab}	0.642±0.001^{c}	26±1.41^{d}	27.10±0.42^{a}	94.30±1.98^{b}	59.90±0.14^{b}	8.43±0.01^{a}	9.85±0.45^{b}
6	13.90±0.14^{bc}	0.828±0.006^{b}	33.50±2.12^{b}	25.25±0.92^{bc}	97.70±0.14^{ab}	60.30±0.57^{ab}	7.42±0.02^{b}	9.30±.0.02^{c}
8	13.85±0.07^{bc}	0.829±0.001^{b}	33.00±1.41^{b}	24.70±0.14^{c}	98.65±1.48^{a}	60.55±0.49^{ab}	7.66±0.54^{ab}	9.50±0.13^{bc}
12	13.70±0.28^{c}	0.833±0.004^{b}	32.50±0.71^{bc}	ND.	ND.	60.95±0.07^{a}	7.78±0.61^{ab}	11.10±0.12^{a}

[a] Dry basis
Means with a same letter within a column are not significantly different ($P \leq 0.05$).

According to the extensographs results *(Table 2),* wheat flour (0% CF) showed greater energy value (cm^2) than composite flours. Both energy and extensibility value decreased with the addition of CF, but no gradual reduction was detected. Maximum resistance to extension (Rm, BU) increased significantly above 4% CF addition. There were no significant differences between 0% and 2% CF samples in terms of the ratio number. With increase in the level of CF from 2% to 12%, the ratio number of dough gradually increased. As proving time is extended (45, 90, and 135 min), energy value, Rm, and ratio number increased while extensibility (mm) decreased. The extended proving times (45, 90, and 135 min) were not affect the energy and extensibility value (P>0.05) in wheat flour (0% CF).

Table 2. Extensogram properties of blended flours

	Carob Flour Level (%)	45 min.	90 min.	135 min.
Energy (cm^2)	0	$133.00±5.20^{aA}$	$162.00±12.58^{aA}$	$146.67±27.39^{aA}$
	2	$122.33±1.53^{bB}$	$152.67±13.58^{abA}$	$147.67±8.08^{aA}$
	4	$120.00±4.36^{bB}$	$139.00±7.81^{bA}$	$146.33±11.15^{aA}$
	6	$118.67±9.87^{bB}$	$146.67±6.66^{abA}$	$145.67±11.06^{aA}$
	8	$104.67±2.08^{cB}$	$134±10.82^{bcA}$	$128.00±5.29^{abA}$
	12	$97.33±3.21^{cB}$	$115.00±13.45^{cA}$	$117.67±3.06^{bA}$
Extensibility (mm)	0	$174.00±7.94^{aA}$	$159.33±21.55^{aA}$	$126.67±43.56^{abcA}$
	2	$175.00±5.20^{aA}$	$149.67±13.20^{aA}$	$149.67±20.60^{aA}$
	4	$160.67±6.81^{bA}$	$117.33±28.68^{aB}$	$134.33±2.89^{abAB}$
	6	$135.33±11.06^{cA}$	$114.67±4.62^{bcB}$	$108.67±2.89^{bcdB}$
	8	$115.67±3.51^{dA}$	$110.33±13.58^{bcAB}$	$94.00±7.21^{cdB}$
	12	$105.00±1.73^{dA}$	$86.67±2.08^{cB}$	$90.33±3.51^{dB}$
Maximum Resistance to Extension (BU)	0	$634.00±12.49^{bB}$	$880.67±41.63^{dA}$	$904.67±20.40^{cA}$
	2	$625.33±7.09^{bC}$	$846.00±42.16^{dB}$	$906.00±25.63^{cA}$
	4	$640.67±37.50^{bB}$	$967.33±22.30^{cA}$	$1006.33±28.43^{bA}$
	6	$700.67±40.38^{aB}$	$1110.67±46.70^{abA}$	$1165.67±40.08^{aA}$
	8	$710.00±18.36^{aC}$	$1104.67±24.17^{bB}$	$1202.33±5.51^{aA}$
	12	$732.33±20.74^{aB}$	$1167.67±25.28^{aA}$	$1171.00±21.70^{aA}$
Ratio Number	0	$2.63±0.23^{deB}$	$4.03±0.68^{dA}$	$4.80±0.85^{cdA}$
	2	$2.50±0.20^{eC}$	$3.77±0.12^{dB}$	$4.70±0.56^{dA}$
	4	$3.10±0.17^{dB}$	$5.83±0.60^{cA}$	$6.07±0.25^{cA}$
	6	$4.20±0.52^{cB}$	$8.30±0.26^{bA}$	$9.37±0.93^{bA}$
	8	$5.47±0.15^{bC}$	$8.93±0.81^{bB}$	$12.30±1.00^{aA}$
	12	$6.47±0.23^{aB}$	$12.67±1.01^{aA}$	$12.93±0.67^{aA}$

