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Effect of partial replacement of wheat flour with three green leaves′ flours in the nutritional and antioxidant properties of sliced bread

Efecto de la sustitución parcial de la harina de trigo con harinas de tres hojas verdes en las propiedades nutricionales y antioxidantes del pan de molde

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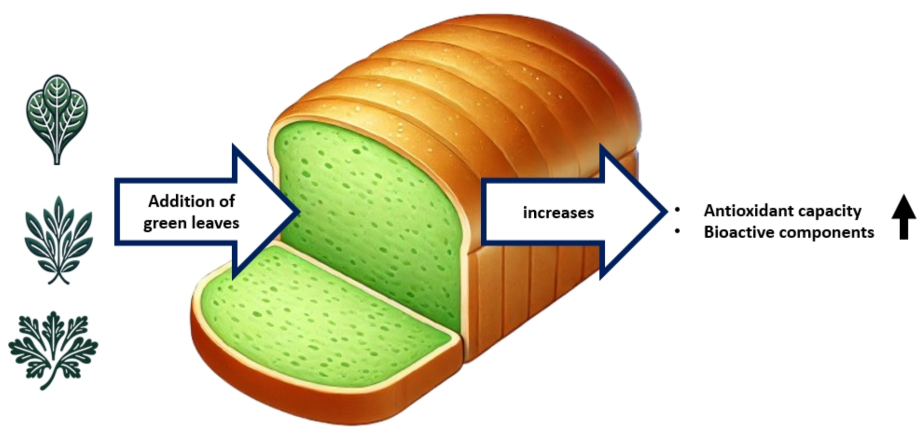

The inclusion of 3% leaf flour increased protein, fiber, and ash content while reducing fat content of sliced bread.
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Short title: GREEN LEAF FLOURS: NUTRITIONAL AND ANTIOXIDANT IMPACT ON BREAD

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ABSTRACT

The leaves of certain cultivated plants can exhibit a high phenolic compound content, demonstrating significant antioxidant activity. Phenols, organic compounds found in many plants, possess antioxidant properties that can protect body cells from oxidative damage caused by free radicals. In this study, four different types of bread were prepared: standard bread, spinach bread, parsley bread, and tarragon bread, using a 3% partial substitution (PS) of wheat flour with flours derived from different types of leaves. The physicochemical, antioxidant, and sensory properties of the bread with this 3% leaf flour addition were evaluated. The results revealed that the inclusion of 3% leaf flour increased protein, fiber, and ash content while reducing fat content. Additionally, a decrease in crumb lightness and a shift in the a* color coordinate were observed. The total phenolic content (TPC) and antioxidant activity (AA) also increased with the addition of these natural ingredients, rising from 51.1 to 101.38 mg GAE/100 g and from 15.48 to 50.48 mg AAE/100 g, respectively. In terms of sensory evaluation, the bread with 3% spinach flour was the most accepted after the standard bread. In summary, the inclusion of 3% leaf flour in the bread influenced its properties, with tarragon being the most notable for increasing fiber, ash content, antioxidant activity, and total phenolic content.

Additional key words: antioxidants; parsley; polyphenols; spinach; tarragon.

RESUMEN

Las hojas de ciertas plantas cultivadas pueden presentar un alto contenido de compuestos fenólicos, demostrando una actividad antioxidante significativa. Los fenoles, compuestos orgánicos presentes en muchas plantas, poseen propiedades antioxidantes que pueden proteger las células del cuerpo del daño oxidativo causado por los radicales libres. En este estudio, se elaboraron cuatro tipos diferentes de pan: pan estándar, pan con espinaca, pan con perejil y pan con estragón, utilizando una sustitución parcial (SP) del 3% de la harina de trigo con harinas derivadas de diferentes tipos de hojas. Se evaluaron las propiedades fisicoquímicas, antioxidantes y sensoriales del pan con esta adición del 3% de harina de hojas. Los resultados revelaron que la inclusión del 3%

de harina de hojas aumentó el contenido de proteína, fibra y cenizas, mientras que redujo el contenido de grasa. Además, se observó una disminución en la luminosidad de la miga y un cambio en la coordenada de color a*. El contenido total de fenoles (TPC) y la actividad antioxidante (AA) también aumentaron con la adición de estos ingredientes naturales, pasando de 51.1 a 101.38 mg GAE/100 g y de 15.48 a 50.48 mg AAE/100 g, respectivamente. En términos de evaluación sensorial, el pan con un 3% de harina de espinaca fue el más aceptado después del pan estándar. En resumen, la inclusión del 3% de harina de hojas en el pan influyó en sus propiedades, destacándose el estragón por su mayor incremento en fibra, contenido de cenizas, actividad antioxidante y contenido total de fenoles.

