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Improvement of the antioxidant capacity of a yogurt enriched with aqueous ginger extract (*Zingiber officinale*)

Mejora de la capacidad antioxidante de un yogur enriquecido con extracto acuoso de jengibre (*Zingiber officinale*)

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Abstract

The search for new foods is related to adding nutraceuticals or ingredients that have a specific function on the human metabolism. These types of foods have been called “functional foods.” Ginger (*Zingiber officinale*) has been studied for its antioxidant capacity. Thus, this ingredient has the potential to confer antioxidant properties to fermented drinks such as yogurt. For this reason, this work was carried out to develop a yogurt with aqueous ginger extract to determine a synergistic effect between compounds that provide antioxidant activity. ABTS, DPPH, and FRAP techniques carried out antioxidant capacity analyses. The concentration of total phenols was calculated, and a sensory test was carried out to determine the amount of aqueous ginger extract that was mostly accepted. Sensory tests showed that the yogurt preparation with 18 % ginger extract increased the chances of having greater antioxidant activity and had the best characteristics. The results of the hedonic test of the prepared yogurt showed that the texture had a value of 3.28, which was close to “I neither like nor dislike”, and the flavor value was 3.63, which was close to "I like". The antioxidant analysis showed that adding ginger extract improved the bioactivity of the enriched yogurt to a natural one. In fact, after fermentation, a significant increase in antioxidant capacity was observed. Ginger in yogurt seems like an alternative to the technological diversification of a well-known product.

Keywords: Ginger, yogurt, antioxidant capacity, sensory analysis, functional food.

Resumen

La búsqueda de nuevos alimentos está relacionada con la incorporación de nutracéuticos o ingredientes que tengan una función específica sobre el metabolismo humano. A este tipo de alimentos se les ha llamado “alimentos funcionales”. El jengibre (*Zingiber officinale*) ha sido estudiado por su capacidad antioxidante. Así, este ingrediente tiene el potencial de conferir propiedades antioxidantes a bebidas fermentadas como el yogur. Por tal motivo, se realizó este trabajo para desarrollar un yogur con extracto acuoso de jengibre para determinar un efecto sinérgico entre compuestos que aportan actividad antioxidante. Se realizaron análisis de capacidad antioxidante mediante técnicas ABTS, DPPH y FRAP. Se calculó la concentración de fenoles totales y se realizó una prueba sensorial para determinar la cantidad de extracto acuoso de jengibre mayoritariamente aceptado. Las pruebas sensoriales demostraron que el preparado de yogur con 18 % de extracto de jengibre aumentó las posibilidades de tener mayor actividad antioxidante y tuvo las mejores características. Los resultados de la prueba hedónica del yogur preparado arrojaron que la textura tenía un valor de 3,28, que se acercaba a “ni me gusta ni me disgusta”, y el valor de sabor era de 3,63, que se acercaba a “me gusta”. El análisis de antioxidantes mostró que la adición de extracto de jengibre mejoró la bioactividad del yogur enriquecido a uno natural. De hecho, tras la fermentación se observó un aumento significativo de la capacidad antioxidante. El jengibre en yogur parece una alternativa a la diversificación tecnológica de un producto muy conocido.

Palabras clave: jengibre, yogurt, capacidad antioxidante, análisis sensorial, alimento funcional.

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1 Introduction

Ginger (*Zingiber officinale*) is a perennial herbaceous plant of the Zingiberaceae family and is contained within the spice group (Dissanayake *et al.,* 2020). It is used in various areas, whether in food preparation, beverages, confectionery, perfumery, or medicinal fields (Dhanik *et al.,* 2017). The rhizome, the underground horizontal stem, is the predominant component of fresh ginger, containing moisture, proteins, lipids, fiber, carbohydrates, minerals, and ash (Moghaddasi & Kashami, 2012). Studies have been conducted on ginger's effects on treating allergic rhinitis and in patients with postoperative nausea and vomiting (Adib-Hajbaghery & Hosseini, 2015; Kawamoto *et al.,* 2016). In addition to these properties, ginger is well known for its antioxidant activity (Atashak *et al*., 2014). The existence of ginger phenolic compounds that give it an antioxidant character has been proven, and when evaluated, their excellent bioavailability and bioaccessibility have been determined (Contreras-López *et al*., 2020; Ramírez-Godínez *et al.,* 2017). This leads to these compounds being considered potentially valid as functional ingredients in food production. The antioxidant activity of carotenoids and phenols in general from ginger has been studied in convection oven drying and freeze-drying processes. Despite losing some phenolic compounds during heating, the antioxidant capacity is maintained in both processes without significant differences. This is why these emerging processes have been widely used in the extraction of compounds and their inclusion in food formulations (Ghafoor *et al*., 2020).

