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Investigating chemical, antioxidant, and sensory properties of chocolate fortified with cactus stems powder

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Objective: The present study aimed to examine the sensory, antioxidant, and chemical characteristics of chocolate that were enhanced with a powder derived from cactus leaves.

Methods: Treatments were prepared by fortified chocolate with cactus stems powder at different levels (0%, 3%, 5%, and 7%) w/w.

Results: The results indicated that fortification of chocolate with cactus stems powder increased significantly ($p \leq 0.05$) moisture, protein, ash, total carbohydrate, and fiber of chocolate treatments with cactus stems powder, in comparison to the control group (0%). On the contrary, there was a notable ($p \leq 0.05$) reduction in fat content in all the treatments that contained cactus leaf powder.

Conclusions: The augmentation of cactus stems powder in chocolate treatments had a positive impact on their antioxidant activity, which increased with increasing the concentration of the powder. In addition, this inclusion of cactus stems powder aided in lowering the oxidative degradation of the chocolate treatments. The sensory analysis findings revealed no significant difference between mean ratings for the overall acceptability of control chocolate and fortified chocolate treatments ($p \leq 0.05$).

KEYWORDS

antioxidant, cactus leaves powder, chemical, chocolate, fortification

Introduction

Chocolate is among the most popular food items worldwide owing to its distinct taste, aroma, and flavor. The essential component of chocolate is cocoa powder or cocoa butter (Deshmukh et al., 2020). Chocolate has certain fascinating properties, consumption of chocolate releases feel-good hormones such as serotonin and dopamine, which suppress anxiety and stress (Nehlig, 2013), and have the potential to offer health benefits that go beyond their typical nutritional value (Tolve et al., 2018). Chocolate is produced using cocoa as its main ingredient, which is a blend of cocoa mass, cocoa butter, and sugar (sucrose), using specialized equipment (Yadav et al., 2011).

In recent years, a surge has been observed in consumer demand and expectations in the food industry, leading to a wider array of options for them to choose from compared to previous times. An example of this is the significant growth of the global chocolate market, which expanded by ∼5% up to the year 2020. Chocolate consumption continues to rank as the top confectionery food product consumed worldwide (Chen et al., 2022).

The elevated levels of fat and sugar in chocolate present a significant issue, as they are linked to a heightened risk of numerous diseases, such as high cholesterol, obesity, and coronary heart disease. The primary cause of spoilage and the development of undesirable flavors in chocolate is lipid oxidation. When chocolate undergoes lipid oxidation, it produces various byproducts, some of which are associated with several health issues such as membrane harm, cancer, heart disease, and premature aging (Yadav et al., 2011).

Consumers are becoming more conscious of the health consequences of consuming foods high in fat and sugar. The focus now is to create healthier options that are lower in fat and sugar, while being high in fiber (Angor, 2018, 2023).

Numerous studies have explored the potential of incorporating various types of plant extracts and powders into chocolate to enhance its nutritional value. Some examples of these include the use of white tea, jackfruit flour, and jujube extracts. The composition of protein, β-carotene, minerals, and dietary fiber is noteworthy (Fitriani Nur et al., 2020; Poliński et al., 2021; Shahbazi et al., 2022). The stems s of the prickly pear known as cladodes have been identified as potential candidates for developing food and supplements that promote good health. Opuntia cactus stems are a wellknown source of various nutritional elements, including dietary fiber, minerals, vitamins, antioxidant compounds, and high protein content (up to 13%). Additionally, traditional medicine has recognized the medicinal properties of prickly pear cactus stems s to treat different conditions such as hyperlipidemia, obesity, gastrointestinal disorders, and diabetes (Feugang et al., 2006).

It is suggested that Opuntia ficus-indica comprises phenolic compounds that possess potential health benefits such as prevention of serious health problems including hypercholesterolemia, arteriosclerosis, and cancer. Moreover, cactus phenolic compounds are believed to have curative properties for hypertension, gastric diseases, and hyperglycemia (El-Mostafa et al., 2014). Cacti are rich sources of flavonoids, which are a type of secondary metabolite polyphenolic compound. Flavonoids possess antioxidant properties and exhibit immunomodulatory and anti-inflammatory activities.