Palabras clave adicionales: antioxidantes; perejil; polifenoles; espinaca; estragón.

INTRODUCTION

Spinach (*Spinacia oleracea*) is a plant cultivated worldwide in tropical and temperate climates. Its leaves provide consumers with a high number of vitamins and minerals, thereby preventing diseases arising from nutritional deficiencies such as anemia (Massa *et al*., 2018). Notably, it contains flavonoids with anticancer properties that inhibit the cell division of cancer cells in the stomach and skin, similar to the effects of its antioxidants (Gutierrez and Velazquez, 2020).

Parsley (*Petroselinum crispum*) is an aromatic herb used in various cuisines around the world, although many people are unaware of its numerous health benefits (Arrascue and Troncoso, 2023). It is a good source of vitamins K, C, and A, and contains flavonoids and phenolic compounds that inhibit free radicals in the body (Farzaei *et al*., 2013).

Originating from Southern Europe and Western Asia, tarragon (*Artemisia dracunculus*) is an aromatic herb used in European cuisines. Its composition helps combat flatulence and rheumatism, and it was historically used as a local analgesic due to its eugenol content (Pripdeevech and Wongpornchai, 2012). Its high antioxidant activity and phenolic content make it a potential substitute for synthetic antioxidants (Mumivand *et al*., 2017).

According to Velez *et al*. (2012), cultivated plant leaves have a higher phenolic content compared to wild plants. Various parts of plants such as leaves, stems, roots, and seeds are rich in antioxidants, dietary fibers, and essential oils (Das *et al*., 2012).

As technology and education advance, today's consumers are increasingly aware that food directly impacts their health. This awareness is reflected in their shift in preference towards foods that offer not only satiety but also additional nutritional benefits. Functional foods contain components that enhance health beyond basic nutrition (Skotnicka *et al*., 2017). In recent years, there has been an increase in research on functional foods with antioxidant properties due to health issues caused by oxygen and free radicals (Jridi *et al*., 2019).

Bread is a staple food known in various regions of the world. With wheat flour as its main ingredient, it is an essential item on every household table. Its daily consumption helps meet the necessary requirements for a balanced diet (Jing *et al*., 2019). In a previous study, it was found that adding 3% leaf powder from the Moldavian dragonhead plant to bread maintained acceptable sensory qualities and increased antioxidant capacity. However, this addition did not affect the bread's texture, although it did result in an increase in green color in the crumb and a decrease in its lightness. Additionally, the total phenolic content (TPC) was also influenced, increasing from 4.8 to 10.1 mg GAE/g dry mass (Dziki *et al*., 2019). Similarly, the addition of parsley to wheat flour reduced the bread's volume and increased its moisture, although these changes had little effect on crumb texture. The crumb color was affected, showing less lightness and more redness due to the parsley. However, partially replacing wheat flour with up to 3% parsley resulted in bread with higher antioxidant activity and acceptable sensory qualities (Dziki *et al*., 2022).

The addition of ground leaves in the production of white bread could increase the content of bioactive compounds and improve its nutritional profile. The aim of this study was to evaluate the physicochemical characteristics, antioxidant properties, and sensory quality of white bread enriched with 3% of three different types of leaves: spinach, parsley, and tarragon.

MATERIALS AND METHODS

Raw materials

For the preparation of bread with green leaves, the ingredients (flour, yeast, sugar, butter, salt, and powdered milk) were purchased from the local market, considering their shelf life and technical specifications. The flour used was from the Bemoti brand, the yeast from Fleischmann, the sugar from the Cartavio brand, the butter from Famosa, the salt from the Emsal–Marina brand, and the powdered milk from Mamfrey. The water used was filtered. Regarding the green leaf flour (spinach, parsley, and tarragon), it was sourced from NutryDia (https://nutrydiaperu.com/).