Alternatively, it is known that peptide fractions with biological activity are generated during the milk fermentation process, including peptides with antioxidant capacity (Tonolo *et al.,* 2020). Antioxidant peptides have gained interest due to their low molecular weight, easy absorption, and high activity (Olvera-Rosales *et al*., 2022). Milk and its products are among the best precursors of bioactive peptides. In fermented dairy products such as yogurt, the antioxidant activity of the peptides will depend on the type of starter culture and the degree of proteolysis, even when these types of products have been enriched with other ingredients different from milk (Aloğlu & Öner, 2011; Ayati *et al.,* 2022). It has been found that some tripeptides containing Trp or Tyr residues at the carbon terminal had intense radical scavenging activity (Saito *et al.,* 2003). In addition, it is known that some peptides exert a synergistic effect with other antioxidants, such as phenolic compounds (Liu *et al.,* 2005).

Furthermore, studies have shown that the difference in starter cultures for milk fermentation causes the release of antioxidant peptides with different sequences (Li *et al.,* 2020). It has recently been revealed that the proteolytic system of lactic acid bacteria is a relevant factor in the type of peptide sequences obtained and that they also have to do with the bioactivity shown (Olvera-Rosales *et al*. 2023). Different studies have demonstrated the release of antioxidant peptides through hydrolysis of whey proteins (Mann *et al*., 2019).In addition, fermentation process has been used to provide functional food to consumers (Ertürkmen *et al*., 2024). Furthermore, it is known that the antioxidant properties of these sequences are related to the composition, structure, and hydrophobicity in such a way that it has been determined that aromatic amino acids such as Tyr, Trp, Met, Lys, and His and amino acids that contain sulfur groups such as Cys. They have excellent antioxidant potential by donating protons to electron-deficient radicals (Olvera-Rosales *et al*., 2023). Thus, it has been shown that adding ginger powder (1 %) to yogurt allows fermented milk to be enriched with phenols due to its increased concentration in this compound (Felfoul *et al.,* 2017). For this reason, the objective of this research was to add aqueous ginger extract to yogurt to determine its antioxidant capacity and verify the synergism that could occur between the extract and the antioxidant peptides formed during milk fermentation.

2 Materials and methods

2.1 Ginger extract preparation

The rhizomes of fresh ginger from the Sierra Norte of the State of Puebla (20° 16' north latitude and 97° 57' west longitude) in Mexico were ground and stored frozen. Ginger was milled, lyophilized, and stored at freezing conditions (-14 ºC), following the Ramírez-Godínez *et al.* (2017) methodology. 100 mL of two 2 % (w / v) aqueous extracts were prepared under different conditions, the first at 55 °C with a time of 5 minutes and the second at 90 °C with an extraction time of 15 minutes (Ramírez-Godínez *et al.,* 2017). Once the extracts were obtained, they were filtered with linen cloth and frozen at 4 °C.

2.2 Preparation of yogurt-type fermented milk

Fermented milk was prepared by adapting the methodology of Donkor *et al*. (2007). A liter of a 12 % (w / v) semi-skimmed (DairyGold®; fat: 12 g / 100 g) powdered milk solution with 0.2 % gelatin was prepared to improve its final texture. In three sterile 250 mL Erlenmeyer flasks, 188, 176, and 164 mL of milk were added in duplicate. The six flasks were subjected to heat treatment at 90 °C for 10 minutes. The flasks were placed in an ice bath until 40 °C was reached. Different volumes of both extracts were added to each Erlenmeyer flask, completing 200 mL of the final volume and 12, 24, and 36 mL of each extract (6, 12, and 18 %, respectively). All flasks were inoculated with 2 % (v / v) of commercial yogurt (Danone®) containing yogurt-specific microorganisms (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and incubated at 42 °C for 5 hours, reaching a pH= 5.5 (near to 60 ºD). After this time, the samples were stored at 4 °C for 24 hours. The initial charge of the microorganism incubated was 10^6 UFC/mL.

