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Use of locust bean flour as a substitute for cocoa in the production of chocolate spread: Quality attributes and storage stability

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SUMMARY: The effects of locust bean (carob) flour (LBF) as a substitute for cocoa in the production, quality attributes, and storage stability of chocolate spreads were investigated. CON (4.5% cocoa), CF15 (3.0% cocoa + 1.5% LBF), CF30 (1.5% cocoa + 3.0% LBF), and CF45 (4.5% LBF) formulations were produced, and stored at 22 and 35 °C for 12 weeks. Appearance, odor, sweetness, color, and overall acceptability scores decreased with increasing LBF, but up to 3.0% LBF did not affect the scores compared to the CON. Replacing cocoa with LBF at a low level resulted in higher hardness and spreadability. Hardness, free fatty acid, and peroxide values (PV) increased, a_w values generally decreased during storage, but PV was still lower than 10 meq O_2/kg. As the LBF ratio increased, darkening occurred in the chocolates. Thus, up to 3% of LBF can be used as a cocoa substitute with minimal quality and sensory changes in the production of chocolate spread.

KEYWORDS: *Chocolate spread; Cocoa; Locust bean flour; Quality attributes; Storage stability; Substitute.*

RESUMEN: ***Uso de harina de algarroba como sustituto del cacao en la elaboración de chocolate para untar: atributos de calidad y estabilidad en almacenamiento.*** Se investigó el efecto de la harina de algarroba (LBF) como sustituto del cacao en la producción, los atributos de calidad y la estabilidad en almacenamiento de las cremas de chocolate. Se produjeron las formulaciones CON (4,5% cacao), CF15 (3,0% cacao + 1,5% LBF), CF30 (1,5% cacao + 3,0% LBF) y CF45 (4,5% LBF), y se almacenaron a 22 y 35°C durante 12 semanas. Las puntuaciones de apariencia, olor, dulzor, color y aceptabilidad general disminuyeron al aumentar el porcentaje de LBF, pero hasta un 3,0 % de LBF no afectó a las valoraciones en comparación con el CON. Reemplazar el cacao con LBF en un nivel bajo resultó en una mayor dureza y capacidad de untar. Los valores de dureza, ácidos grasos libres y peróxido (PV) aumentaron, los valores de a_w generalmente disminuyeron durante el almacenamiento, pero el PV aún era inferior a 10 meq O_2/kg. A medida que aumentó la proporción LBF, se produjo un oscurecimiento en los chocolates. Por lo tanto, hasta un 3% de LBF se puede utilizar como sustituto del cacao con cambios sensoriales y de calidad mínimos en la producción de chocolate para untar.

PALABRAS CLAVE: *Atributos de calidad; Cacao; Chocolate para untar; Estabilidad en almacenamiento; Harina de algarroba; Sustituto.*

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1. INTRODUCTION

Chocolate is one of the most preferred confectionery products in the world due to its attractive taste, wonderful smell, and beautiful appearance (Koca, 2011; Yadav *et al*., 2011; Barisic *et al*., 2021). This product is a complex emulsion based on cocoa, and it activates the pleasure centers of the human brain when consumed, thanks to its flavor. Chocolate, besides cocoa, usually contains milk and powdered milk; it may also contain sugar and nuts, depending on the product category (Mexis *et al*., 2010). Today, chocolates can be produced in various colors and shapes. The main types of chocolate are dark, milk, and white, which differ in their cocoa solids, milk fat, and cocoa butter contents (Afoakwa *et al*., 2007; Rossini *et al*., 2011). In addition, there are many types of chocolate, such as plain, hazelnut, pistachio, milk, coconut, grape, liqueur, and croquet (Koca, 2011).

Cocoa, the primary raw material of chocolate and similar products, is a product derived from the beans of the fruit of the cocoa tree (*Theobroma cacao*), which is native to South America (Bhattacharjee and Kumar, 2007; Yadav *et al*., 2011; Loullis and Pinakoulaki, 2018). Cocoa powder is one of the most valued commodities worldwide thanks to its characteristic pleasant flavor and aroma. Among its applications in the food industry, the formulation of beverages, confectionery, bakery, and pastry products stands out. Besides taste and aroma, cocoa is highly appreciated as a natural coloring agent, partly because of the current tendency to restrict artificial colors (Quelal-Vasconez *et al*., 2018). Also, cocoa polyphenols are bioactive compounds with antioxidant and anticarcinogenic properties (Pawłowska *et al*., 2018). On the other hand, cocoa contains substances that can cause health problems, such as theobromine and caffeine. Theobromine is a stimulant for muscular activity, and its high consumption was reported to cause harmful symptoms such as excessive stimulation of the kidneys, heart, and smooth muscles (Bhattacharjee and Kumar, 2007). In addition, the constant increase in prices and resource constraints of cocoa because of high worldwide demand prompted some researchers to develop cocoa substitutes (Loullis and Pinakoulaki, 2018; Akdeniz *et al*., 2021).