Bread formulation and production

A standard recipe was used to prepare sandwich bread, with a partial substitution of green leaves at 3% (Tab. 1). This percentage was determined based on previous studies indicating that up to 3% of green leaves maintains acceptable sensory and technological properties without affecting texture, volume, or consumer acceptance (Dziki *et al.*, 2019; Lim *et al*., 2020; Dziki *et al.*, 2022). The ingredients were accurately weighed using a digital scale and placed in a Blackline BM-6301 bread-making machine. The preparation followed a standard three-step process: kneading, where the dough was mixed and developed to achieve proper gluten formation; fermenting, allowing the dough to rise and develop its structure; and baking, where controlled heat ensured proper texture, crust formation, and internal crumb development.

Table 1. Bread formulation with different leaves

Components	Control bread (%)	Green leaves bread (%)
Wheat flour	100	97
Herbs substitution	0	3
Sugar	9.6	9.6
Yeast	2	2
Butter	6.7	6.7
Salt	1.8	1.8
Milk powder	1.6	1.6
Water	57	57

Proximate composition analysis

For each bread sample, the following analyses were conducted: Moisture content, using oven drying at 105 ± 0.03ºC for 4 h (AOAC, 2010 method 925.10); ash content, by calcination in a muffle furnace at 500°C (AOAC, 2010 method 923.03); fat content, using the Soxhlet extraction method (AOAC, 2010 method 945.16); protein content, using the Kjeldahl method (AOAC, 2010

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method 981.10); and carbohydrate content, calculated by subtracting the total from the other components (AOAC, 2010 method 991.43).

Color properties

Color measurements (CIELAB) in the experiments will be determined using image analysis in ImageJ software version 1.53e. Color indicators L* (Luminosity) and redness degree (s*/b* ratio, where a* represents red-green and b* represents yellow-blue) will be extracted from the RGB information of the ROIs. These data will be used to calculate the color in CIELAB coordinates. For this research, an image acquisition system will be used with: (1) 20 cm walls and matte black internal color; (2) four equidistant white LED lights at 10 cm from the edge; (3) a camera with 52-megapixel image resolution; and (4) a computer with an Intel Core i7 processor and 12GB of RAM. The extracted samples will undergo image acquisition in *.jpg format (Joint Photographic Experts Group, Ginebra).

Total phenolic content and antioxidant properties

Determination of phenolic compounds

For the determination of phenolic compounds, the Folin-Ciocalteu method (Singleton and Rossi, 1965) was used with some modifications. 70% methanol, Folin-Ciocalteu phenol in a 1:5 ratio, 10% sodium carbonate, and gallic acid in a 1:10 ratio were used.

First, 0.4±0.001 g of the sample was weighed into a test tube, which was then placed in a water bath at 60°C. Simultaneously, the sample was agitated in a magnetic stirrer for 30 min. Afterwards, the samples were centrifuged at 3,500 rpm for 10 min to separate the remaining extract, which was decanted with a pipette.

Then, 100 µL of the sample extract was transferred in triplicate to separate tubes, to which 400 µL of Folin-Ciocalteu phenol reagent was added. After a period of 3 to 8 min, 2 mL of a 10% sodium carbonate solution was added, the tubes were capped and mixed. The tubes were then placed in a vortex shaker at 300 rpm for 30 min before measuring the absorbance in a spectrophotometer, adjusting the wavelengths to 725, 750, and 765 nm.

Antioxidant capacity

The antioxidant activity will be evaluated using the DPPH radical scavenging method according to Brand-Williams *et al*. (1995) with some modifications. A stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of ethanol and stored at 6°C. Then, the working solution was obtained by mixing 10 mL of the stock solution with 45 mL of ethanol to reach an absorbance of 1.1±0.02 units at 515 nm. For the reaction, 150 μL of the extract was combined with 2850 μL of the DPPH solution and the mixture was shaken for 30 min. A spectrophotometer (ThermoSpectronic, Model Genesys 10 UV, Massachusetts) with ethanol as a blank was used to measure the absorbance at 515 nm. The antioxidant capacity is expressed as μmol equivalents of ascorbic acid (AA) per 100 g of bread based on a standard curve of ascorbic acid (0-200 μM).