2.3 Preference ordering test

The sensory evaluation was conducted with 30 semi-trained judges with previous experience with ginger flavor and designed the experiment according to the protocol of Yang *et al.* (2012). The test was ordered by preference, which consisted of collecting from 1 to 6 according to lowest to highest preference, respectively. The evaluating judges were in an age range of 20 to 50 years of both genders, and it was considered that they consumed yogurt in their daily diets. A Friedman test for non-parametric data was applied, and analysis was performed with $p < 0.05$ to check for significant differences.

2.4 Preparation of preferred yogurt-type with ginger extract

According to the preference ordering test results, two liters of the most preferred Yogurt-type preparation was carried out according to Donkor *et al*. (2007) methodology. It was hydrated semi-skimmed milk powder (DairyGold®; fat: 12 g / 100 g) to prepare 12 %, adding to 250 mL Erlenmeyer flasks with 170 mL of the final milk solution. All experimental milk solutions were heat treated at 90 °C for 10 minutes. Then, they were cooled to 40 °C in an ice bath. Once cooled, 30 mL of the ginger, extracted according to the best conditions evaluated in the preference test, were added to each flask. They were inoculated with 2 mL of natural commercial yogurt without sugar (Danone®), incubated at 42 °C for 5 hours, and finally stored in the refrigerator at 4 °C. Milk controls were prepared in the same conditions without ginger extract in a final volume of 200 mL.

2.5 Hedonic ginger yogurt test

The test's objective was to know potential consumers' possible product acceptability. Due to the spicy sensation of ginger and its texture, it was necessary to carry out the test on people who consume yogurt regularly. The methodology used was that of Aamir *et al.* (2023), modifying the scale from 9 points to 5. The evaluation was conducted with 100 judges, aged 14 to 73 years, 39 men and 61 women. The test was carried out in two sessions. The first 50 people belonging to the Autonomous University of the State of Hidalgo were primarily students, and the other 50 evaluators were employees and owners of premises in a shopping center. During the test, the flavor and texture were evaluated. The hedonic scale consisted of 5 points, where "I dislike it very much" was given the value of 1 and "I like it very much" was given the value of 5. The judges evaluated the yogurt's taste and texture and recorded their product observations.

2.6 Determination of total phenols by the Folin & Ciocalteu method

The methodology used by Ramirez-Godínez *et al*. (2017) was adapted. A calibration curve was constructed from 0 to 15 mg L^{-1} from a 1000 mg L^{-1} gallic acid standard solution. To each standard solution, 2 mL of 7.5 % Na_2CO_3 and 2 mL of the Folin & Ciocalteu reagent were added and brought to a final volume of 10 mL with distilled water, and the absorbances of each standard were measured at 760 nm. The gallic acid was replaced with 1 mL of sample to determine the total phenolics of the dairy beverages and the extract. The concentration of gallic acid was calculated with Equation 1.

$$\frac{\text{Gallic acid}}{100\text{ g}} = -1.3703[\text{Galic acid}] + 100.62 \quad (1)$$

The results were expressed in mg of gallic acid/100 g of sample.

2.7 Determination of antioxidant activity

All experiments on the antioxidant activity were performed according to the Ramírez-Godínez *et al.* (2017) methodology, and all reagents for this part of the experimentation were from Sigma Aldrich (St.Louis, MO, USA). Five samples and two controls without ginger were prepared: unfermented milk (UM) and fermented milk (FM). The three study samples were fermented milk with ginger extract (FMG), unfermented milk with ginger extract (UMG), and ginger extract (G). The samples with ginger were prepared the same way as in the sensory analysis. 5 mL of samples were taken at the beginning and the end of the fermentation, and 5 mL of the samples without ginger. The ginger extract sample was excluded from this preparation since it is an extract without protein. samples were centrifuged at 10,000 rpm for 10 min at 4 °C to separate proteins and bacteria. The supernatant was separated from the pellet and stored in microbials in the dark at -2 °C for later analysis.