The locust bean (LB) (*Ceratonia siliqua*), also known as carob, does not contain substances that can cause health problems, such as theobromine and caffeine, and is cheaper than cocoa; it has been used in human nutrition for centuries (Avallone *et al*., 1997). It is considered a natural sweetener with an appearance and flavor similar to chocolate, so it is widely applied in food professions as a cocoa substitute in different products such as drinks, ice cream, cakes, and candies. LB powder has a high dietary fiber concentration and can be categorized as a product with high fiber (Akdeniz *et al*., 2021). Few studies have shown that locust bean flour (LBF) can used as a cocoa substitute in chocolate production. For example, Salem and Fahad (2012) reported that adding LB pod powder to milk chocolate enhanced its nutritional value and functional and sensory properties, and LB powder is a valuable ingredient that can replace cocoa pod powder in the manufacturing of chocolate products. Likewise, Akdeniz *et al*. (2021) determined that it is possible to utilize LB powder to replace cocoa powder in chocolate production to improve nutritional values (higher fiber and fewer calories) with pleasant sensory attributes.

Food processors increasingly seek to efficiently use unconventional raw materials of plant origin that are undervalued as food ingredients. This work will help further research into the valorization of LBF, and therefore contribute to developing strategies to produce innovative value-added chocolate formulations. In addition, this is important because the generation of new products such as chocolate spreads, where the LBF is used as a substitute for cocoa in the production of chocolate, would meet consumer expectations and ensure a more sustainable use of resources.

As mentioned above, although the quality characteristics of chocolates containing LB have been examined in very few studies, there needs to be a study about these products' storage stability. Because chocolates are fatty products, their shelf-life is limited due to lipid oxidation and other changes during storage. Lipid oxidation produces off-flavors and decreases the foods' nutritional quality, safety, and shelf-life (Rossini *et al*., 2011). Therefore, in our experiment, we have focused on monitoring the effect of LBF as a substitute for cocoa in producing chocolate spread on the quality attributes and storage stability. For this purpose, we determined the chocolates' proximate composition and sensory properties immediately after production along with hardness, spreadability, water activity, color properties, free fatty acid (FFA), and peroxide values during storage at 22 and 35 °C.

2. MATERIALS AND METHODS

2.1. Materials

In producing chocolate spreads, cocoa butter, palm oil, powdered sugar, milk powder, whey powder, hazelnut paste, starch, lactose, cocoa, soy lecithin, hazelnut flavor, chocolate flavor, and vanillin were used. These materials were obtained from Nutpa Gıda Sanayi ve Ticaret A.Ş (Ordu, Turkey), where the chocolates were produced. The LBF used in the chocolate production was purchased from Aşçı Baharatları (Ordu, Turkey). All the chemicals used in the analyses were of analytical purity and obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation and storage of chocolate spreads

Chocolate spreads were produced in Nutpa Gıda Sanayi ve Ticaret A.Ş., according to the procedure suggested by the company, using a ball mill with a 20-kg mixer. Four different chocolate spread formulations were included in the experimental design, and the first formulation, which contained 4.5% cocoa but not LBF, was taken as the control formulation (CON). The second formulation was composed of 3.0% cocoa + 1.5% LBF (CF15), the third formulation was 1.5% cocoa + 3.0% LBF (CF30), and the fourth formulation was 4.5% LBF (CF45) (Table 1). For the preparation of chocolate spreads, the cocoa butter was mixed in a ball mill at 42 °C until melted, and palm oil and lecithin, then sugar, milk powder, cocoa, whey powder, starch, hazelnut paste, and lactose were added. After mixing for one hour, the thickness of the mixture was measured, and when the particle size fell below 30 µm, hazelnut flavor, chocolate flavor, and vanillin were added. The chocolate spreads, prepared for 3 hours, were mixed for about 10 more minutes, filled into 350g glass jars, and after cooling to room temperature, stored for 12 weeks in warehouses at 22 and 35 °C. The chocolate spreads were analyzed for moisture, protein, fat, and sensory properties on the day of production, texture, water activity, color, free fatty acidity, and peroxide value at the beginning of storage and at the 4th, 8th, and 12th weeks.