Sensory evaluation

It was conducted using a hedonic test with 66 panelists aged 18 to 56, including 39 men and 27 women, who were regular consumers. Each panelist was given a sensory evaluation sheet containing bread samples, including a control and the samples under study. To avoid bias, the samples were cut, bagged, and coded before being given to the panelists. Participants were asked to rate the smell, color, taste, texture, and overall appearance of each sample according to their preferences. The sensory attributes were evaluated using a nine-point hedonic scale, where "1" indicated "dislike extremely," "5" meant "neither like nor dislike", and "9" represented "like extremely."

Statistical analysis

A completely randomized design (CRD) was used, with four treatments, each with five replicates. Each treatment consisted of a variety of dried leaf flour, designated as control (0% dried leaf flour), tarragon (3% dried tarragon leaf flour), parsley (3% dried parsley leaf flour), and spinach (3% dried spinach leaf flour). Data on physicochemical, physical, chromatic, and functional properties such as moisture, ash, fat, carbohydrates, crude fiber, protein, area, and total number of pores, instrumental color L* (white to black), a* (red to green), b* (yellow to blue), antioxidants, and polyphenols were evaluated using an analysis of variance (ANOVA). Means were compared using Tukey's test with a significance level set at $P<0.05$. All statistical analyses were performed using Minitab 17 software.

For the sensory evaluation of smell, color, taste, texture, and overall appearance, the means were compared using statistical analysis performed in Excel 2016 (Microsoft, Washington).

RESULTS AND DISCUSSION

Proximate analysis

The physicochemical analyses are shown in table 2. All treatments report data below 40% humidity without statistical significance among the samples. On the other hand, it was found that spinach bread had a higher protein content (9.5%) than the control (7.54%). The tarragon (8.64%) and parsley (9.30%) samples showed intermediate protein levels between the spinach and control bread, as they share statistical similarity with both groups. The percentage of fat varies among the samples, with the highest being the standard sample (13.42%) and the lowest being the tarragon sample (10.03%). The percentage of crude fiber varies among the samples, with the highest being the tarragon-containing sample (1.71%) and the lowest being the standard sample (0.69%). The parsley sample has the highest ash percentage (1.76%), while the tarragon-containing sample (1.50%) and the control sample (1.42%) show the lowest ash percentage.

Table 2. Proximal composition of control bread and those produced with formulations of green leaves′ flours.

Components	Control	Tarragon	Spinach	Parsley
Moisture (%)	34.50±2.85 a	30.20±2.28 a	32.84±6.33 a	34.20±3.6 a
Protein (%)	7.54±1.03 b	8.64±0.32 ab	9.50±0.57 a	9.30±1.23 ab
Fat (%)	13.42±0.94 a	10.03±1.55 b	12.34±1.83 ab	11.70±1.78 ab
Carbohydrate (%)	42.45±2.8 b	47.92±1.71 ab	42.59±6.23 b	41.53±5.09 ab
Crude fibre (%)	0.69±0.14 b	1.71±0.76 a	1.05±0.47 ab	1.48±0.28 ab
Ash (%)	1.42±0.04 c	1.50±0.02 c	1.67±0.05 b	1.76±0.08 a

All means are expressed as mean±SD (n=3). The means that do not have the same letters are significantly different at the 0.05 significance level according to the Tukey test.

The high ash content in bread is influenced by the mineral content of the ingredients used. Parsley leaf flour has a mineral content rich in calcium and potassium, according to Saavedra and Maldonado (2021). The calcium content of parsley leaf is 202 mg/100 g. The increase in the protein level of the bread is influenced by the protein content of the raw material used; thus, the more spinach flour is added, the higher the protein content in the bread (Waseem *et al*., 2021). This fact would be important considering that the number of non-polar side chains of the proteins that bind to the hydrocarbon chains of the fat is related to the fat absorption capacity of a food (Sgarbieri, 1998). Regarding fiber content, breads substituted with leaf flour presented a higher fiber content compared to the control bread. This increase was attributed to the high fiber content of the leaf powder used. Additionally, the progressive addition of leaf powder showed a significant improvement in proteins, minerals, and fiber in baked products, as observed in this study, and similar results have been reported by Filip and Vidrih (2015).