The concept of the "diet hypothesis" proposes that consuming healthy diets, rich in vegetables and fruits, can contribute significantly to the enhancement of antioxidants in the human body. This is because foods that are rich in antioxidants possess the capability of reducing or avoiding degenerative diseases (Hegazy et al., 2020).

Improving the bio-functionality of food is a top priority for the food industry, as the demand for healthier food options continues to grow. To meet this demand, various methodologies and techniques are developed over the past few decades, such as nutrient fortification and coating films. These methods aim to enhance the nutritional value and overall quality of food, making them more appealing and beneficial for consumers (Angor, 2016). Fortifying the chocolate with nutrient supplements increases the certain nutrients content in chocolate resulting in a healthier and more nutritious chocolate product, this fortification will provide essential nutrients to the consumers, without compromising the unique characteristics of the chocolate such as aroma, flavor, and taste (Deshmukh et al., 2020).

There is limited research available on industrial applications of cactus stems powder as a source of dietary fiber and antioxidants. The present study aimed to investigate the development of chocolate fortified with cactus stems powder from prickly pear and its effect on chemical, antioxidant, and sensory properties.

Material and methods

Preparing cactus stems powder from prickly pear

Cactus stems were obtained from a local farm (Al-Juri Nurseries, Jarash, Jordan) (Figure 1) on April 2022. The process of preparing cactus stems for further use involved cleaning them thoroughly, manually removing the outer layer with a knife, cutting them lengthwise, and then drying them in an air oven (Memmert, 854, Schwapach, Germany) at 45 ± 5°C for 72 h. After drying the cactus leaves, they were ground into a fine powder with the help of a coffee grinder machine (Grind & Brew, Philips, The Netherlands). The ground cactus stems powder was then passed through a sieve to obtain particles with a size of 355 μm. Finally, the resulting powder was kept at room temperature (∼25°C) in sealed bags.

Preparing chocolate samples

Plain chocolate (Avanti, Elmer Candy Corporation, Ponchatoula, Los Angeles, United States of America) was purchased from the local market and dissolved in a hot water bath for 15 mins at 50°C. Then, the cactus leaf powder was added at levels 3, 5, and 7% weight/weight (W/W) and mixed until homogenization.

After the sample was prepared, it was promptly moved to a plastic mold and kept in a chilled environment at 15°C for half an hour. Following this, it was carefully wrapped in aluminum foil and stored under refrigeration.

Proximate analysis

Chemical methods

Moisture, fat, ash, carbohydrates, protein, and fiber content (nitrogen-free extract, NFE) were analyzed for both cactus stems to powder and chocolate samples. AOAC (2000) (Association of Official Analytical Chemists) guidelines were followed for each analysis, and each analysis was conducted twice to ensure accuracy.

The air circulation oven drying method was employed for determining the moisture content of each sample. The samples were dried in an oven (Memmert, 854 Schwapach, Germany) at 105°C to get a constant weight. The content of nitrogen in the sample was measured by using the Rapid Kjeldahl method (Labconco, Model 652, Labconco Corp., Kansas City, MO, USA). The factor of 6.25 was used to multiply the % N to obtain the protein content. The ash content was calculated by incinerating the sample using a Muffle Furnace (Automatic Muffle Furnace,