2.7.1 *Determination of antioxidant activity by the 2,2-diphenyl-1-picrihydrazyl (DPPH) method*

A calibration curve was made from 0 to 33 μM from a standard trolox solution (1 mM in methanol). To each standard solution, 2.9 mL of the DPPH solution (0.1 mM in methanol) was added (Ramírez-Godínez *et al.,* 2017). The standard solutions were brought to a final volume of 3 mL with methanol. Together with the standards, a control containing only 0.1 mL of methanol and 2.9 mL of DPPH was prepared. All standards were allowed to react for 50 minutes in the dark, absorbances were measured at 515 nm, and methanol was used as a blank. The antioxidant activity of the samples was measured concerning the calibration curve, replacing the trolox with 100 μL of the sample. The calibration curve was constructed with the concentration of Trolox against the percentage of remaining DPPH, which was calculated using Equation 2

$$\%DPPH_{remainder} = \frac{A_{sample - A_{blank}}}{A_{control}} \times 100 \quad (2)$$

Where

A_{sample} = Absorbance obtained from the sample,

A_{blank} = Absorbance obtained from methanol

$A_{control}$ = Absorbance obtained from the control sample

Antioxidant activity was expressed in mg of Trolox/100 g of sample.

2.7.2 *Determination of antioxidant activity by the FRAP (Ferric ion Reducing Antioxidant Power) method*

The FRAP reagent was prepared with a buffer solution of 300 mM acetates with a pH of 3.6, 20 mM ferric chloride hexahydrate, and 10 mM TPTZ (dissolved in 40 mM HCl). These three solutions were mixed in proportions of 10:1:1 (v /v / v). A calibration curve from 0 to 100 mM was constructed with a solution of ferrous chloride tetrahydrate in 40 mM HCl. For each standard solution, 1 mL of the FRAP reagent was added and brought to a final volume of 10 mL with distilled water. The solutions were incubated at 37 °C for 4 minutes, and their absorbance was measured at 593 nm; only the FRAP reagent was used as a blank. The ferric chloride hexahydrate was replaced with 250 μL of each sample to measure the antioxidant activity of the samples. Equation 3 measured the antioxidant activity (Ramírez-Godínez *et al.,* 2017).

$$Fe^{2+}/100\ g = 3.8791\left[Fe^{2+}\right] + 0.0071 \quad (3)$$

The antioxidant activity by the FRAP method was expressed in mg of Fe^{2+}/100 g of sample.

2.7.3 *Determination of antioxidant capacity by the ABTS [2,2'-azino-bis(3 ethylbenzothiazoline-6-sulphonic acid) method*

The method used by Ramírez-Godínez *et al.* (2017) was used in which a solution of 7 mM ABTS with 2.45 mM potassium persulfate was prepared, incubated for 16 h at room temperature in the dark, and after time, it was diluted with deionized water until obtaining an absorbance of 0.7 ± 0.01. Subsequently, 20 μL of the sample and 980 μL of the ABTS dilution were taken and allowed to rest for 7 min. The absorbance was measured at 754 nm, which was compared with a standard curve of Trolox at concentrations of 0, 10, 20, 30, 40, and 50 μM. The antioxidant capacity was expressed as % ABTS remaining with Equation 4:

$$\%ABTS_{\ remainder} = \frac{A_{sample - A_{blank}}}{A_{control}} x\ 100 \quad (4)$$

Where

$A_{\ sample}$ = Absorbance obtained from the sample,

$A_{\ blank}$ = Absorbance obtained from methanol

$A_{\ control}$ = Absorbance obtained from the control sample

The antioxidant capacity was expressed in mg of Trolox/100 g of sample.

2.8 *Statistical analysis of results*

In each experiment, the antioxidant activity was determined in triplicate, an ANOVA analysis of variance was performed, and a comparison of means was carried out using the Tuckey method with a significance level of 0.05. The chi-square test was performed for $p < 0.05$ to analyze the results.