Table 3 and figure 2 shows the results obtained from image analysis. The tarragon sample exhibited the highest number of gas cells, followed by the control, parsley, and spinach samples. However, there were no significant differences between the means of the tarragon and parsley samples. However, there were no significant differences between the means of the tarragon and parsley samples.

The leaves influenced the CIE LAB* color space according to the analysis. In the L* coordinate, brightness decreased as the standard sample value (76.83±1.62) was higher than spinach (67.38±3.73). On the other hand, in the a* coordinate, all four treatments had the characteristic green color, with the control (-4.60±0.19) having the least coloration and parsley (5.89±0.48) having the highest value. Finally, for the b* coordinate, all values were in the yellowish hue range, with the lowest value for the control (26.12±2.39) and the highest for tarragon (42.98±2.83).

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Table 3. Results of the determination of physical properties of control bread and those produced with formulations of green leaves′ flours.

Components	Control	Tarragon	Spinach	Parsley
N°. of gas cells	894.8±3.53 ab	954.2±6.36 a	714.6±2.82 b	867.8±2.12 ab
Area occupied by gas cells (mm)	119.05±11.5 bc	117.27±11.1 c	126.01±15.7 a	121.77±13.6 ab
Instrumental colour				
L* (White to black)	76.83±1.62 a	70.25±4.64 b	67.38±3.73 c	71.17±4.01 b
a* (red to green)	-4.60±0.19 a	-5.35±0.48 c	-4.87±0.47 a	-5.89±0.48 c
b* (yellow to blue)	26.12±2.39 c	42.94±2.26 a	41.17±3.29 a	42.94±2.26 a

All means are expressed as mean±SD (*n*=3). The means that do not have the same letters are significantly different at the 0.05 significance level according to the Tukey test.

Figure 2. Bread crumb appearance of control bread and those produced with formulations of green leaves′ flours.

In a similar study Skendi *et al*. (2019), the number of cells and total area determined that the addition of oregano, savory, and thyme at 1 and 2% in enriched breads showed comparable total cell size to the control group, except when dried savory was added, where a notably lower value was observed. The addition of powdered leaves strongly influences the crumb color, which is related to the chlorophyll and carotenoid pigments present in the herbs (Dimov *et al*., 2018). Similar findings were reported for color changes in cookies supplemented with powdered spinach (Galla *et al*., 2017). Color change is not necessarily a negative factor in consumer preference for new product development (Zhu *et al*., 2008).

Phenolic determination and antioxidant capacity

The total amount of phenolic compounds (TPC) in the four treatments can be seen in figure 3. It is observed that the bread with partial substitution of tarragon flour at 3.00% showed a significant increase in total phenolic content (101.38 mg eq. GAE/100 g) and antioxidant activity (50.48 mg eq. AA/100 g), compared to the breads with partial substitution with spinach and parsley flour. Likewise, it was observed that the bread with partial substitution of tarragon showed an increase of 98.16% for TPC and 226% for antioxidant activity. On the other hand, the breads with partial substitution of spinach and parsley flours at 3.00% showed total phenolic contents of 81.55 and 74.68 mg eq GAE/100 g, respectively. Also, the antioxidant activity of these formulations showed values of 33.82 and 44.54 mg eq. AA/100 g, respectively.