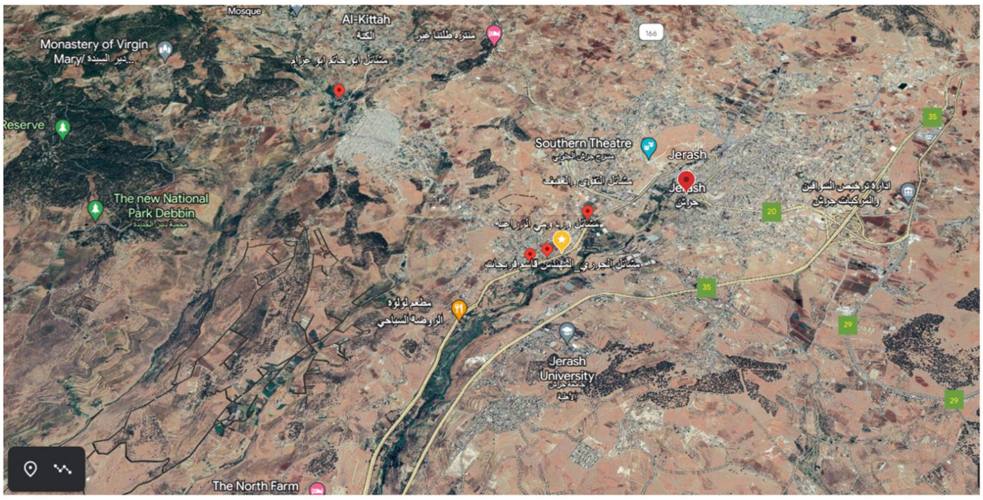

FIGURE 1
Location of Al-Juri Nurseries, Jarash, Jordan.

cat.no. OSK405) at 550°C for 4 h, and then weighing the residue (automatic muffle furnace, cat.no. OSK405). The AOAC method was used to quantify the crude fiber in each sample. Following this, each sample underwent a 30-min process of acidic digestion (using 0.255 N H_2SO_4) and then alkaline digestion (using 0.322 N NaOH). To determine the total lipid content, a 2:1 solution of chloroform and methanol was used to extract lipids from the samples through the chemical extraction Soxhlet method. The Soxhlet extraction method used a sample of 4 gm. The process included acid digestion and 4-h Soxhlet reflux at a boiling range of 40 to 60°C was done in petroleum ether at ISO grades (Bracco, Milan, Italy). The carbohydrate content was determined by subtracting the sum of all other ingredients from the total (100 gm).

The chocolate samples were analyzed for peroxide value (PV) and antioxidant activity (DPPH scavenging activity). The fat was first extracted from the samples, and then the peroxide value was determined at two different time points—zero time and after one month of storage. The PV was determined as per the AOAC guidelines and the sample value was calculated through PV= titer value*molarity*100 divided by sample weight as mentioned by Desta et al. (2020). This helped evaluate the oxidation status of the chocolate (Msaddak et al., 2015). The chocolate sample's DPPH scavenging activity was also assessed to determine its antioxidant capacity at two different time points—zero time and after one month of storage. The method used was based on a modification of Sulistyo and Haryant (2020) procedure. After incubating the sample for 30 mins at 37°C, the absorbance at 517 nm was measured using a spectrophotometer at both the start and after one month of storage.

Sensory evaluation

The assessment was conducted by employing a five-point hedonic ranking test to measure overall acceptability, flavor, taste, texture, appearance, and color. The study recruited 55 individuals aged between 17 and 55, comprising an equal distribution of 50% males and 50% females. Participants were selected from the student body and members of the Department of Nutrition and Food Processing at Al-Huson College. Before conducting the sensory analysis, the evaluators were introduced to the assessment process and instructed on how to use the rating systems. The sensory assessment was conducted at room temperature under white light. The assessors were requested to rate the hedonic attributes based on a 5-point ranking scale, where 5 indicated "like very much," 4 indicated "like moderately," 3 indicated "like slightly," 2 indicated "dislike slightly," and 1 indicated "dislike very much" (Thirumalaiselvi et al., 2023). A small quantity of water and bread were utilized to eliminate any remaining taste from the sample before assessing the next one.

Statistical analysis

The data analysis was conducted with the help of Statistical Analysis Software (SAS, 2002). The statistical analysis of the data involved a Completely Randomized Design to investigate the impact of different treatments on various parameters, including moisture, fat, protein, ash, fiber content, carbohydrates, peroxide value, antioxidant activity, and sensory scores. The data were further compared using the Least Significant Difference (LSD) method at a 0.05 significance level.

Results and discussion

Proximate analysis

Table 1 presents the chemical characteristics of the powder obtained from cactus leaves.

