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Araştırma Makalesi

Reduced-Sugar Cocoa Hazelnut Spread: The Effect of Stevia-Inulin Substitution on Physicochemical, Microbiological, and Sensory Properties

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ABSTRACT

This study aimed to develop cocoa hazelnut spread with reduced sugar content by partially or fully substituting sucrose with a stevia-inulin (90:10, w/w) mixture. The control sample contained no inulin or stevia, while other formulations had sucrose replaced at 50, 60, 70, 80, and 100% levels. The impact of these substitutions on oil and ash content, total aflatoxins, aflatoxin B1 (AFB1), peroxide value (PV), free fatty acid (FFA) content, total aerobic plate counts (APC), yeast/mold counts were assessed over a 3-month storage period (SP). Sensory evaluation was also conducted. The oil content averaged 32.8%, and the inulin-stevia addition led to a slight rise in ash content. Aflatoxin levels stayed within acceptable safety limits. PVs ranged from 0.085 to 0.248 meq O_2/kg, while FFA content varied between 0.304 to 0.365%. The PVs increased over time, particularly in control and samples with lower inulin-stevia content. Samples with higher levels of inulin-stevia (80, 100%) demonstrated greater oxidative stability, showing lower PV and FFA content. The addition of inulin-stevia also enhanced microbial stability, as seen in the reduced APC and yeast/mold counts in samples with higher substitution levels. Sensory evaluation showed that all samples performed similarly in terms of color, consistency, appearance, and spreadability, with the control scoring highest overall. Samples with a 50% sucrose replacement closely resembled the control across all sensory attributes.

Keywords: Consumer acceptability, functional food, lipid oxidation, self-life, sugar reduction

Azaltılmış Şeker İçeriğine Sahip Kakaolu Fındık Ezmesi: Stevia-İnülin İkamesinin Fizikokimyasal, Mikrobiyolojik ve Duyusal Özellikler Üzerindeki Etkisi

ÖZ

Bu çalışma, sakkarozun kısmen veya tamamen stevia-inülin (90:10, w/w) karışımı ile ikame edilmesi yoluyla azaltılmış şeker içeriğine sahip kakao-fındık ezmesi geliştirmeyi amaçlamıştır. Kontrol örneği inülin veya stevia içermemekte olup, diğer formülasyonlarda sakkarozun %50, %60, %70, %80 ve %100 oranlarında ikame edilmesi sağlanmıştır. Bu değişimlerin yağ ve kül içeriği, toplam aflatoksin ve aflatoksin B1 (AFB1) düzeyleri, peroksit değeri (PV), serbest yağ asidi (FFA) içeriği, toplam aerobik mezofilik bakteri sayısı (APC) ve maya/küf sayıları üzerindeki etkileri 3 aylık depolama süreci (SP) boyunca değerlendirilmiştir. Ayrıca duyusal analiz gerçekleştirilmiştir. Çalışmada, yağ içeriği ortalama %32.8 olarak belirlenmiş, inülin-stevia ilavesi kül içeriğinde hafif bir artışa neden olmuştur. Aflatoksin seviyeleri kabul edilebilir güvenlik limitleri içinde kalmıştır. Peroksit değerleri 0.085 ile 0.248 meq O_2/kg arasında değişirken, serbest yağ asidi (FFA) içeriği %0.304 ile %0.365 arasında bulunmuştur. Depolama süresi boyunca PV değerleri artış göstermiş, özellikle kontrol grubu ve daha düşük inülin-stevia içeriğine sahip örneklerde bu artış daha belirgin olmuştur. Yüksek inülin-stevia içeriğine (%80, %100) sahip örnekler, daha düşük PV ve FFA içeriği ile daha yüksek oksidatif stabilite sergilemiştir. İnülin-stevia ilavesinin mikrobiyal stabiliteyi de artırdığı, özellikle yüksek ikame seviyelerine sahip örneklerde APC ve maya/küf sayılarının daha düşük olduğu gözlemlenmiştir. Duyusal analiz sonuçları, tüm örneklerin renk, kıvam, görünüm ve sürülebilirlik açısından benzer performans gösterdiğini, ancak kontrol örneğinin genel olarak en yüksek puanı aldığını ortaya koymuştur. Sakkarozun %50 oranında ikame edildiği örnekler, duyusal özellikler açısından kontrole en yakın sonuçları vermiştir.