Table 1. Formulations of chocolate spreads produced with different proportions of locust bean flour (%)

	Formulations			
	CON	CF15	CF30	CF45
Cocoa butter	9.00	9.00	9.00	9.00
Palm oil	15.00	15.00	15.00	15.00
Powdered sugar	38.50	38.50	38.50	38.50
Milk powder	6.00	6.00	6.00	6.00
Whey powder	4.90	4.90	4.90	4.90
Hazelnut paste	15.00	15.00	15.00	15.00
Starch	2.50	2.50	2.50	2.50
Lactose	4.00	4.00	4.00	4.00
Cocoa	4.50	3.00	1.50	-
Locust bean flour	-	1.50	3.00	4.50
Soy lecithin	0.50	0.50	0.50	0.50
Hazelnut flavor	0.04	0.04	0.04	0.04
Chocolate flavor	0.04	0.04	0.04	0.04
Vanilla	0.02	0.02	0.02	0.02

CON, 4.5% cocoa; CF15, 3.0% cocoa + 1.5% locust bean flour; CF30, 1.5% cocoa + 3.0% locust bean flour; CF45, 4.5% locust bean flour.

2.3. Proximate composition and sensory evaluation

The proximate composition, including moisture, protein, and fat contents in CON, CF15, CF30, and CF45 formulations was measured using the official standard method (AOAC, 2000). Briefly, moisture percentage was calculated with a weight loss experiment in the sample maintained in the oven at 103 ± 2 °C until constant weight. According to Kjeldahl's total nitrogen method, protein content was determined by multiplying the total nitrogen content by 6.25. For the determination of fat content, the samples were subjected to a liquid-solid extraction using diethyl ether in an extractor apparatus at 60 °C for six h. The fat content was obtained based on gravimetric difference. Proximate analyses were carried out in triplicate.

A panel consisting of faculty members and research assistants from the Food Technology Department of Ondokuz Mayıs University performed the sensory evaluation of chocolate spreads. The panel was asked to rate the nine attributes of the chocolate samples: appearance, granularity and roughness, spreadability, stickiness in the mouth, oiliness, odor, sweetness, color, and overall acceptability. Each attribute was scored from 1 (unacceptable) to 9 (excellent). All sensory evaluations were performed under fluorescent light and at room temperature.

2.4. Texture and water activity (a_w) measurement

Within the scope of the textural properties of chocolate spreads, the hardness and spreadability properties were determined using the TA XT Plus Texture Analyzer (Stable Micro Systems, UK) device equipped with an HDP/SR probe and connected with the computer program (Texture Expert Exceed 2.3., Stable Micro System, Godalming, Survey, UK). Analyses were made on a 15 g sample at room temperature (21 ± 2 °C), and 3 mm/s test speed and hardness values were given in kg and spreadability values in kg.s. Analyzes were performed in three replicates for each chocolate formulation.

The water activity of the chocolate samples was determined at 25 ºC using the Aqualab Dewpoint Water Activity Meter 4TE USA. Measurements were made in 3 replicates for each chocolate formulation.

2.5. Determination of color properties

Color properties were measured on the surface of chocolate formulations at room temperature using a colorimeter (ColorFlex EZ spectrometer, Reston, VA, USA). Three readings were taken from each chocolate sample. Color measurement included CIE L^*, a^*, and b^* parameters, where L^* represents lightness with a scale from 0 (black) to 100 (white), a^* represents redness with a scale from -60 (green) to +60 (red), and b^* represents yellowness with a scale from -60 (blue) to +60 (yellow). Also, chroma (C^*) and hue (h^*) were calculated from a^* and b^* parameters according to the following Equations (Becerra *et al.*, 2023):

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad \text{[Eq. 1]}$$

$$h^* = arctan\frac{b^*}{a^*} \quad \text{[Eq. 2]}$$

2.6. Determination of free fatty acid (FFA) and peroxide value (PV)

Firstly, the fats were extracted from the chocolate samples using a chloroform/methanol solvent system (2/1, v/v), and the lipid extract was used in FFA and PV analyses. The amount of FFA was determined for all samples according to the official standard method Ca 5-40 (AOAC, 2000). For this purpose, 5 g of lipid extract was dissolved in 50 mL of solvent mixture (diethyl ether/ethanol, 1/1, v/v) and titrated with 0.1 M KOH solution using a phenolphthalein indicator until the pink color disappeared. The percentage FFA content was calculated according to the Equation:

$$\%\,FFA = \frac{V.M.28.2}{m} \quad \text{[Eq. 3]}$$

where % FFA represents the percentage of free fatty acids, V is the volume of solvent used, M corresponds to the molarity of the KOH solution, and m is the mass of the lipid extract. The results were expressed as % oleic acid, and all analyses were conducted in triplicate.