Means with a same lower-case letter within a column are not significantly different (P ≤ 0.05).
Means with a same upper-case letter within a row are not significantly different (P ≤ 0.05).

3.2. Chemical and Microbiological Characteristics of Sourdough

Six sourdoughs were produced at different carob flour percentage ranging from 0% to 30% in order to ensure the carob flour level of 0% to 12% in the final bread dough. The pH of the sourdoughs during refreshment procedure was shown in *Figure 1*. The sourdough containing 5% of CF showed the highest pH (~5.50) in all on the day 0. Esteve et al. (1994) suggested that pH of well-developed sourdough ranged from 3.5 to 4.3. The pH of all sourdoughs was in this range on the 2nd day except from the sourdough containing 30% CF. The sourdough containing from 0% to 10% CF reached the constant the pH after two refreshment procedure referring day 3rd while the sourdoughs including higher levels of CF (15% - 30%) became constant on the day 4th. The variations between the flours caused such differences in the pH of the sourdough. Complementary studies reported that sourdoughs achieved constant pH after 5 to 7 days of propagation (Ercolini et al. 2013). In a study of conducted by Rizzello et al. (2014), five days were required for wheat–legume sourdough to become almost constant. The pH of faba bean sourdoughs also stabilized from the day 5 onward (Coda et al. 2017).

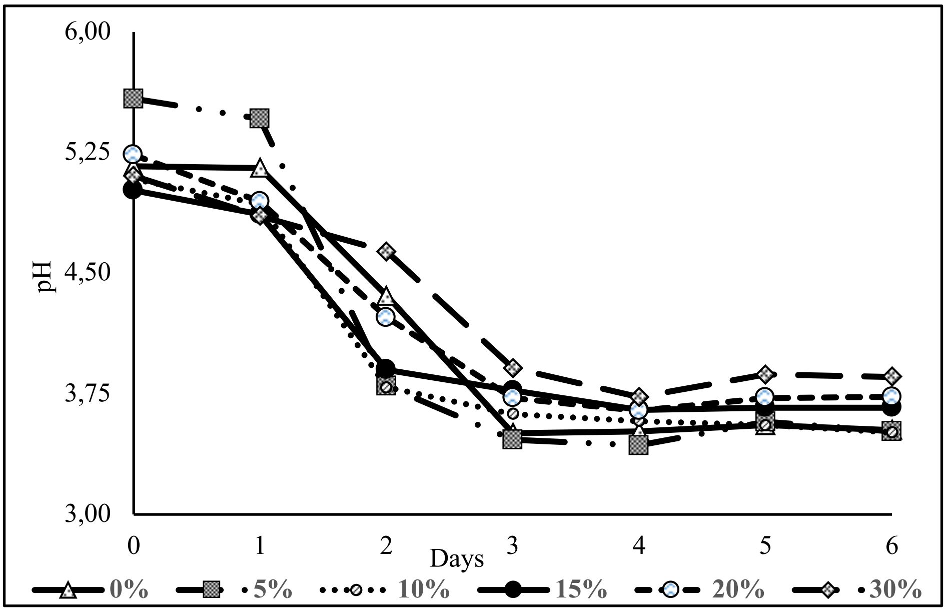

Figure 1. The pH of the sourdoughs during refreshments

Fermentation steps determine the microbial stability of sourdough since adaptation of sourdough LAB and yeast depended the environmental conditions. Several studies demonstrated that numbers of LAB ranged from 6 to 9 log CFU/g and yeasts from 5 to 8 log CFU/g in the mature sourdough (Minervini et al. 2012). Yeast cell densities of this study ranged from 5.10±0.02 log CFU/g (30% CF sourdough) to 7,43±0,08 log CFU/g (0% CF sourdough) in accordance with the results of previous studies (Lattanzi et al. 2013; Minervini et al. 2012). CF addition of sourdough caused a significant reduction of yeast cell densities of sourdough *(Table 3).*