Anahtar Kelimeler: Tüketici kabulü, fonksiyonel gıdalar, lipit oksidasyonu, raf ömrü, şeker azaltımı

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Reduced-Sugar Cocoa Hazelnut Spread: The Effect of Stevia-Inulin Substitution on Physicochemical, Microbiological, and Sensory Properties

Introduction

Hazelnuts (*Corylus avellana* L.) are widely recognized for their rich nutritional profile, containing high levels of monounsaturated fatty acids, proteins, dietary fiber, vitamins (particularly vitamin E), minerals, phytosterols, and a variety of polyphenols (Özdemir and Devres, 1999; Karaosmanoğlu, 2022). Hazelnut consumption has been linked to several health benefits, including improved heart health, reduced inflammation, and better cholesterol regulation (Brown et al., 2022; Shataer et al., 2021). Given these nutritional benefits, hazelnuts have become a core ingredient in a variety of food products.

The demand for hazelnut-based products is primarily driven by the confectionery industry, which uses a significant portion of the world's hazelnut supply. Cocoa hazelnut spread is a sweet, creamy confection made primarily from roasted hazelnuts, cocoa, sugar, and vegetable oils. Cocoa hazelnut spreads are gaining global popularity, particularly due to their rich, creamy texture and sweet, chocolate-like taste, making them a staple in many households. However, one of the major concerns with these products is their high sugar content. As consumers become more aware of the detrimental effects of high sugar diets (Arshad et al., 2022), there has been a growing demand for reduced-sugar alternatives in food products. To address this concern, researchers and food manufacturers have explored strategies to reduce sugars in foods without compromising taste or texture (Gomes et al., 2023; McKenzie and Lee, 2022).

Stevia, a natural sweetener derived from the Stevia rebaudiana plant, has emerged as a popular alternative to sugar due to its zero-calorie nature and high sweetness intensity-up to 200-300 times sweeter than sucrose (Gasmalla et al., 2014). Stevia's use in food products has been extensively studied, showing promise as a sugar replacement that can maintain the sensory properties of the final product (Schiatti-Sisó et al., 2023). Inulin, a dietary fiber found in plants such as chicory root, has also been explored as fat and sugar replacers and ability to improve the texture and mouthfeel of food products (Jackson et al., 2023; Shoaib et al., 2016). Studies suggest that inulin can enhance gut health by promoting the growth of beneficial bacteria, making it an attractive ingredient for developing healthier food products (Shoaib et al., 2016).

Previous studies have explored the potential of incorporating stevia and inulin into various food products (Schiatti-Sisó et al., 2023; Shoaib et al., 2016). Inulin and stevia have been used as ingredients in chocolate formulations by several researchers (Konar et al., 2018; Aidoo et al., 2015; Shourideh et al., 2012; Shah et al., 2010). However, there is limited research focused on reducing sugar content in cocoa hazelnut spreads. In chocolate spread formulations, alternatives to sucrose, such as inulin-maltitol, apple pomace, and grape pomace, have been explored for their potential in sugar reduction (Büker et al., 2021; Acan et al., 2021; Tolve et al., 2021). Given the rising health concerns and the need for functional foods with lower sugar content, this study seeks to contribute to the literature by formulating a dietary fiber-enriched, reduced-sugar cocoa hazelnut paste using stevia and inulin.

The aim of this study is to produce cocoa hazelnut paste with reduced sugar content by partially or fully replacing the sugar with stevia and inulin. Specifically, the study will examine the chemical, physical, microbiological, and sensory properties of the reformulated spreads, as well as monitor changes during storage. This research not only provides valuable insights into sugar reduction in cocoa hazelnut spreads but also contributes to the development of functional food products that align with modern health trends.

Materials and methods

Materials

Hazelnut puree, hazelnut oil, and whey powder generously supplied by Karimex Gıda (Ordu, Türkiye). Skim milk powder (Enka, Konya, Türkiye), cocoa powder (Altınmarka, İstanbul, Türkiye), inulin sourced from chicory root (Orafti® HSI, Beneo-Orafti, Tienen, Belgium), and stevia (Egepak, İzmir, Türkiye) were used in the formulations of cocoa hazelnut spreads. The sweetness level of inulin is 30%.