3 Results and discussion

3.1 *Sensory evaluation of ordering by preference*

A sensory test was carried out before the antioxidant activity evaluations to assess its acceptability in the market due to the intense sensations typical of ginger. The results in Table 1 determined the appropriate concentration in preparing the fermented milk. The analysis showed that according to the extract added in different proportions to the fermented milk drink, there are no apparent differences in the flavor intensity. After statistical analysis, it was found that there were no significant differences ($p>0.05$) in the flavor intensity concerning the different thermal treatments applied for each extract. However, differences in texture related to the extract concentration were identified in each judge's observations.

Table 1. Arrangement of the different samples of yogurt with ginger extract.

Judge number	Yogurt with ginger extract obtained at 55 ºC for 5 minutes (% v/v)			Yogurt with ginger extract obtained at 95 ºC for 15 minutes (% v/v)		
	6	12	18	6	12	18
1	1	3	6	2	4	5
2	3	4	5	2	6	1
3	1	2	3	6	5	4
4	2	4	6	5	3	1
5	3	5	6	1	2	4
6	6	5	3	2	4	1
7	1	4	3	6	5	2
8	6	4	3	5	1	2
9	2	3	5	4	1	6
10	4	6	3	5	2	1
11	3	3	2	1	6	5
12	6	3	2	5	4	1
13	6	3	2	5	4	1
14	4	5	3	6	1	2
15	1	3	4	5	6	2
16	6	4	3	5	2	1
17	5	6	2	1	4	3
18	6	5	4	2	3	1
19	4	6	1	3	5	2
20	4	6	5	2	3	1
21	1	2	4	3	5	6
22	5	3	2	1	4	6
23	4	2	1	3	6	5
24	6	5	4	3	2	1
25	6	4	3	1	2	5
26	2	6	1	3	5	4
27	5	3	1	6	4	2
28	4	6	5	2	3	1
29	5	3	2	1	4	6
30	5	1	2	3	6	4

The following results emerged from the judge's comments: 70 % of the judges observed a slight sensation of spice, and 20 % assured that the drink had a "freshness" impact. In comparison, 98 % of the population recommended an increase in the concentration of the extract to perceive the difference in the intensity of the flavor. Therefore, the increase in the proportion of added extract and the heat treatment did not influence the intensity of the final flavor of the drink. Thus, the yogurt preparation was carried out with 18 % ginger extract to increase the chances of having greater antioxidant activity.

Some studies have reveled contrary results to those of this analysis. For example, Larasati *et al.* (2018) conducted a descriptive analysis of yogurt with ginger extracts. They found some aspects that influenced the differentiation of this yogurt from a natural one (without ginger). The factors that had the most influence were color, aroma, and pungency. These were more evident as the ginger concentration increased. On the contrary, the attributes with the most negligible influence and no significant difference were the sour taste and smell, the aftertaste, and the foam sensation. Additionally, adding ginger to yogurt positively affected the sweetness sensation, improving this attribute's perception. However, sensory studies must be established with this objective to have an adequate addition. In a study with consumers, they carried out a preference mapping between yogurt added with ginger extracts in concentrations from 2% to 10%. They determined that a concentration of 4% was adequate for general product acceptance. Higher concentrations have a negative impact (Yang *et al*., 2012).

Recently, Aamir *et al.* (2023) determined the sensory properties of yogurt added to ginger powder. They determined that adding ginger gives the yogurt a unique flavor and increases its health benefits by increasing its antioxidants and total viable cells. Furthermore, using ginger powder in yogurt improves the sensory characteristics of yogurt, although no substantial impact on the overall acceptability of yogurt was recorded. These same results were reported by Felfoul *et al*. (2017), who found that an addition of 1% ginger powder is adequate for the acceptance of an enriched yogurt.