The PV was determined according to the official standard method Cd 8-53 (AOAC, 2000) and expressed as meq O_2/kg lipid. Briefly, 2 g of lipid extract were dissolved with 25 mL of a glacial acetic acid and chloroform mixture (3/2, v/v) and shaken vigorously to achieve complete dissolution. Then, 1 mL of saturated potassium iodide solution was added to the mixture and kept in the dark for 5 minutes. At the end of that time, 75 mL of distilled water and 1 milliliter of starch solution (1%, w/v) were added as indicator, and the oxidized iodine released from the potassium iodide as a result of the reaction was titrated with 0.002 M sodium thiosulphate solution and the POV was calculated according to the following Equation:

$$PV\ (meq\ O_2/kg)\frac{V.M.1000}{m} \quad \text{[Eq.4]}$$

where V is milliliters of sodium thiosulfate solution (corrected to account for the blank test), M corresponds to the molarity of the sodium thiosulfate solution, and m is the mass of the lipid extract. All analyses were carried out in triplicate.

2.7. Statistical analysis

The whole trial was replicated twice, with each replication corresponding to a different production day. The data were analyzed with the SPSS 21 statistical software (IBM, Chicago, IL, USA) and normal distribution and homogeneity of variances were first checked. While data from texture, a_w, color, FFA, and PV analyses were analyzed using a randomized complete block design, those from proximate composition and sensory evaluation were analyzed by one-way ANOVA. Duncan's multiple comparison tests were used to evaluate the differences between the mean values found to be significant ($p < 0.05$). All results were expressed as mean value ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. Proximate composition and sensory evaluation

The proximate composition of chocolate spreads produced with different proportions of LBF is presented in Table 2. Replacing cocoa with LBF significantly affected the chocolates' moisture and fat content ($p < 0.05$), whereas its effect on the protein content was not significant ($p > 0.05$). The highest moisture content was determined in the CF15 chocolates as 0.73% ($p < 0.05$), and the fat content in the chocolates partially decreased as the addition of LBF increased ($p < 0.05$). These differences in the moisture and fat content of the chocolate formulations could be due to the proximate composition of LBF. Despite these differences, the proximate composition of all the chocolate formulations was similar to those reported by Cağındı and Otles (2007) in dark chocolate and by Ali *et al*. (2021) in low-calorie dark chocolate using different intense sweeteners and wheat fiber isolate.

The popularity of hedonic foods, like chocolate, mainly depends on their sensory properties, which constitute the key to the acceptance of food products on the market (Roda and Lambri, 2019). Replacing cocoa with LBF significantly affected the chocolates' appearance, odor, sweetness, color, and overall acceptability scores ($p < 0.05$). In contrast, its effect on other sensory attributes was insignificant ($p > 0.05$), as shown in Figure 1. Generally, the highest appearance, odor, sweetness, color, and overall acceptability scores were observed in the CON and CF15 containing 1.5% LBF. The scores decreased with more LBF