Cocoa Hazelnut Spread Preparation

Production of cocoa hazelnut spreads were performed in the pilot production plant of Karimex Gıda company. The control sample (C) contained neither inulin nor stevia. To study the effects of the use of stevia and inulin, 50, 60, 70, 80, and 100% of the sucrose in control formulation was replaced with inulin-stevia (90:10, w/w) mixture. Formulation of each sample was given in

Table 1. The production of cocoa hazelnut spreads began with the mixing of oil, powdered sugar, and hazelnut puree at 40°C for 20 min. Afterward, inulin, stevia, lecithin, cocoa powder, whey powder, and skim milk powder were added, and mixing continued for an additional 40 min. The mixture was then transferred to a ball mill and processed at 60°C for 1.5 h. Once the milling was complete, the mixture was cooled to 40°C and tempered at 27-29°C. After tempering, the spread formulations were filled into jars at 29°C.

Table 1. Composition of cocoa hazelnut spread samples

Ingredients	Quantity (g)					
	C*	IS50	IS60	IS70	IS80	IS100
Palm oil	20	20	20	20	20	20
Hazelnut puree	13	13	13	13	13	13
Whey powder	7.1	7.1	7.1	7.1	7.1	7.1
Cocoa powder	5	5	5	5	5	5
Hazelnut oil	5	5	5	5	5	5
SMP*	4	4	4	4	4	4
Lecithin	0.9	0.9	0.9	0.9	0.9	0.9
Sucrose	45	22.5	18.0	13.5	9.0	-
Inulin	-	20.25	24.3	28.35	32.40	40.5
Stevia	-	2.25	2.70	3.15	3.60	4.50
Total (%)	100	100	100	100	100	100

*C: control, SMP: skim milk powder

Methods

The physicochemical analyses were carried out according to the reference method of the Turkish Standard Institute (TSE), namely the ash content (TS 2131 ISO 928, 2001), the peroxide value (TS EN ISO 3960, 2010), the free fatty acid content (TS EN ISO 660, 2010). Determination of fat content and aflatoxin analysis were carried out according to AOAC official methods 991.36 and 999.07, respectively (AOAC, 2000).

Microbiological Analysis

A 10 g portion of the sample was aseptically transferred into 90 mL of sterile peptone water and homogenized into a sterile stomacher bag. Serial dilutions were performed as necessary. For the enumeration of total mesophilic aerobic bacteria, an aliquot of the homogenized sample was inoculated onto Plate Count Agar (PCA) plates (Merck, 105463) and incubated at 37°C for 24 h under aerobic conditions. Similarly, for the enumeration of yeast and mold, an aliquot of the homogenized sample was inoculated onto Dichloran Rose Bengal Chloramphenicol (DRBC) agar plates (Merck, 100466) and incubated at 25°C for 72 h. Following the respective incubation periods, colony counts were performed, and the results were expressed as colony-forming units per gram (CFU/g) of the sample.

Sensory analysis

The sensory properties of the samples were evaluated by a semi-trained panel comprising 10 individuals (6 females, 4 males) aged between 20 and 40 years. Panelists were selected from male and female candidates who neither smoke nor have allergies to hazelnuts or other components. The panelists were instructed to evaluate the color, taste, odor, consistency, appearance, flavor, aroma, and spreadability of the samples using a 5-point hedonic scale (5: Very good, 4: Good, 3: Neither good nor bad, 2: Bad, 1: Very bad). The mean scores given by the panelists for each sample were calculated.

Storage stability

The storage stability of hazelnut spread samples was assessed by storing them at 19°C (±1) and 65% (±5) relative humidity in capped glass containers for a period of three months. To evaluate the impact of storage on product quality, free fatty acid (FFA) content, peroxide value (PV), and microbiological parameters were analyzed at monthly intervals throughout the storage period.

Statistical Analysis

The data were assessed using a one-way and/or two-way analysis of variance (ANOVA). Differences among individual means were compared by using Tukey Comparison test ($p \leq 0.05$) (Minitab, Version 17).