Fat (1.92%), total carbohydrates (64.46%), and crude fiber contents (28.55%) of cactus leaf powder were reported in the present study. Previous studies by Albergamo et al. (2022) and Sáenz et al. (2010) reported higher total dietary fiber of cactus

TABLE 1 Proximate chemical characteristics of cactus stem powder (g/100 g) of the sample.

Chemical parameters (%)	Mean ± SD
Moisture	7.10 ± 0.31
Protein	3.64 ± 0.58
Fat	1.92 ± 0.21
Ash	22.88 ± 0.87
Total carbohydrate	64.46 ± 2.24
Crude fiber	28.55 ± 1.76

stems powder (43%) in comparison to our study. Nevertheless, the findings of this study indicated a relatively elevated proportion of ash content (22.88%) and protein (3.64%) compared to the data reported by Albergamo et al. (2022). After analyzing the findings, it was discovered that powdered cactus stems are an excellent source of crude fiber. The results indicated that it contains more than 3% of dietary fiber, which meets the standard set by the European Commission (2006). This implies that cactus leaf powder can be labeled as a "natural fiber source."

Table 2 depicted a close chemical breakdown of chocolate samples prepared with different levels of cactus stems powder (3, 5, and 7%) and control. Chocolate samples that contained cactus stems powder at 3, 5, and 7% levels showed a noticeable ($p \leq 0.05$) rise in their protein, ash, crude fiber, and overall carbohydrate contents. In comparison to the control product, these findings suggest a significant increase in the nutritional value of the chocolates.

Protein, ash crude fiber, and total carbohydrate content of control and chocolate samples prepared with varying levels of cactus stems powder (3, 5, 7%) ranged between 4.94 to 5.83, 2.57 to 3.53, 1.49 to 2.40, and 48.36 to 54.96% respectively. The observed changes in the chocolate samples were attributed to the increased protein, ash, and crude fiber levels. These desirable components are naturally abundant in cactus leaf powder, as confirmed by the findings of Jana (2012). The results obtained are consistent with those of Al-Marazee (2018), which established that fortifying dark chocolate with wheat germ is an effective way of boosting the fiber intake of consumers and augmenting ash content. In a related study, Msaddak et al. (2015) stated that the substitution of wheat flour with cactus stems powder yielded enriched cookies with superior nutritional value. The researchers did not report significant improvements in the protein, moisture, fat, and total carbohydrate amount ($p>0.05$) but a significant increase in dietary fiber and ash content of the cookies ($p<0.05$) with an overall increase in dietary fiber content from 0.40 to 2.42% after incorporation of 2.5, 5, and 7.5% cactus stems powder.

In our study, chocolate samples prepared with the addition of cactus stems powder at different levels (3, 5, 7%) reported significantly reduced fat content from 37.92 to 28.70% in comparison to the control product ($p \leq 0.05$). This has a healthy positive effect by decreasing lipid content by 9.22%. According to a recent study by Al-Marazeeq et al. (2023), adding cactus cladodes powder to low-fat beef and chicken burgers had a marked effect on the reduction of lipid levels. The researchers found that the use of this ingredient significantly lowered the lipid content in the burgers. Tlili et al. (2011) reported improved protein content storage in cactus stems (between 2007 to 2009) from 3 to 6% and 8% respectively with a mean protein content of 6.05 ± 0.23. Another study reported crude *Opuntia ficus-indica* content of protein as 71 g/kg under field and 264 g/kg under greenhouse conditions (Mayer et al., 2019). These studies are concerning our results of increased protein content in chocolate samples.

Albergamo et al. (2022) revealed that cactus stems powder contains significant amounts of total polyphenol content, which may be attributed to the active compounds for their antioxidant properties. Furthermore, the polyphenols in cactus stems powder hold their antioxidant activity even after undergoing heat processing, which may be beneficial for humans. These findings are in agreement with the study by Dziki et al. (2014). Antioxidant activity was assessed by DPPH radical-scavenging activity, and oxidation was assessed by the peroxide value of chocolate samples prepared with different levels of cactus stems powder at zero time, and one month of storage as illustrated in (Table 3). The results showed that the control chocolate sample had the lowest DPPH radical scavenging activity, with values of 32.21% at zero time and 24.92% after one month. On the other hand, the chocolate sample with a 7% level of cactus stems powder had the highest DPPH radical-scavenging activity, with values of 56.29% at zero time and 52.23% after one month.