Table 3. Yeast and LAB counts of sourdoughs enriched of carob flour (Log cfu/g)

		Carob Flour Level (%)					
Days		**0**	**5**	**10**	**15**	**20**	**30**
	0	6.04±0.06^{cA}	5.38±0.22^{dB}	4.05±0.02^{cC}	3.81±0.09^{eC}	3.50±0.09^{eD}	3.46±0.11^{dD}
	1	6.26±0.20^{cA}	5.37±0.13^{dB}	4.19±0.01^{cCD}	3.99±0.08^{eDE}	3.74±0.06d^{eE}	4.34±0.17^{cC}
	2	6.98±0.20^{bA}	6.16±0.25^{cB}	6.35±0.23^{bB}	4.96±0.12^{dC}	3.92±0.13^{dD}	4.56±0.12^{bcC}
	3	7.19±0.22^{abA}	6.60±0.25^{bcB}	6.16±0.10^{bC}	5.25±0.11^{cD}	4.73±0.16^{cE}	4.74±0.11^{bE}
	4	7.34±0.13^{abA}	6.87±0.06^{abB}	7.17±0.02^{aA}	5.62±0.13^{bC}	5.15±0.11^{bD}	4.82±0.11^{abE}
	5	7.43±0.08^{aA}	7.26±0.03^{aAB}	7.20±0.05^{aB}	6.59±0.13^{aC}	5.63±0.13^{aD}	5.10±0.02^{aE}
LAB (Log cfu/g)	**0**	6.09±0.08^{cD}	7.62±0.19^{cA}	7.46±0.25^{bA}	7.18±0.04^{cAB}	6.86±0.01^{cBC}	6.44±0.36^{cCD}
	1	7.23±0.04^{bcD}	9.23±0.26^{bA}	8.96±0.11^{aAB}	8.52±0.41^{bBC}	8.42±0.01^{bC}	8.15±0.19^{bC}
	2	7.78±0.99^{bB}	9.26±0.04^{bA}	9.26±0.37^{aA}	9.17±0.08^{aA}	9.09±0.49^{aA}	9.04±0.01^{aA}
	3	8.07±0.08^{bB}	9.46±0.37^{abA}	9.37±0.37^{aA}	9.25±0.10^{aA}	9.13±0.31^{aA}	9.09±0.28^{aA}
	4	8.22±0.02^{abB}	9.49±0.33^{abA}	9.45±0.02^{aA}	9.36±0.28^{aA}	9.22±0.06^{aA}	9.20±0.12^{aA}
	5	9.33±0.75^{aA}	9.97±0.25^{aA}	9.57±0.52^{aA}	9.51±0.04^{aA}	9.41±0.26^{aA}	9.25±0.03^{aA}

Means with a same lower-case letter within a column are not significantly different ($P \leq 0.05$).
Means with a same upper-case letter within a row are not significantly different ($P \leq 0.05$).

The growth of LAB were stimulated by the addition of CF according to the control (P>0.05) but no significant differences were observed among sourdoughs with the increasing level of CF. LAB cell densities were between 9.25±0.03 and 9.97±0.25 log CFU/g on the day 5 *(Table 3)* as reported by Ercoloni et al. (2013) that presumptive LAB reached stable values above 9.0 log CFU g^{-1} in mature sourdough. Some studies also indicated that the cell densities of LAB was 9 log CFU/g and stayed almost constant after the second day of sourdough. This was a quite common observation for cereal sourdoughs or cereal-legume mixtures (Van der Meulen et al. 2007). In a similar manner, addition of CF to the sourdough ecosystem promoted LAB counts significantly after 1st fermentation referring day 1. The LAB cell numbers of these doughs above 9.0 log CFU g^{-1} on the day 2 while 0% CF sourdough reached on the day 5 *(Table 3)*. Coda et al. (2017) previously observed during sourdough-type propagation of faba bean flour and the cell density of LAB was found 9.7 ± 0.1 and 9.9 ± 0.1 log CFU/g for Italian and Finish cultivar faba bean sourdoughs on the day 2, respectively. On the other hand, the cell densities of LAB reached the values of 8.6, 9.0, 8.7, and 9.5 Log CFU/g in spontaneous legume fermentations for bean, chickpea and wheat-legume sourdoughs after 5 days (Rizzello et al. 2014).