Results and Discussion

The oil and ash contents of the cocoa hazelnut spread samples were presented in Table 2. Cocoa hazelnut samples had about 32.8% oil content, and ash content ranging between 1.21 and 1.27 %. According to Turkish Standard Institute 'Hazelnut Paste' standard (TS 8371, 2022), the minimum oil content in cocoa hazelnut paste is specified as 28%, while the maximum ash content is specified as 1.5%. Both the oil content and ash content of the samples remained within the specified standards published by Turkish Standards Institute (TSE). The reduction of sugar and the addition of inulin and stevia increased the ash content. Ash content represents the total mineral content in food. Inulin, derived from chicory root, naturally contains minerals such as calcium, sodium, potassium, magnesium, iron which contribute to the overall ash content (Mudannayake et al., 2015).

Table 2. Oil content, ash content, total aflatoxin and aflatoxin B1 (AFB1) contents of cocoa hazelnut spread samples

Formulation	Oil content (%)	Ash content (%)	Total Aflatoxin (µg/kg)	Aflatoxin B1 (AFB1) (µg/kg)
Control	32.81±0.02A	1.21±0.00D	2.67±0.02A	1.13±0.00AB
IS50	32.83±0.03A	1.23±0.01C	2.69±0.00A	1.16±0.05A
IS60	32.83±0.03A	1.25±0.01B	2.69±0.00A	1.13±0.00AB
IS70	32.83±0.05A	1.25±0.00B	2.58±0.00A	1.12±0.00AB
IS80	32.83±0.00A	1.26±0.01B	1.73±0.29B	1.11±0.01AB
IS100	32.85±0.03A	1.27±0.00A	1.56±0.01B	1.10±0.00B

*Means ± standard deviation within a column followed by different letters is significantly different ($p \leq 0.05$).

Aflatoxin contamination in hazelnuts is a significant concern for food safety, particularly due to the health risks associated with aflatoxins, especially aflatoxin B1 (AFB1) (Savatore et al., 2023). AFB1 is classified as human carcinogen belonging to Group 1 by the International Agency for Research on Cancer (IARC, 2002). Long-term exposure to AFB1 through food can lead to liver cancer, especially in populations with high dietary intake of aflatoxin-contaminated foods (Cao et al., 2022). Most countries enforce strict limits on aflatoxin levels in food products to safeguard public health. In the European Union (EU), for hazelnuts intended for direct human consumption or use as ingredient in foodstuffs, the maximum allowable level for aflatoxin B1 (AFB1) is set at 5 µg/kg (ppb), while the total aflatoxin limit (including B1, B2, G1, and G2) is 10 µg/kg (EU, 2010). In this study, the total aflatoxin levels in cocoa hazelnut spread samples ranged from 1.558 to 2.689 µg/kg, while AFB1 levels ranged from 1.101 to 1.158 µg/kg. Aflatoxin levels of the samples remained within the specified standards.

Peroxide Value (PV)

Cocoa hazelnut spread samples contain hazelnut oil which is rich in unsaturated fatty acids (Taş and Gökmen, 2015). Unsaturated fatty acids are particularly susceptible to oxidation through a process known as autoxidation (Shahidi and Zhong, 2010; Frankel, 1991). This is a self-sustaining reaction where unsaturated fats react with oxygen to form lipid peroxides. Peroxide value (PV) is one of the most commonly used quality indicators for monitoring oil oxidation during storage (Zhang et al., 2021). It is an indicator of the initial stages of oxidation. Given the high unsaturated fat content in cocoa hazelnut spreads, monitoring the PV during storage is essential for evaluating product quality and stability. PVs of cocoa hazelnut spread samples were presented in Table 3. The storage period and formulation were identified as significant factors affecting the PV (Table 4). PV of samples increased with increasing storage time. PV of the samples stored for 1, 2, and 3 months ranged from 0.085 to 0.180, 0.092 to 0.213, and 0.110 to 0.248, respectively. According to Turkish Standard Institute 'Hazelnut Paste' standard (TS 8371, 2022), the maximum PV is specified as 5 meq O_2/kg. The PV levels of the cocoa hazelnut spread samples remained well below the specified standard during the 3-months storage period. Similar findings have been obtained in various studies regarding the effect of storage on the peroxide values of nut pastes. Gamlı and Hayoğlu (2007) showed that packaging type and storage period affected PV of pistachio nut paste. For the paste stored in a sealed glass jar, they observed a steady increase in PV from an initial value of 0.05 to 0.23 meq O_2/kg at 4°C and to 0.29 at 20 °C after 3 months of storage. Ciftci and Ozilgen (2019) observed an increase in PV of tightly covered almond paste stored for 31 days at various temperatures (4, 20, 30, and 60 °C). In their study, they found that the PV increased from an initial value of 0.00 meq O_2/kg to around 1.2-1.6 meq O_2/kg at 20°C, and to around 3.0-4.0 at 30°C. In a different study, Shakerardekani et al. (2015) reported an increase in PV of pistachio spread (with palm olein) from 0.05 to 4.9 meq O_2/kg after being stored in capped glass containers under accelerated autoxidation conditions (60 °C) for 25 days. Even if packed, small amounts of oxygen can remain in the headspace of the container. This residual oxygen can initiate the oxidation of unsaturated fats in the samples (Johnson et al., 2015). Johnson et al. (2017) investigated the impact of oxygen reduction on lipid oxidation kinetics in a 1.0% fish oil-in-water emulsion and reported that nearly complete oxygen removal is required to protect oxygen-sensitive ingredients. Once initiated, the free radical reactions can continue to drive up the peroxide values, even in low-oxygen conditions, leading to peroxide formation over time. In their study, Mexis et al. (2010) used active and modified atmosphere packaging to reduce oxygen level for the preservation of dark chocolate with hazelnuts and recorded PV ranging from 0.8 to 4.38 meq O_2/kg chocolate fat after 12 months of storage in the dark at 20°C.