Table 3 also shows reduced antioxidant activity after a month of storage for all treatments. During the storage of chocolate samples, the control sample showed the greatest reduction in antioxidant activity, which could negatively impact the storage stability of the chocolate. However, chocolate samples treated with cactus leaf powder were able to maintain most of their antioxidants, as they showed only a slight reduction in antioxidant activity after one month of storage. This is an exciting finding that suggests cactus leaf powder can help to preserve the antioxidant activity of chocolate during storage.

For determining the amount and types of antioxidants present in various food products supplemented with cactus leaf powder, further research is needed. Several studies, including those by Msaddak et al. (2015), Hegazy et al. (2020), and Albergamo et al. (2022), have suggested that adding cactus stems powder to food products like cookies may help to reduce oxidative damage and improve product stability. The findings imply that incorporating cactus leaf powder into chocolate enhances its nutritional value and aids in protecting it from oxidative damage due to its antioxidant potential.

Thus, to determine the impact of cactus stems powder on the oxidative stability of chocolate, its antioxidant properties were assessed using peroxide value (PV) measurements at the initial stage and after one month of storage (Table 3). PV of the control sample (1.52 mEq/kg) at zero time was the highest among all treatments. Throughout the storage period, the PVs of the control and cactus stems powder chocolate treatments increased gradually. After the storage period, the control chocolate had a PV of 9.95 mEq/kg, while the 7% cactus stems powder chocolate treatment had a PV of 5.01 mEq/kg. This indicates that incorporating cactus leaf powder into chocolate significantly reduced oxidation ($p \leq 0.05$). Msaddak et al. (2015) found that cookies enriched with prickly

TABLE 2 A proximate chemical composition (g/100 g) of chocolate samples prepared with different levels of cactus stems powder.

Treatments	Moisture	Protein	Fat	Ash	Total carbohydrate	Crude fiber
Control	6.21 ± 0.02[d]	4.94 ± 0.15[d]	37.92 ± 0.82[a]	2.57 ± 0.13[d]	48.36 ± 1.45[d]	1.49 ± 0. 15[d]
3%	6.55 ± 0.25[c]	5.25 ± 0.30[c]	34.44 ± 0.70[b]	2.93 ± 0. 31[c]	50.83 ± 1.20[c]	1.7 ± 0.10[c]
5%	6.76 ± 0.20[b]	5.41 ± 0.15[b]	31.98 ± 0.95[c]	3.20 ± 0.05[b]	52.65 ± 0.87[b]	2.30 ± 0.64[b]
7%	6.98 ± 0.20[a]	5.83 ± 0.15[a]	28.70 ± 0.55[d]	3.53 ± 0.35[a]	54.96 ± 0.98[a]	2.40 ± 0.10[a]

[a−d]represent significant differences ($p \leq 0.05$) between means in the same column.

TABLE 3 Antioxidant activity, oxidation of chocolate samples prepared with different levels of cactus stems powder.

	Scavenging activity[f]		PV (mEq/kg)	
Treatments	Zero time	One month	Zero time	One month
Control	32.21 ± 0.35[a]	24.92 ± 1.05[a]	1.52 ± 0.21[a]	9.95 ± 0.24[a]
3%	38.95 ± 0.44[b]	29.56 ± 1.33[b]	1.25 ± 0.02[a]	7.19 ± 0.21[b]
5%	43.52 ± 0.30[c]	35.01 ± 1.41[c]	1.19 ± 0.04[a]	6.05 ± 0.19[c]
7%	56.29 ± 0.16[d]	52.23 ± 1.44[d]	1.11 ± 0.06[a]	5.01 ± 0.90[d]

[a−d]represent significant differences ($p \leq 0.05$) between means in the same column.
[f]to determine the fDPPH radical-scavenging activity (%) and reducing power (absorbance at 700 nm), cookies were tested at a concentration of 50 mg/ml.