3.3. Quality Characteristic of Breads

Bread characteristics were evaluated on carob flour yeasted bread (CFYB), carob flour sourdough yeasted bread (CFSYB) and carob flour sourdough bread (CFSB). Specific volume of CFYB samples prepared by blended flour showed higher values than CFSYB and CFSB. The specific volume of all breads decreased with increase in the addition level of CF *(Fig. 2A)*.

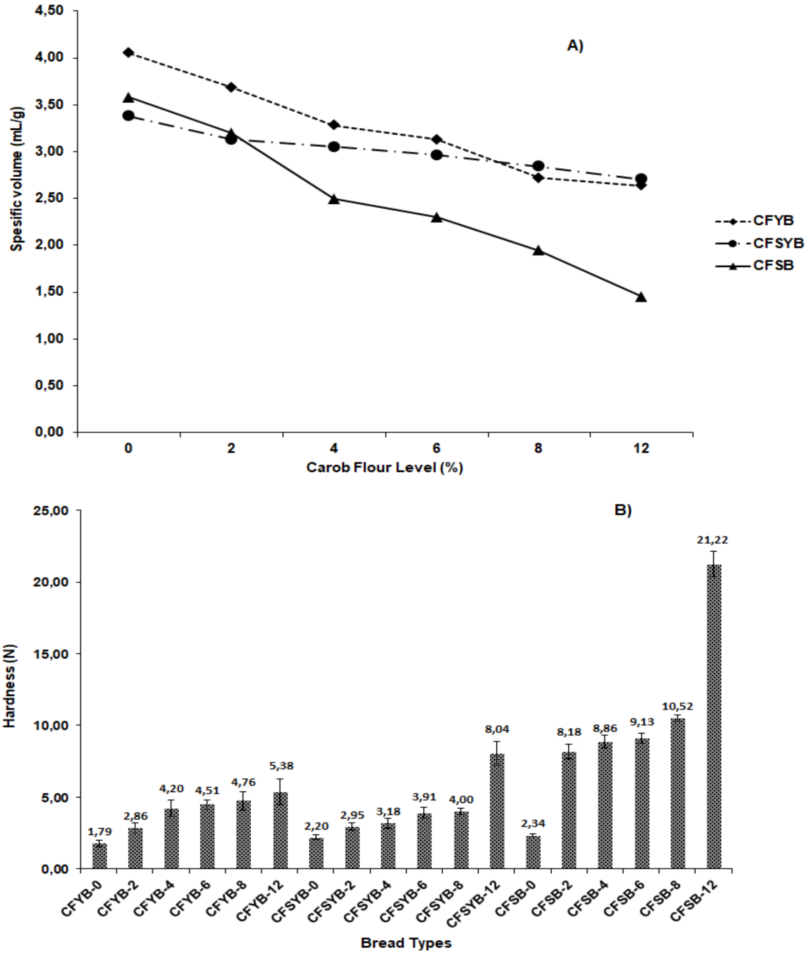

Figure 2. Physical properties of breads

A) Specific volume (ml/g) according to carob flour level, **B)** Hardness value (N) according to bread types. **CFYB:** Carob flour yeasted bread, **CFSYB:**Carob flour sourdough yeasted bread, **CFSB:** Carob flour sourdough bread. Means with a same letter are not significantly different (P ≤ 0.05). Each mean has been compared among its own bread types.