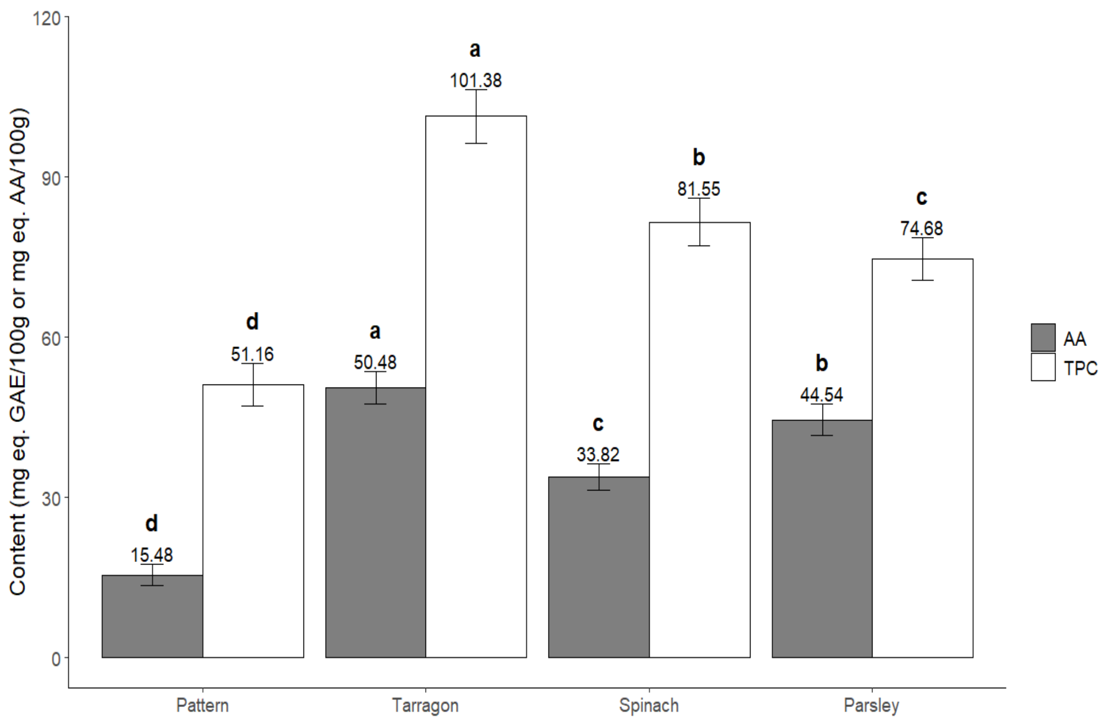

Figure 3. Total phenolic content (mg eq. GAE/100 g) and antioxidant activity (mg eq. AA/100 g) of bread formulations enriched with tarragon, spinach, and parsley flours. The means that do not have the same letters are significantly different at the 0.05 significance level according to the Tukey test.

The total phenolic content (101.38 mg eq. GAE/100 g) and antioxidant activity (50.48 mg eq. AA/100 g) of bread with partial substitution of tarragon flour at 3% is higher than the results reported by Lim *et al*. (2020), who indicated TPC (10.3 mg eq. GAE/100 g) and AA (6.97 mg eq.

T/100 g) in breads with partial substitution of kenaf flour at 4%, respectively. The antioxidant properties of tarragon could be attributed to the inclusion of phenolic compounds such as luteolin and specifically derivatives of caffeoylquinic acid, which are known to have proven antioxidant activity (Tajner-Czopek *et al*., 2020). Although high doses of tarragon may offer antioxidant benefits, it is important to note that its application in large quantities can carry health risks. This is due to the known mutagenic and hepatotoxic effects of estragole; however, it has been concluded that its consumption represents a minimal risk to human health (Nesslany *et al*., 2010).

The TPC values are high for parsley as in a study under similar conditions (Dziki *et al*., 2022) mentions that by substituting 3% parsley leaf flour in bread, a value of 0.61 mg eq. GAE/g was obtained, and for spinach, its resulting values were lower when compared to a similar study where TPC 110.56 mg eq. GAE/g and AA 210.88 mg eq. T/100 g were found in partial substitutions of spinach leaf flour at 2% and 3% respectively (El-Sayed, 2020; Junejo *et al*., 2021).

The variation in TPC and AA values in plant leaves can be attributed to a combination of genetic, physiological, and environmental factors, including plant variety, maturity at the time of harvest, and growing conditions (Howard *et al*., 2002). Also, variations in TPC values may be due to differences in raw materials, processing conditions, and extraction procedures (Mpofu *et al*., 2006). It is important to consider these factors when interpreting and comparing levels of phenolic compounds in different products.

Sensory evaluation

Table 4 and figure 4 present sensory evaluation data for different components and substitution levels in the product, including odor, color, taste, texture, and overall appearance. The average scores for odor, color, and taste were similar for tarragon and parsley, with lower statistical significance. The standard sample had the highest scores in these categories, followed by spinach. The control sample stood out in texture with an average of 7.26, showing a significant difference from the other treatments. Spinach, averaging 6.55, grouped with tarragon, indicating no significant difference between them. However, tarragon, with an average of 5.9, formed a distinct group, significantly different from the control and spinach but not from parsley (5.7), which differed from the rest.