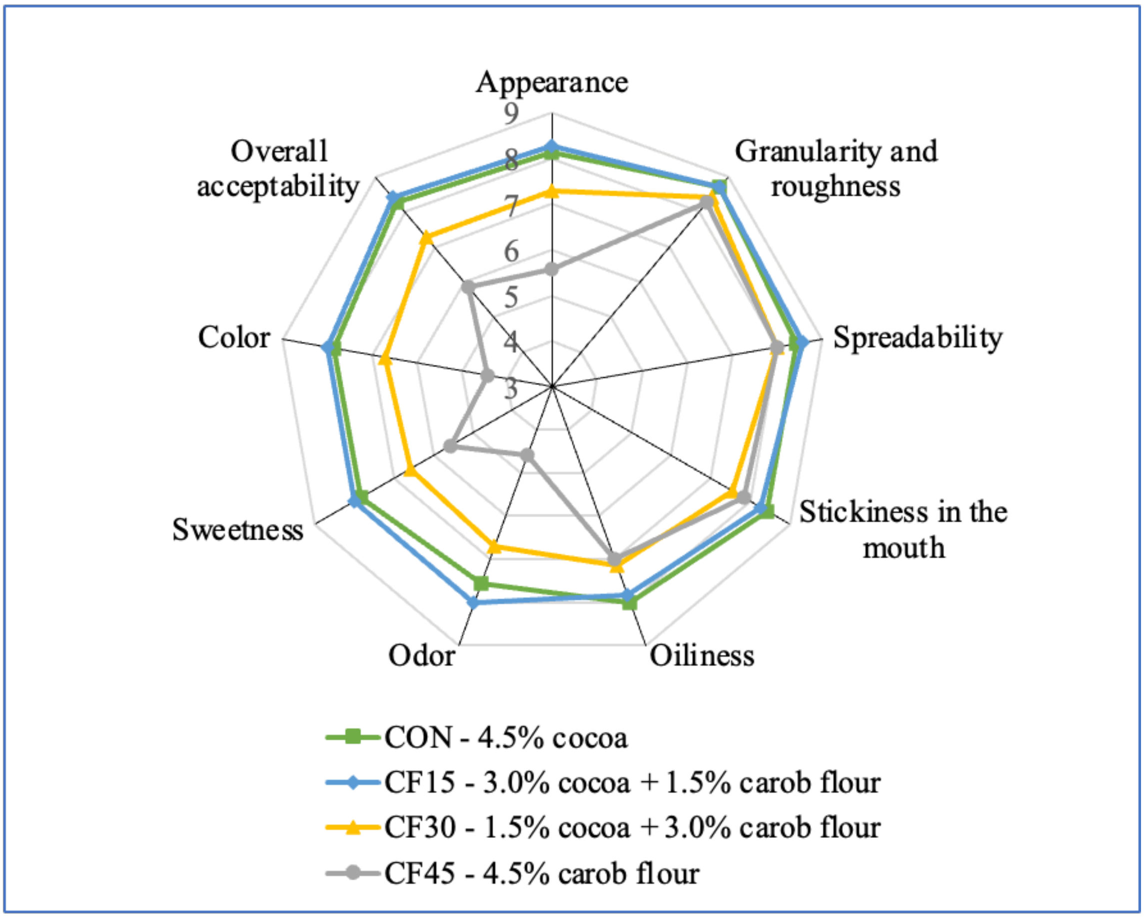

Figure 1. Sensory scores of chocolate spreads produced with different proportions of locust bean flour. Results are presented as means ± standard deviation of duplicate experiments.

Table 2. Proximate composition of chocolate spreads produced with different proportions of locust bean flour

Formulations	Moisture (%)	Protein (%)	Fat (%)
CON	0.33 ± 0.01c	5.71 ± 0.23	35.63 ± 0.03a
CF15	0.73 ± 0.01a	5.76 ± 0.41	34.92 ± 0.24b
CF30	0.34 ± 0.01c	5.84 ± 0.07	33.48 ± 0.79c
CF45	0.41 ± 0.01b	5.38 ± 0.08	33.69 ± 0.39c
Significance	*	ns	*

Results are presented as means ± standard deviation of duplicate experiments. Means with different letters within the column indicate differences at $p < 0.05$ level as assessed by Duncan's multiple range test. ns: not significant ($p > 0.05$); *$p < 0.05$. CON, 4.5% cocoa; CF15, 3.0% cocoa + 1.5% locust bean flour; CF30, 1.5% cocoa + 3.0% locust bean flour; CF45, 4.5% locust bean flour.

Table 3. Hardness, spreadability, and water activity values of chocolate spreads produced with different proportions of locust bean flour

	Hardness (kg)	Spreadability (kg·s)	a_w
Formulation			
CON	11.51 ± 12.78ab	4.62 ± 5.14bc	0.327 ± 0.028b
CF15	8.68 ± 8.19b	3.04 ± 2.87c	0.348 ± 0.046a
CF30	16.19 ± 10.93a	7.54 ± 4.93a	0.350 ± 0.040a
CF45	16.12 ± 10.85a	5.86 ± 4.46ab	0.328 ± 0.029b
Significance	*	**	**
Temperature (°C)			
22	12.01 ± 11.12	4.92 ± 4.71	0.342 ± 0.037
35	14.25 ± 11.11	5.61 ± 4.66	0.335 ± 0.038
Significance	ns	ns	ns
Time (week)			
0	9.01 ± 12.66c	4.86 ± 6.34	0.386 ± 0.037a
4	10.93 ± 8.81bc	4.36 ± 3.42	0.314 ± 0.012c
8	17.12 ± 10.58a	6.03 ± 4.15	0.321 ± 0.017c
12	15.45 ± 10.74ab	5.79 ± 4.37	0.333 ± 0.027b
Significance	*	ns	**

Results are presented as means ± standard deviation of duplicate experiments. Means with different letters within the column indicate differences at $p < 0.05$ level as assessed by Duncan's multiple range test. ns: not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$. CON, 4.5% cocoa; CF15, 3.0% cocoa + 1.5% locust bean flour; CF30, 1.5% cocoa + 3.0% locust bean flour; CF45, 4.5% locust bean flour.