Hazelnuts contain natural antioxidants, such as tocopherols, that protect the fats from oxidation (Frankel, 1991; Shahidi and Zhong, 2010; Pycia et al., 2019). However, these antioxidants can degrade or become less effective over time, leaving the fats more vulnerable to oxidation and contributing to a rise in PV as storage progresses. In their study, Lobuono et al. (2023) detected a reduction in tocopherol content (particularly α- and δ-tocopherols) in the oil phase of hazelnut paste samples stored at 40°C in open containers, likely due to the antioxidant action of tocopherols, which reacted with oxygen and free radicals over time.

IS80 and IS100 samples had lower PVs compared to the other samples and the control. The PVs of these samples remained stable after the second month of storage. The PVs of IS50 and IS70 samples were statistically similar to that of the control. Lower PVs in cocoa hazelnut spreads with high inulin content suggests that inulin may contribute to oxidative stability. This aligns with studies indicating that inulin can act as a prebiotic fiber with some antioxidant activity (Shang et al., 2018; Pasqualetti et al., 2014; Mu et al., 2021), potentially slowing lipid oxidation in food systems. Li et al. (2020) prepared emulsion gels containing olive oil and Jerusalem artichoke inulin, showing that inulin exhibited antioxidant activity by delaying or inhibiting the formation of conjugated dienes in linoleic acid oxidation. Stevia may also contribute to oxidative stability (Singh et al., 2012). Stoyanova et al. (2011) compared the ROS (reactive oxygen species) scavenging capacities of inulin and stevioside with that of sucrose and showed that both inulin and stevioside are superior scavengers of both hydroxyl and superoxide radicals, demonstrating greater effectiveness than sucrose.

Free Fatty Acid (FFA)

The increase in free fatty acid (FFA) content in oil during storage is a key indicator of oil quality degradation. The FFA contents of cocoa hazelnut spread samples were presented in Table 3. All FFA values, ranging between 0.304 to 0.365 %, remained well below the maximum permissible FFA level of 1.5% set by the Turkish Standards Institute (TS 8371, 2022). The FFA values were influenced by the formulation (Table 4). The FFA values of IS50 sample were similar to those of control during storage while all other samples had lower FFA values compared to both the control and IS50 sample. In general, increasing the inulin-stevia ratio was associated with a decrease in FFA level. This indicates that the extent of sucrose replacement may play a role in formation of free fatty acids during cocoa hazelnut spread processing.