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Table 4. Sensory evaluation on hedonic scale of breads with different green leaves.

Components	Control	Tarragon	Spinach	Parsley
Smell	6.75±1.17 a	4.30±1.68 c	5.68±1.69 b	4.61±1.74 c
Color	7.25±1.23 a	4.96±1.80 c	4.96±1.47 b	4.78±1.96 c
Flavor	7.08±1.25 a	4.57±1.97 c	5.08±1.61 b	4.78±1.92 c
Texture	7.26±1.16 a	5.95±1.71 ab	6.55±1.32 bc	5.75±1.95 c
General appearance	7.05±1.45 a	5.57±2.14 b	5.81±1.41 b	5.31±1.94 c

All means are expressed as mean±SD (*n*=3). The means that do not have the same letters are significantly different at the 0.05 significance level according to the Tukey test.

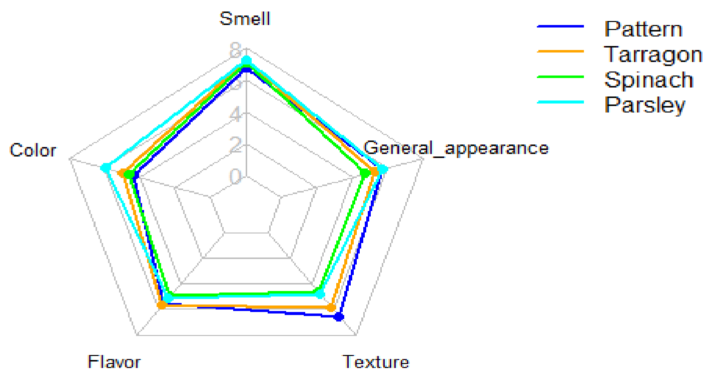

Figure 4. Sensory evaluation results of breads with different green leaves.

The Tukey test identifies significant differences among the control, spinach, tarragon, and parsley for smell, color, and flavor. The control and spinach do not differ significantly from each other, but both tarragon and parsley are significantly different from the other treatments. Regarding overall appearance, significant differences are also evident between the control and the other three treatments (spinach, tarragon, and parsley). However, there are no significant differences between spinach, tarragon, and parsley among themselves, as all three significantly differ from the control.

Đurović *et al*. (2020) stated that a 5% increase in nettle leaf incorporation had an impact on the sensory characteristics of bread. These included a darker crumb, distinctive flavor, pronounced grassy aroma, and firmer texture in the bread crumb. On the other hand, Dziki *et al*. (2022) found that the addition of parsley leaves in the range of 1 to 3% was well received by the evaluated individuals in terms of bread odor and taste. However, as the amount of parsley leaves added beyond this range increased, the panelists no longer showed acceptability in the sensory characteristics of the bread. The low acceptability values of parsley and tarragon in terms of odor and taste, may be due to the oil content of parsley: felandrene, α-terpinolene, 1.3.8-p-mentatriene, myristicin, and elemicin and in the case of tarragon it is too strong and pungent (Sany *et al*., 2022).

CONCLUSIONS

The antioxidant content and low-fat index make spinach, parsley, and tarragon leaves a good alternative to current foods as they would prevent diseases from an unhealthy and disorderly lifestyle. This study revealed that the addition of the aforementioned leaf flours in bread showed an improvement in protein, fiber, and ash compared to the control bread, which can help reduce constipation and cholesterol. High levels of phenols and antioxidant capacity were also showed, especially in tarragon, which could inhibit oxidation reactions in the body and reduce cardiovascular and neurodegenerative diseases. The substitution of spinach leaf at 3% had the highest overall acceptance in terms of sensory aspects. This research could help emphasize the development of baked products with substitution of wheat flour by leaves powders, as they are healthier than conventional baked foods.

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Industries Engineering, where the institution supports research by providing salaries for their time dedicated to conducting this study.

Open source research data repository: we have shared the data and code to ensure the transparency and reproducibility of our work. We kindly request that if these resources are used for academic purposes, the original article be cited. The experimental data used and obtained can be found in the open-access repository: https://github.com/dannychambi/Datos-de-panes-con-hojas, to guarantee the reproducibility and transparency of the results presented in this study.

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