TABLE 4 Sensory characteristic evaluation of chocolate samples prepared with different levels of cactus stems powder.

Treatment	Overall acceptability	Texture	Flavor	Color	Taste	Appearance
Control	4.00 ± 0.85[a]	3.94 ± 0.92[ab]	3.92 ± 0.87[a]	3.64 ± 1.20[b]	3.81 ± 1.06[a]	4.06 ± 0.98[ab]
%3	4.19 ± 1.06[a]	4.00 ± 1.10[ab]	3.97 ± 1.03[a]	3.83 ± 0.91[ab]	4.00 ± 1.07[a]	4.00 ± 1.10[ab]
5%	3.97 ± 0.88[a]	4.14 ± 0.80[a]	4.00 ± 1.04[a]	4.11 ± 1.06[a]	3.83 ± 1.06[a]	4.28 ± 0.97[a]
7%	4.08 ± 0.87[a]	3.72 ± 1.06[b]	4.08 ± 0.81[a]	3.78 ± 1.02[ab]	3.75 ± 1.11[a]	3.75 ± 1.01[b]

[a−c] indicate a significant difference ($p \leq 0.05$).
5-point hedonic score: 1 = dislike very much, 5 = like very much.

pear cladodes experienced reduced oxidative degradation during storage, which supports the idea that cactus-derived ingredients can help to stabilize food products.

Sensory evaluation

Sensory evaluation scores of the control and experimental chocolate samples prepared with different levels of cactus leaf powder were found to be attractive to consumers. Table 4 presented the results of a sensory evaluation that was carried out on both the control chocolate samples and the chocolate samples that had been treated with cactus leaf powder. The evaluation was based on a 5-point hedonic scale, which considered various factors such as texture, flavor, color, taste, and appearance. Upon analysis, no significant difference was found between the average scores of the overall acceptability, flavor, and taste of the control samples and the treated samples. The scores for both groups were between the range of "slightly liked" to "moderately liked." These results disagreed with De Wit et al. (2015) who reported that increasing inclusion levels of cactus pear (Opuntia ficus-indica L. Mill and Opunt robusta Wendl) cladode flour in different baked products had a significantly decreased effect on all the sensory attributes evaluated. However, Msaddak et al. (2015) concluded that cookies fortified with cladodes at a 5% level did not elicit any negative sensory responses, indicating that the product remained acceptable to consumers. The sensory results for texture and appearance showed that the cactus stems powder treatment (7%) had the least scores (3.72 and 3.75) respectively compared with all other treatments and control.

While the color of control treatment had the lowest significant ($p \leq 0.05$) score (3.64) compared to all treatments. According to Fitriani Nur et al. (2020), the sensory evaluation conducted on chocolate made using a combination of jackfruit flour and red palm olein showed that it was wellreceived by the panelists.

Conclusion

The present study aimed to enrich the nutritional value of chocolate by incorporating cactus stems powder at various concentrations (0, 3, 5, and 7%) w/w. The study also assessed the antioxidant and chemical properties of the chocolate as well as evaluated its sensory characteristics. Fortification of chocolate with cactus stems powder increased the moisture, protein, ash, total carbohydrate, and fiber of chocolate treatments, whereas fat content decreased. Moreover, the results demonstrated that the addition of cactus stems powder at different levels (3, 5, and 7% w/w) enhanced the antioxidant activity of chocolate treatments and reduced their oxidative degradation. Importantly, the sensory evaluation showed that all levels of fortification had no impact on the chocolate product's sensory properties. These findings suggest that cactus stems powder could be a promising source of ash, antioxidants, and dietary fiber in the food processing industry, offering potential health benefits to consumers. In conclusion, this study highlights the potential of cactus stems powder as an innovative and functional ingredient in food formulations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

MA: conception, design, correspondence, analysis, editing, and results. NA: drafting and analysis. KA-M: drafting and analysis. WA-R: drafting and analysis. RA: drafting and analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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