Table 3. Free fatty acid (FFA) and peroxide value (PV) of the cocoa hazelnut spread samples

Property	SP	Control	IS50	IS60	IS70	IS80	IS100
FFA (%)	0	0.361±0.001A*a**	0.359±0.000Bc	0.342±0.000Cb	0.331±0.001Da	0.321±0.001Eb	0.305±0.001Fa
	1	0.362±0.001Aa	0.361±0.001Ab	0.343±0.000Bb	0.332±0.001Ca	0.322±0.000Da	0.304±0.001Ea
	2	0.364±0.002Aa	0.363±0.001Ab	0.346±0.001Ba	0.332±0.003Ca	0.323±0.001Da	0.304±0.001Ea
	3	0.365±0.002Aa	0.365±0.001Aa	0.347±0.001Ba	0.333±0.001Ca	0.324±0.001Da	0.304±0.000Ea
PV (meq O_2/kg)	0	0.000±0.000Ad	0.000±0.000Ad	0.000±0.000Ad	0.000±0.000Ac	0.000±0.000Ab	0.000±0.000Ac
	1	0.120±0.009BCc	0.137±0.003Bc	0.180±0.013Ac	0.127±0.018Bb	0.108±0.003CDa	0.085±0.010Db
	2	0.148±0.012BCb	0.160±0.005Bb	0.213±0.016Ab	0.158±0.018Bb	0.117±0.008CDa	0.092±0.015Dab
	3	0.195±0.009Ba	0.207±0.016Ba	0.248±0.008Aa	0.208±0.008Ba	0.117±0.008Ca	0.110±0.005Ca

SP: Storage period (month), Values represent mean±standard deviation.

*For each property, capital letters shown in the same row compare the effect of the formulations for the given storage period (P ≤ 0.05).

**For each property, lowercase letters in the same column compare the effect of storage period for the given formulation (P ≤ 0.05).

Table 4. Two-way ANOVA results for formulation (F) and storage period (SP) effects on free fatty acid and peroxide value

	Free fatty acid				Peroxide value			
Source	DF	Adj MS	F-value	P-value	DF	Adj MS	F-value	P-value
F	5	0.006197	5021.48	0.000*	5	0.012186	136.03	0.000
SP	3	0.000034	27.71	0.000	3	0.113012	1261.53	0.000
F X SP	15	0.000004	3.38	0.001	15	0.001789	19.97	0.000
Error	47	0.000001			48	0.000090		
Total	70				71			
R-sq	99.82%				99.00%			

*$p \leq 0.05$ denotes significant effect of main factors (F, SP) or interaction (FXSP)

During storage, FFA contents of the control, IS70, and IS100 samples remained nearly constant, while a slight increase in FFA levels was observed in the other samples. FFA formation is primarily due to the hydrolysis of triglycerides, which breaks down into free fatty acids and glycerol, usually catalyzed by enzymes from the source or contaminating microorganisms or external factors like moisture, temperature, and light exposure (Talbot, 2016). The activity of lipase is substantially reduced by the roasting process (Özdemir and Devres, 1999). Any trace amounts of lipase enzyme may lead to a slow increase in FFA during storage. Our results align with the data reported by Covaliov et al. (2022), who observed a slight increase in FFA in walnut paste, prepared using roasted walnuts, powdered sugar, cocoa and dark chocolate, from 0.10 to 0.17 g oleic acid/ 100 g during storage in closed jars kept in the dark. Our results are also in agreement with the data obtained by Shakerardekani et al. (2015), who reported a slight increase in FFA values (from 0.53 to 0.68 % oleic acid) of pistachio spread prepared with 58.3% pistachio paste, 25% icing sugar, and 16.7% red palm olein after being stored in capped glass containers at 60 °C for 25 days.

Microbiological Analysis

Table 5 shows the total aerobic plate counts (APC) (CFU/g) of cocoa hazelnut spread samples over a 3-month storage period. Initially, APC levels across all samples were similar, showing no significant differences until the end of the first month. However, after the second month, variations in APC levels became prominent, with samples containing higher percentages of inulin-stevia (IS80 and IS100) showing stable APC, whereas others exhibited an increase. By the end of the third month, samples containing inulin-stevia generally exhibited lower APC than the control, with the control sample having the highest APC and the IS80 and IS90 samples the lowest.

The yeast/mold counts of cocoa hazelnut spread samples were presented in Table 6. Yeast and mold counts showed no significant differences by the end of the first month, but by the end of the second month, significant differences emerged. Similar to the APC results, yeast and mold counts remained stable in the IS80 and IS100 samples throughout storage, while they increased in other formulations. At the end of the storage period, the control sample exhibited the highest yeast/mold counts, whereas the IS80 and IS100 samples had the lowest.