Salinas et al. (2015) reported that the increment of carob seed germ diminished the specific volume of breads gradually. Contrary to these results, Turfani et al. (2017) reported that the bread blended with 10% refined CF produced a statistically significant higher loaf volume than control bread. Different parts of the carob fruit such as germ and pulp cause different influences on wheat dough performance and bread quality (Salinas et al. 2015). This could be the reason of dissimilar findings in some research. CFSYB samples showed higher specific value than CFSB samples, probably due to the effect of bakery yeast in formulation. *Figure 2* presents also hardness value of breads. Highest concentration of the CF in formulation caused the raise of hardness *(Fig. 2B),* which in turn was associated with the decrease in the specific volume of the bread. This was not in agreement with the results of Wang Jinshui et al. (2002) who found the crumb softness effect produced by the 3% carob fibre supplementation in wheat bread. This difference is due to using carob flour in this study rather than fiber. Carob flour may contain many components that affect the dough and bread structure such as phenolic compounds, sugar and proteins besides fibres (Durazzo et al. 2014; Youssef et al. 2013). Total effect of these nutrients especially phenolic compounds might be the description of the variability of dough structure and bread properties (Han and Koh 2011).

The effect of legume flours (chickpea, lentil, bean, and carob) on the texture of baked goods has been well studied and generally associated to a decreased volume of the bread and an increased hardness of the loaves (Kohajdová et al. 2013; Salinas et al. 2015). Rizzello et al. (2014) performed a study in order to enhance the nutritional, texture and sensory characteristics of white bread by using sourdough prepared with wheat, chickpea, lentil and bean flours. Softer breads were obtained by using 15% (w/w) of legume flours as a consequence of the sourdough fermentation. Similarly, carob flour usage in bread formulation via sourdough fermentation (CFSYB) revealed the softer breads as compared to CFYB (Figure 2B) except for the sample CFSYB-2. CF addition of yeasted bread samples (CFYB) decreased the lightness (L*) of the bread crust *(Table 4).*

Table 4 Crust and crumb characteristics of the breads

	Crust Color			**Crumb Color**		
Bread Types	**L***	**a***	**b***	**L***	**a***	**b***
CFYB0	47.81±2.22^{cd}	11.29±0.21^{d}	14.52±2.27^{c}	69.70±0.75^{b}	-1.78±0.06^{i}	12.96±0.29^{cd}
CFYB2	43.12±0.39^{f}	9.08±0.30^{f}	12.47±0.44^{def}	47.69±0.71^{e}	4.45±0.18^{j}	12.12±0.39^{def}
CFYB4	43.17±1.96^{f}	8.58±1.17^{f}	11.67±1.12^{ef}	41.01±1.43^{g}	6.12±0.23^{h}	11.71±1.00^{fg}
CFYB6	43.22±1.68^{f}	6.92±0.48^{g}	11.59±0.68^{ef}	40.86±3.41^{g}	7.10±0.37^{f}	11.53±2.06^{fg}
CFYB8	42.95±1.71^{f}	6.74±0.29^{g}	11.31±0.66^{fg}	35.20±0.67^{i}	7.65±0.13^{e}	8.30±0.48^{i}
CFYB12	42.07±1.44^{f}	6.68±0.45^{g}	9.78±1.50^{gh}	32.01±1.02^{j}	8.13±0.27^{d}	6.29±0.95^{j}
CFSYB0	54.39±3.11^{a}	14.03±1.09^{b}	21.84±1.96^{a}	76.84±0.91^{a}	-0.69±0.06^{k}	13.85±0.34^{bc}
CFSYB2	50.21±2.72^{b}	12.62±0.65^{c}	17.51±1.92^{b}	57.57±1.15^{c}	4.84±0.32^{i}	16.57±0.21^{a}
CFSYB4	48±1.26^{cd}	11.52±0.21^{d}	14.61±1.09^{c}	50.84±1.27^{d}	7.44±0.21^{e}	16.70±0.48^{a}
CFSYB6	46.39±2.24^{de}	11.49±0.12^{d}	13.58±0.84^{cd}	43.09±1.83^{f}	9.11±0.45^{c}	14.46±1.01^{b}
CFSYB8	46.3±2.09^{de}	11.41±0.27^{d}	12.93±1.31^{de}	41.92±2.85^{fg}	9.16±0.39^{c}	14.22±1.59^{b}
CFSYB12	42.04±1.00^{f}	11.31±0.25^{d}	8.98±1.07^{hi}	37.12±0.99^{h}	10.49±0.08^{a}	11.97±0.47^{efg}
CFSB0	49.39±2.32^{bc}	15.92±1.20^{a}	23.31±2.94^{a}	68.14±1.08^{b}	-0.81±0.16^{k}	12.17±0.70^{def}
CFSB2	45.45±0.78^{e}	12.47±1.86^{c}	14.00±0.72^{cd}	46.41±1.11^{e}	6.49±0.14^{g}	12.93±0.43^{cd}
CFSB4	38.38±0.86^{g}	11.25±0.16^{d}	7.51±0.79^{i}	40.72±0.11^{g}	8.14±0.10^{c}	12.82±0.27^{de}
CFSB6	35.90±0.36^{h}	10.41±0.15^{e}	4.59±0.72^{j}	37.23±0.30^{h}	8.96±0.17^{d}	11.17±0.24^{gh}
CFSB8	35.50±0.43^{hi}	10.09±0.18^{e}	3.66±0.55^{j}	35.85±0.61^{hi}	9.20±0.23^{bc}	10.24±0.65^{h}
CFSB12	33.61±0.33^{i}	7.19±0.26^{g}	-0.23±0.36^{k}	33.22±0.31^{j}	9.45±0.09^{b}	8.16±0.35^{i}