addition. However, the use of up to 3.0% LBF did not affect the appearance, odor, sweetness, color, and overall acceptability scores compared to the CON formulation, and the lowest appearance, odor, sweetness, color, and overall acceptability scores were noted for the CF45 formulation due to an astringent taste and a lighter color formation after high-level LBF addition. Because the whole LB fruit contains a high level of tannins, causing excess astringency, this factor limits its use in human consumption (Avallone *et al*., 1997). Similarly, Akdeniz *et al*. (2021) reported that chocolates produced using 40% LBF as a cocoa substitute had the highest odor, aroma, appearance, sweetness, melting, and overall acceptability scores, but the use of higher rates of LBF reduced sensory acceptability. Also, Rosa *et al*. (2015) observed that the cakes with up to 75% replacement of cocoa powder by LBF showed no difference in flavor, odor, or texture, demonstrating that replacement up to this level did not influence sensory attributes. In another study, Aydın (2012) reported that adding LBF at up to 20% to biscuits did not negatively affect general appeal and taste. Still, the sensory scores generally decreased at higher rates when adding LBF. The current study and literature findings show that the use rate of LBF alone or as a cocoa substitute varies depending on the product type, and up to 3% LBF can be used with minimal compositional and sensory changes as a cocoa substitute in the production of chocolate spread.

3.2. Texture and water activity (a_w)

The effect of the formulation, temperature, and time on the hardness, spreadability, and a_w values for chocolate spreads produced with different proportions of LBF is shown in Table 3. As seen, LBF formulation and storage time affected the hardness ($p < 0.05$), whereas storage temperature effect on hardness was not significant ($p > 0.05$). Among the chocolate formulations, the highest hardness values were determined for CF30 and CF45 formulations, but the differences between CF30 and CF45 formulations and CON formulation were insignificant ($p > 0.05$). The lowest hardness value was determined for the CF15 formulation. As a result, adding LB at a high ratio to chocolate caused a slight increase in hardness. Hardness, which depends on the content and type of fat, sugar, and cocoa particles used in chocolate production (Konar *et al*., 2014; Barisic *et al*., 2021), is one of the essential mechanical properties that affects the sensory acceptability of chocolate (Machalkova *et al*., 2015; Hrivna *et al*., 2021). In the present study, LBF replaced some of the cocoa mass in the chocolate samples, resulting in lower total fat content (Table 2). This may be the reason for the increase in the hardness of chocolates containing high ratios of LBF. In addition, the differences in hardness can also be associated with the internal structure and more intermolecular bonds in chocolate samples prepared with LB powder. Since LB powder addition enhanced the fiber and carbohydrates of the produced samples, these compounds may act with the other compounds in chocolate and make more intermolecular bonds (Akdeniz *et al*., 2021). As in our findings, Akdeniz *et al*. (2021) also reported that adding LBF affects chocolates' hardness values. The hardness values for chocolates produced using LBF increased until the 8th week of storage, then decreased, but this decrease was insignificant ($p > 0.05$).

Spreadability is a key textural attribute affecting consumer acceptance (Aydın and Özdemir, 2017). As observed in Table 3, only LBF formulation affected the spreadability values for chocolate spreads ($p < 0.01$). As in the hardness values, the highest spreadability values were determined for the CF30 and CF45 formulations. However, the differences between the spreadability values of CF45 and the CON formulations were insignificant ($p > 0.05$), and the lowest values were obtained for the CF15 formulation. This situation could be attributed to the chemical composition of the spreadable chocolates.

LBF formulation and time affected the a_w values for chocolate spreads ($p < 0.01$), whereas the storage temperature effect on a_w was not significant ($p > 0.05$) (Table 3). The CF15 and CF30 formulations showed higher a_w values than the CON formulation ($p < 0.05$), and the differences between the a_w values of CF45 and CON formulations were not significant ($p > 0.05$). The highest a_w value determined at the beginning of the storage decreased to the lowest value in the 4th week of storage and tended to increase again from the 12th week. Despite these changes, the a_w values during storage did not reach 0.6, thus prohibiting microbial growth. However, several factors, such as the raw materials used, the surface area of the materials, and the temperature and humidity of refining and conching, may influence this parameter. Chocolate is protected from external water by its fatty surface, which makes it difficult to uptake moisture. However, the presence of amorphous sugars in chocolates should be considered. Amorphous sugar is in metastable form and tends to crystallize under several factors, mainly temperature and moisture (Rossini *et al*., 2011).