The findings suggest that replacing part of the sucrose in cocoa hazelnut spreads with inulin-stevia enhanced microbial stability. This effect may be partly attributed to the antimicrobial properties of stevia. Research has demonstrated that stevia exhibits antimicrobial effects, potentially due to its phytochemical components, such as rebaudioside and stevioside (Kumari and Chandra, 2015; Puri and Sharma, 2011; Debnath, 2008; Singh et al., 2012). These compounds may inhibit microbial growth to some extent, which could explain the lower APC and yeast/mold counts in formulations with higher stevia content. Inulin also likely contributes to microbial stability, depending on its concentration. As a prebiotic fiber with water-binding properties, inulin can lower water activity, as demonstrated by Berk et al. (2024) who found that replacing sugar with inulin-stevia in cocoa hazelnut paste formulations led to lower water activity values. This reduction in water activity helps limit microbial growth, as microorganisms need available water to thrive. Studies have shown that reduced water activity can extend the shelf life of food products, making inulin an effective sugar substitute for improving microbial stability. The combination of inulin and stevia may have had a cumulative effect on microbial stability, creating a less favorable environment for aerobic bacteria, yeast, and molds especially in higher substitution levels like IS80 and IS100.

Table 5. Changes in total aerobic plate count (APC) (CFU/g) in cocoa hazelnut spread samples during storage

Formulation	Storage period (month)			
	0	1	2	3
Control	193.33±2.89A*c**	193.33±2.89Ac	216.67±2.89Ab	235.00±0.00Aa
IS50	193.33±2.89Ac	193.33±2.89Ac	203.33±2.89Bb	226.67±2.89Aa
IS60	185.00±5.00Ab	185.00±5.00Ab	195.00±5.00Bab	205.00±5.00Ba
IS70	186.67±2.89Ab	186.67±2.89Ab	195.00±5.00Bab	196.67±2.89Ba
IS80	186.67±2.89Aa	186.67±2.89Aa	185.00±0.00Ca	183.33±2.89Ca
IS100	186.67±2.89Aa	186.67±2.89Aa	183.33±2.89Ca	185.00±5.00Ca

Values represent mean±standard deviation.
*Capital letters shown in the same column compare the effect of the formulations for the given storage period ($P \leq 0.05$).
**Lowercase letters in the same row compare the effect of storage period for the given formulation ($P \leq 0.05$).

Table 6. Changes in yeast/mold counts in cocoa hazelnut spread samples during storage

Formulation	Storage period (month)			
	0	1	2	3
Control	12.50±0.50A*c**	12.50±0.50Ac	14.67±0.29Ab	16.50±0.50Aa
IS50	12.33±0.29Ab	12.33±0.29Ab	13.67±0.29Ba	14.50±0.50Ba
IS60	11.67±0.58Ab	11.67±0.58Ab	13.33±0.29BCa	13.67±0.29Ba
IS70	11.50±0.00Ac	11.50±0.00Ac	12.50±0.50CDb	13.50±0.00BCa
IS80	11.83±0.29Aa	11.83±0.29Aa	11.67±0.29DEa	12.33±0.29Da
IS100	11.83±0.29Aab	11.83±0.29Aab	11.50±0.00Eb	12.50±0.50CDa

Values represent mean±standard deviation.
*Capital letters shown in the same column compare the effect of the formulations for the given storage period ($P \leq 0.05$).
**Lowercase letters in the same row compare the effect of storage period for the given formulation ($P \leq 0.05$).