Means with a same lower-case letter within a column are not significantly different ($P \leq 0.05$).

This can be associated with the increase in browning (Purlis and Salvadori 2007). No significant effect was observed with the increasing level of CF in opposition to the results reported by Turfani et al. (2017), who observed that browning increased significantly upon increasing the content of raw CF in bread formulation. On the other hand, dramatic decreases as a regular trend was seen for L* value of both CFSYB and CFSB samples. Redness (a*) value of the crust of all breads containing CF decreased as compared to control (0% CF), but the reduction depending on CF amount was insignificant for CFYB6-CFYB12, CFSYB4-CFSYB12, and CFSB6-CFSB8. Similar results were also obtained for yellowness (b*) of the bread crust. CF addition cause a reduction of L* value while a* value of the crumb color of all breads increased, significantly. High level of CF addition (8%-12%) cause a drastic reduction of b* value of breadcrumbs.

3.4. Sensory Evaluation

The sensory attributes (crust and crumb colour, taste, odour, chewiness and overall acceptance) of the breads are presented in *Figure 3*.

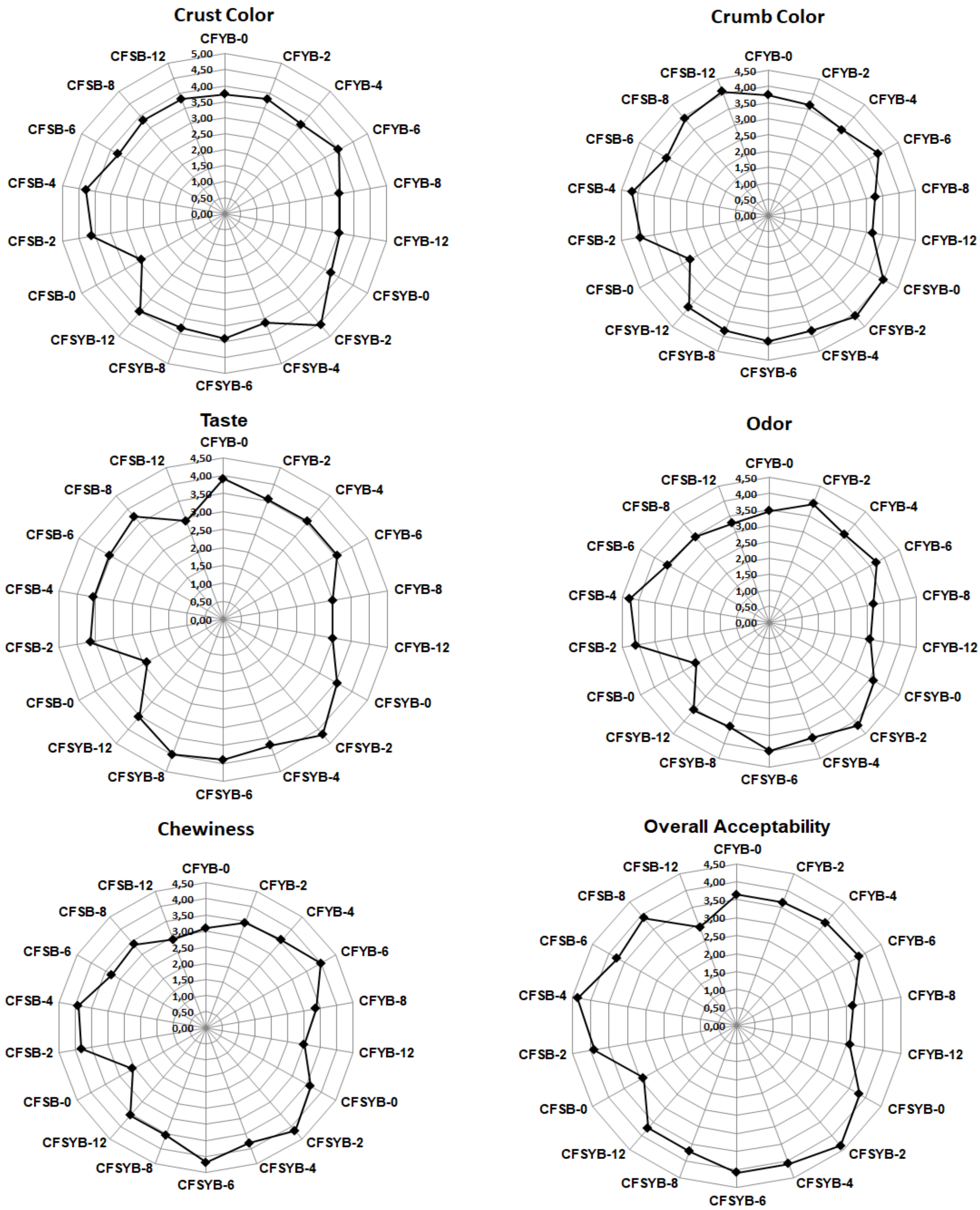

Figure 3. Sensory evaluation of breads.

CFYB: Carob flour yeasted bread, **CFSYB:**Carob flour sourdough yeasted bread, **CFSB:** Carob flour sourdough bread.

Breads produced in this study were considered as acceptable if their mean scores for overall acceptability were above 3 (neither like nor dislike). All breads were considered acceptable by the panellists with the exception of sourdough bread containing no carob flour (CFSB-0) and high-level carob flour (CFSB-12).

All level of carob flour yeasted bread (CFYB) was also acceptable and CFYB-6 sample was mostly preferred in all since it was scored 3.82. Wang Jinshui et al. (2002) reported that addition of carob fibre with the level of 3% to wheat bread considered as acceptable for each specific sensory characteristic and overall acceptability. Low level of carob flour sourdough bread