3.2 Hedonic test

The hedonic test was carried out to determine the acceptance of the selected fermented milk (18% ginger extract 95 ºC/15 min). This concentration of ginger guarantees greater antioxidant activity, taking advantage of the results in the ordering test. Because only one sample was presented to determine the product's possible acceptance, the values were given on the scale (1 to 5) were averaged for each attribute of the beverage, in this case, flavor and texture. For taste, an average value of 3.28 was obtained; when approximated to the scale used, it belongs to "I neither like nor dislike". The evaluators concluded that the flavor of ginger was not perceived. Some of them knew the sensory characteristics and expected a more intense flavor. In contrast, others observed that the flavor was bitter and were not accustomed to the spice of the rhizome. For both cases, 80 % of the judges said they would prefer a drink with added sugar since they did not like it, as it only had an acidic flavor. They advised the preparation of a sweetened yogurt to improve the flavor of ginger and its spice.

In the case of texture, an average value of 3.63 was obtained. This number is close to "like" within the scale. However, it is not a value as close as in flavor; the prepared drink was related to a drinkable one due to the fluidity observed after fermentation. The judges concluded that the texture was suitable for a product of this type but that they would prefer a thicker yogurt with a high viscosity, because extract addition decreased this physical parameter. This effect has been observed by Moulaei *et al*. (2022) but they did not found impact in acidification of the yogurt added with ginger after refrigerated storage.

In the sensory analysis by Melia *et al.* (2022), judges identified that the taste in the hedonic test readily accepted yogurt with a concentration of 3 % ginger powder. The authors observed changes in the texture related to the ginger extract added because the mouthfeel decreased as the concentration of the extract increased.

Furthermore, it has been determined that the spice flavor of ginger is one of the characteristics that determine its low acceptability by consumers (Aamir *et al.,* 2023). One of the strategies to increase the acceptance of yogurt added with ginger is to add it previously fermented (Seo, 2017). Aamir *et al*. (2023). determined through a 9-point hedonic scale that a mint flavor in a yogurt enriched with different herbal extracts was the most preferred compared to a control yogurt which was added with 0.5% ginger powder, and which had previously been evaluated as the favorite among consumers.

3.3 Determination of total phenols by the Folin & Ciocalteu method

Table 2 shows the results obtained for total phenols in the dairy drink samples and the ginger extract. The results show that the extract's total phenols concentration is significantly higher (up to 5 times) than the analyzed milk's. This concentration is due to the very composition of ginger. Ramírez-Godínez *et al.* (2017) determined that the extraction temperature influences the antioxidant capacity of an aqueous extract of ginger. However, the authors found a high concentration of total phenols in all the experiments.

As observed, the phenol concentration of the fermented dairy drink with extract is significantly higher compared to non-fermented drinks with and without the extract. Additionally, the drink with the ginger extract has a higher phenolic content than the fermented milk that does not contain it. However, this is not statistically different for a $p<0.05$. It is sure that to increase the phenol content, the concentration of the ginger extract would have to be increased to the extent that the product's sensory properties are unaffected. The formulation of fermented milk with ingredients such as ginger, commonly used as an infusion, is becoming more common daily. Muniandy *et al.* (2016) studied the effect of adding green, white, and black tea infusions on the total phenol content in the yogurt preparation. They individually analyzed the phenol content in each infusion, obtaining values from 250 to 322 mg AG/100 mL of extract, which were very close to the values obtained in this study. In the yogurt samples added with the different extracts, values of 47 (green tea), 36 (white tea), and 27.8 (black tea) mg AG/100 mL were reported, showing that the phenols came from the infusions and were not lost when interacting with the dairy matrix, as in the case of this study. Recently, infusions or aqueous extracts of spices such as ginger have become relevant to be added to different foods, including fermented milk, due to their biological value and sensory characteristics (Omieljanowicz & Malinowska-Pańczyk, 2020).

Table 2. Total phenols in dairy beverages and ginger extract

Sample	mg GA (100 mg sample)
UM	65.91 ± 0.95^{a}
FM	77.76 ± 1.36^{ab}
UMG	69.82 ± 0.38^{a}
FMG	97.30 ± 1.15^{b}
G	345.54 ± 18.90^{c}

**Different letters in the subscript indicate differences between the measurements of each group (p<0.05). GA (galic acid), UM (unfermented milk); FM (fermented milk without ginger extract); UMG (unfermented milk with ginger extract); FMG (fermented milk with ginger extract); G (ginger extract).*