Sensory Analysis

Sensory evaluation was conducted to assess the color, taste, odor, consistency, appearance, flavor, aroma, and spreadability of the cocoa hazelnut spread samples. The results (Table 7), based on a 5-point hedonic scale, revealed that the control sample generally received the highest scores across all attributes. However, formulations with up to 50% sucrose replacement (IS50) demonstrated sensory properties comparable to the control, making them the most acceptable reduced-sugar alternatives. The color scores of the samples did not show significant differences ($p > 0.05$), indicating that replacing sucrose with the stevia-inulin mixture did not have a major impact on the visual appeal of the spreads. The control (4.40 ± 0.52) and IS50 (4.20 ± 0.42) samples had the highest color scores, while IS60, IS70, and IS80 samples showed slightly lower values. The appearance scores followed a similar trend, with control (4.30 ± 0.48) receiving the highest rating, while IS60 (3.60 ± 0.52) had the lowest. The taste scores revealed that increasing the inulin-stevia substitution beyond 50% negatively impacted overall acceptability. The control sample had the highest taste rating (4.50 ± 0.71), followed by IS50 (4.30 ± 0.82). The taste scores decreased in higher substitution levels, with IS80 (3.60 ± 0.52) receiving the lowest score. A similar trend was observed for aroma, where the control sample (4.70 ± 0.48) was rated the highest, while IS60, IS70, and IS100 received lower scores.

Consistency and spreadability were not significantly affected by the sugar replacement levels ($p > 0.05$). All samples showed comparable spreadability, with values ranging from 4.20 (control) to 3.50 (IS70). The IS80 and IS100 samples retained their consistency and spreadability, indicating that the inulin-stevia mixture did not adversely impact textural attributes. Among the reduced-sugar samples, IS50 exhibited the most similar sensory profile to the control, maintaining high ratings for taste, aroma, consistency, and spreadability. In contrast, formulations with higher substitution levels (IS70, IS80, IS100) exhibited noticeable declines in taste and aroma scores, suggesting that excessive inulin-stevia substitution may impact consumer acceptability.

Table 7. Sensory analysis

Sensory parameter	Control	IS50	IS60	IS70	IS80	IS100
Color	4.40±0.52A*	4.20±0.42A	3.90±0.74A	4.00±0.00A	4.00±0.67A	4.10±0.74A
Taste	4.50±0.71A	4.30±0.82AB	3.90±0.32AB	3.70±0.48AB	3.60±0.52B	4.00±0.67AB
Smell	4.40±0.52A	3.90±0.57ABC	3.50±0.53BC	3.20±0.42C	4.20±0.63AB	4.10±0.74AB
Consistency	4.20±0.63A	4.20±0.79A	3.70±0.48A	3.40±0.97A	3.90±0.57A	4.10±0.99A
Appearance	4.30±0.48A	4.00±0.00A	3.60±0.52A	3.80±0.63A	4.00±0.67A	3.80±1.03A
Flavor	4.00±0.47A	4.00±0.67A	3.50±0.53A	3.70±0.48A	4.10±0.88A	4.20±0.79A
Aroma	4.70±0.48A	4.30±0.48AB	3.60±0.52B	3.90±0.32B	4.00±0.67AB	3.80±0.92B
Spreadability	4.20±0.42A	4.10±0.74A	3.90±0.57A	3.50±0.53A	4.20±0.42A	4.20±0.79A

Values represent mean±standard deviation.

*Capital letters shown in the same column compare the effect of the formulations for the given sensory parameter ($P \leq 0.05$).

Conclusion

The formulation with the highest inulin-stevia ratio (IS100) demonstrated the lowest peroxide values and stable free fatty acid content over the storage period, suggesting enhanced oxidative stability. Incorporating inulin-stevia as a sucrose replacement in cocoa hazelnut spreads significantly impacted microbial stability, specifically in terms of aerobic plate counts (APC) and yeast/mold counts, during storage. Increasing the inulin-stevia ratio in the formulation appears to be effective in suppressing bacterial growth, particularly in the IS80 and IS100 samples. Sensory evaluation revealed that all samples scored similarly on attributes such as color, consistency, and spreadability, with the control sample achieving the highest overall sensory scores. Samples with 50% sucrose replacement were closest to the control in terms of all sensory attributes. These findings suggest that up to 50% sucrose replacement with inulin-stevia can be achieved without significantly compromising sensory attributes, making it a promising formulation for reduced-sugar cocoa hazelnut spreads. However, higher substitution levels may require optimization in formulation to maintain consumer acceptability, particularly in terms of taste and aroma.

The results of this study indicate that substituting sucrose with an inulin-stevia mixture not only reduces sugar content but also contributes to the oxidative and microbial stability of the cocoa hazelnut spread. This has implications for producing healthier spreads with lower sugar and improved stability, appealing to consumers seeking reduced-sugar options without compromising shelf life or quality.

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