for both yeasted (CFSYB-2) and non-yeasted (CFSB-4) breads were scored higher values in overall acceptability parameter. Results from sensory evaluation indicated that the panellists preferred carob flour sourdough bread (CFSYB and CFSB) rather than carob flour yeasted bread (CFYB) in all the sensory parameters, maybe due to flavour contribution of sourdough. Sourdough bread containing no carob flour (CFSB-0) was poorly accepted although this bread exhibited higher specific volume *(Fig. 2A)* and softer texture *(Fig. 2B).* This can be explained that carob flour conferred a distinctive taste in sourdough bread and the panellists preferred this taste. This is also a good result since showing that low level of carob flour usage (from 2% to 6%) in sourdough making was not detrimental to any of the sensory parameters in this study.

4. Conclusions

Sourdough fermentation was used in this study as an option to improve the sensorial and technological quality of breads containing carob flour. Carob flour blends showed good performances in terms of water absorption and stability value. The addition of CF caused no gradual reduction for both energy and extensibility value. The microbial growth during refreshment procedure of sourdough made with CF was also evaluated for the first time in this study. LAB growth was promoted by CF incorporated sourdoughs while significant reduction were observed for yeast. Carob flour addition revealed the softer breads thanks to sourdough fermentation (CFSYB) as compared to directly usage of CF in bread formulation (CFYB). Moreover, CF addition to bread by sourdough fermentation was more acceptable in terms of sensory analyses. Consequently, there is a great interest to enrich the flour used in sourdough making with nutritious foodstuffs especially legumes (bean, lentil, carob, etc.) to improve the bread organoleptic and functional quality. The present study focused on the pre-requirements of carob flour sourdough bread making before the application of industrial level.

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