3.3. Color properties

The color of chocolate is the first characteristic that influences consumers and their desire to consume chocolate (Barisic *et al*., 2021). The effects of the formulation, temperature, and time on the color properties of chocolate spreads produced with different proportions of LBF are shown in Table 4. As seen, LBF formulation and storage time affected all measured (L^*, a^*, and b^*) and calculated color properties (C^* and h^*) at the $p < 0.01$ level, while the storage temperature affected the L^* value at $p < 0.05$. However, the effect of storage temperature on the h^* value was insignificant ($p > 0.05$). All color values generally increased with the addition of LBF, and the highest values were determined for the CF45 formulation containing 4.5% LBF. High values for a^* and C^* indicated chocolates with a dark color. As the substitution of LBF for cocoa increased, a^* and C^* values increased, and accordingly, the darkening of the chocolates occurred. It can be concluded that the compositional differences play a crucial role in the color properties of the final product as they directly affect the product's color. Absorptivity and scattering factors can also affect the color properties of foods (Rad *et al*., 2019). Many researchers also reported that substituting LBF for cocoa affects the color properties of different products. For example, Rosa *et al*. (2015) showed that using LBF (0, 25, 50, 75, and 100%) as a substitute for cocoa in the production of gluten-free cake increased the C^* and a^* values, resulting in darker cakes.

Chocolate spreads stored at 22 °C exhibited higher L^* and C^* values than those held at 35 °C, while those kept at 35 °C showed higher a^* and b^* values than those stored at 22 °C ($p < 0.05$). Similarly, it was also reported by Machalkova *et al*. (2015) that storage temperature affected the color properties of chocolates.

The effect of the storage time on the color properties differed depending on the color properties. For example, a^* values decreased ($p < 0.05$) until the 8th week of storage and then did not change ($p > 0.05$), while b^* and C^* values decreased until the 8th week of storage and then increased slightly ($p < 0.05$). On the other hand, L^* values increased until the 4th week

TABLE 4. Color properties of chocolate spreads produced with different proportions of locust bean flour

	L*	***a****	***b****	***C****	***h****
Formulation					
CON	24.41 ± 0.83[d]	10.72 ± 0.31[b]	14.59 ± 0.56[d]	18.10 ± 0.60[d]	53.68 ± 0.68[d]
CF15	28.88 ± 0.89[c]	10.78 ± 0.46[b]	16.18 ± 0.73[c]	19.44 ± 0.80[c]	56.29 ± 0.98[c]
CF30	33.03 ± 1.17[b]	10.59 ± 0.33[c]	17.61 ± 0.69[b]	20.55 ± 0.74[b]	59.01 ± 0.60[b]
CF45	44.16 ± 1.50[a]	10.92 ± 0.51[a]	24.55 ± 1.01[a]	26.87 ± 1.13[a]	66.00 ± 0.29[a]
Significance	**	**	**	**	**
Temperature (°C)					
22	32.70 ± 7.55[a]	10.67 ± 0.45[b]	18.10 ± 3.92[b]	21.08 ± 3.53[a]	58.74 ± 4.62
35	32.55 ± 7.41[b]	10.83 ± 0.39[a]	18.36 ± 3.90[a]	21.39 ± 3.46[b]	58.75 ± 4.75
Significance	*	**	**	**	ns
Time (week)					
0	33.48 ± 7.73[b]	11.18 ± 0.33[a]	18.92 ± 4.13[a]	22.05 ± 3.70[a]	58.68 ± 4.62[b]
4	33.86 ± 7.76[a]	11.04 ± 0.20[b]	18.70 ± 1.07[b]	21.79 ± 3.63[b]	58.69 ± 4.67[b]
8	31.60 ± 7.20[c]	10.38 ± 0.15[c]	17.20 ± 3.78[d]	20.17 ± 3.30[d]	58.14 ± 4.99[c]
12	31.54 ± 7.24[c]	10.40 ± 0.17[c]	18.11 ± 3.57[c]	20.95 ± 3.13[c]	59.48 ± 4.56[a]
Significance	**	**	**	**	**

Results are presented as means ± standard deviation of duplicate experiments. Means with different letters within the column indicate differences at p < .05 level as assessed by Duncan's multiple range test. ns: not significant (p > 0.05); *p < 0.05; **p < 0.01. CON, 4.5% cocoa; CF15, 3.0% cocoa + 1.5% locust bean flour; CF30, 1.5% cocoa + 3.0% locust bean flour; CF45, 4.5% locust bean flour.

of storage and then decreased, while after the 4th week, *h** values first reduced and then increased (p > 0.05). A high fat percentage probably made the chocolates more susceptible to oxidation during storage, resulting in color changes. Similar to our findings, in a study by Bui and Coad (2014), it was also noted that some color properties of chocolate changed during storage. The authors found that storage time affected the *L** and *b** values but not the *a** value.