3.4 Determination of antioxidant capacity by DPPH, FRAP, and ABTS methods

3.4.1 Determination of antioxidant capacity by the DPPH method

Table 3 shows the antioxidant activity of the different samples, in which significant differences are observed between unfermented and fermented dairy drinks. The antioxidant activity increased after the fermentation process; however, when adding the ginger extract, the antioxidant power was greater than that of the drinks that did not contain it and were not fermented. This phenomenon is due to the metabolism of lactic acid bacteria (*L. bulgaricus and S. thermophilus*), which is related to the release of antioxidant and bioactive peptides in general (Contreras-López *et al*., 2021; Castañeda-Ovando *et al*., 2022; Gallardo-Martínez *et al*., 2024; Olvera-Rosales *et al*., 2023).

Our results agree with those reported by Felfoul *et al*. (2017), who found that the antioxidant capacity increased according to the degree of fermentation and days of refrigerated storage in yogurt added with ginger extracts. In fact, the increase in the antioxidant capacity of ginger-fermented milk is proportional to the storage time. Actually, the antioxidant activity measured by the DPPH method has shown that the enrichment of yogurt with a concentration of 3% ginger extract generates an increase of more than 55%, which decreases after refrigeration (48.4%). According to the results obtained, in the scavenging of the DPPH radical, there was a synergistic effect between the fermented milk drink and the addition of ginger extract (Melia *et al*., 2022).

In addition, Zhang *et al.* (2011) reported that cells of *Lactobacillus delbruekii* subsp. *bulgaricus* LJJ has significant antioxidant properties. These properties could be related to antioxidant peptides released during fermentation, which synergize with ginger essential oil (Afsaneh *et al.,* 2021). An example of this kind of effect was reported by Njoya *et al.* (2020), who determined that the extracts obtained after the maceration of ginger powder and paste by cooking above 80 ºC generate a greater antioxidant capacity measured by DPPH in yogurt enriched with 5% of this macerate.

Taking advantage of the bioactive activity of ginger, mainly its antioxidant capacity, it has been used to enrich fermented foods. An example is the highest antioxidant activity measured *in-vitro* in 6-gingerol (88.93 ± 0.03% DPPH; 88.23 ± 0.98% ABTS) (Tanweer *et al.*, 2020). Other important antioxidant compounds are shogaols. The higher antioxidant potential of 6-shogaol compared to other ginger phenols is due to a unique functional group (α,β-unsaturated carbonyl), which effectively regulates glutathione. Glutathione is the most available type of thiol in animal cells and participates in the cellular detoxification mechanism against harmful substances and in intracellular redox management (Shaukat *et al.*, 2023). This marks the advantage of using ginger as a functional ingredient in yogurt manufacturing.

On the other hand, milk has antioxidant activity due to different compounds that give it these characteristics, such as peptides and amino acids, enzymatic systems, such as the lactoperoxidase system, and vitamins (Stobiecka & Brodziak, 2022). Fermented dairy drinks generally have a greater antioxidant capacity than other dairy products, such as butter, cheese, etc. (Fardet & Rock, 2018). Furthermore, it is known that lactic acid bacteria release antioxidant peptides. A study comparing the antioxidant activity of different genera of *Lactobacillus*, such as *L. plantarum*, *L. acidophilus,* and *L. casei* showed that the percentage of inhibition of the DPPH radical was higher (64 – 87 %) than in *L. bulgaricus* (41 – 52 %) after milk fermentation (Najgebauer-Lejko & Sady, 2015).

Likewise, the antioxidant capacity is affected by the chemical composition of the fermented milk drinks, which have been analyzed according to their chemical composition. It has been observed that a high content of proteins with sulfur groups and hydrophobic amino acids favors antioxidant activity over the inhibition of the DPPH radical (Fardet & Rock, 2018).

3.4.2 Determination of antioxidant capacity by the FRAP method

Table 3 identifies the antioxidant activity expressed in the mg Fe^{2+}/100 g sample. Significant differences were found between dairy drinks that were not fermented and those in which this process was carried out by adding the extract. However, for the drink to which the extract was added, an increase in the concentration of Fe^{2+} is observed but not significantly different from that of the drink fermented without it.

Table 3. Antioxidant activity of dairy drinks with and without the addition of ginger extract.