3.4. Free fatty acid (FFA) and peroxide value (PV)

The effects of the formulation, storage temperature, and storage time on the FFA and PV values for chocolate spreads produced with different proportions of LBF are shown in Table 5. As observed, LBF formulation and storage time affected the FFA values for chocolates (p < 0.01). While CF15 and CF45 formulations showed lower FFA values than the CON formulation, the CF30 formulation showed a higher value (p < 0.05), and FFA values increased slightly during storage (p < 0.05). The increase in FFA during storage can be attributed to the activity of the lipase enzyme, which is activated due to the high temperature of the storage medium. Similar to

TABLE 5. Free fatty acid (FFA) and peroxide value (PV) of chocolate spreads produced with different proportions of locust bean flour

	FFA (%)	**PV (meq O_2/kg)**
Formulation		
CON	0.35 ± 0.04[b]	0.97 ± 0.08[b]
CF15	0.32 ± 0.03[c]	0.98 ± 0.06[b]
CF30	0.43 ± 0.02[a]	0.85 ± 0.04[c]
CF45	0.32 ± 0.04[c]	1.23 ± 0.17[a]
Significance	**	**
Temperature (°C)		
22	0.36 ± 0.07	0.99 ± 0.20
35	0.35 ± 0.05	1.02 ± 0.13
Significance	ns	ns
Time (week)		
0	0.35 ± 0.06[b]	0.92 ± 0.11[c]
4	0.33 ± 0.05[c]	1.00 ± 0.13[b]
8	0.35 ± 0.06[b]	1.04 ± 0.13[ab]
12	0.38 ± 0.05[a]	1.06 ± 0.24[a]
Significance	**	**

Results are presented as means ± standard deviation of duplicate experiments. Means with different letters within the column indicate differences at p < 0.05 level as assessed by Duncan's multiple range test. ns: not significant (p > 0.05); **p < 0.01. CON, 4.5% cocoa; CF15, 3.0% cocoa + 1.5% locust bean flour; CF30, 1.5% cocoa + 3.0% locust bean flour; CF45, 4.5% locust bean flour.

our findings, Yadav *et al*. (2011) and Ali *et al*. (2021) also reported that the FFA values for dark chocolates increased during storage.

The formation of primary oxidation products was monitored by PV, and similar to FFA, only LBF formulation and storage time affected the PV values for chocolates ($p < 0.01$). As observed in Table 5, the CF45 formulation showed the highest PV value. In contrast, the CF30 formulation exhibited the lowest PV value ($p < 0.05$), and the differences between the PV values for CON and CF15 formulations were not significant ($p > 0.05$). The PV values for chocolate formulations increased during storage, and the highest value was determined to be 1.06 meq O_2/kg in the 12th week. Despite this increase, it was still lower than 10 meq O_2/kg, indicating that alterations related to lipid degradation were still in the initial stage (Rossini *et al*., 2011). A high percentage of fat probably made the control group and chocolates produced with different proportions of LBF more susceptible to oxidation during storage. Similar results were obtained by Mexis *et al*. (2010) in dark chocolate with hazelnuts during 12 months of storage at 20 °C, by Rossini *et al*. (2011) in white chocolates during ten months of storage at 20 and 28 °C and by Ali *et al*. (2021) in low-calorie dark chocolates during the storage period of up to 90 days.

4. CONCLUSIONS

Chocolate is one of the most preferred confectionery products for people from many parts of the world. Due to the fact that cocoa contains substances that can cause health problems, such as theobromine and caffeine, and due to the constant increase in prices and resource constraints, chocolate spreads containing LBF as a substitute for cocoa were developed and analyzed for quality and storage stability. The results showed that replacing cocoa with LBF affected the hardness, spreadability, a_w, color properties, FFA, and PV values of chocolates. Replacing cocoa with LBF at a low level showed higher quality in hardness and spreadability. Appearance, odor, sweetness, color, and overall acceptability scores decreased with more LBF addition. Storage temperature affected only the color properties among the parameters examined. Storage time affected hardness, a_w, color properties, FFA, and PV values, but after 12 weeks of storage at 22 and 35 °C, the peroxide value was still lower than 10 meq O_2/kg. Thus, up to 3% of LBF can be used with minimal quality and sensory changes as a cocoa substitute in the production of chocolate spread. This work will help further research into the valorization of LBF and therefore contribute to developing strategies to produce innovative value-added chocolate formulations. As a whole, the nutritional and economic advantages of LB make it a promising option for the replacement of cocoa.

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DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

AUTHORSHIP CONTRIBUTION STATEMENT

B Parlatır: Conceptualization, Formal analysis, Investigation, Methodology, Writing–original draft. N Ş Üstün: Project administration, Conceptualization, Methodology, Writing–review & editing. S Turhan: Statistical analysis, Writing–review & editing.

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