Sample	DPPH mg Trolox (100 g sample)	ABTS mg Trolox (100 g sample)	FRAP mg Fe^{2+} (100 g sample)
UM	51.10 ± 11.66a	32.40 ± 1.93a	149.16 ± 8.68a
FM	70.73 ± 9.37a	68.71 ± 3.05b	210.34 ± 5.7ab
UMG	71.83 ± 3.29a	41.24 ± 1.45a	130.45 ± 5.50a
FMG	95.02 ± 7.71b	86.40 ± 3.42b	263.13 ± 2.91b
G	418.93 ± 11.11c	795.05 ± 43.04c	1178.56 ± 86.37c

**Different letters in the subscript indicate differences between the measurements of each group ($p<0.05$). GA (galic acid), UM (unfermented milk); FM (fermented milk without ginger extract); UMG (unfermented milk with ginger extract); FMG (fermented milk with ginger extract); G (ginger extract).*

In the case of ginger extract, a greater antioxidant capacity was found compared to all drinks. Thus, the fermented dairy with ginger extract has phenolic compounds from ginger that can reduce oxidized substances (in this case, Fe^{3+}). Still, a higher concentration is needed for the antioxidant activity to be statistically more significant than the fermented drink that does not contain the extract. The results indicate an increase in antioxidant capacity once the milk has been fermented. Values of 210.34 ± 5.7 and 263.13 ± 2.91 were observed in fermented milk without ginger and with ginger, respectively. These values were significantly higher than in unfermented samples and higher than those reported by other authors.

Najgebauer and Sady (2015) studied fermented milk, such as kefir, natural yogurts, and yogurts with fruit, and added flavorings derived from cocoa, coffee, and infusions. They found that yogurts added with fruits and flavored ones had a greater antioxidant capacity than natural yogurts, with values from 0.90 to 7.48 mM Fe^{2+}/100 mL, which came from the phenolic compounds of the plant matter. They also studied the antioxidant activity of natural yogurts with different chemical compositions and lactic acid bacteria used for fermentation. They concluded that yogurts with *Bifidobacterium*, *L. acidophilus*, and *L. casei defenses* had more excellent antioxidant activity than those fermented with conventional bacteria, such as *L. bulgaricus* and *S. thermophilus* (yogurt bacteria). These results are influenced by the fermented milk's protein content and the high protein content of each yogurt.

In the case of ginger, the compounds present in the extract that increase the antioxidant capacity of yogurt could be geraniol, butanol, 3-methyl butanol, terpineol, nerodinol, and gingerol, among others. Notably, these phenols are part of the oil fraction of ginger but could still be found in the aqueous extract (Ramírez-Godínez *et al.,* 2017). In this way, it has been observed that the use of at least 1% of the ginger powder allows the sensory characteristics of the product to be improved, also allowing the functional characteristics to be improved, especially those involved in the antioxidant capacity (Shaukat *et al.*, 2023).

3.4.3 Determination of antioxidant capacity by the ABTS method

In this evaluation, the calculated values are represented in mg Trolox/100 g sample before and after adding the extract to each drink. The results in Table 3 show no significant difference between the fermented dairy drinks with and without the addition of the extract. However, there was a difference concerning the drinks that were not fermented. In this case, UM and UMG obtained a lower antioxidant activity value than those calculated for FM and FMG.

Furthermore, when analyzing the results, no synergistic effect was observed when adding ginger extract to dairy drinks. No significant changes would allow us to conclude that adding the extract increases the antioxidant potential. Thus, the antioxidant activity is attributable only to compounds derived from milk fermentation, such as bioactive peptides from caseins and whey proteins.

Several authors have reported that the antioxidant capacity in fermented milk is due to the presence of peptides released during protein fractionation during the fermentation process (De Lima *et al.,* 2018; Fardet & Rock, 2018; Habibi *et al.,* 2019; Li *et al.,* 2020; Stobiecka *et al.,* 2022). Rahmawati & Suntornsuk, 2016 studied the effect of milk fermentation time and its storage for 21 days. In this study, the inhibition percentage of the ABTS radical was raised by up to 20 % during the storage time, concluding that the antioxidant activity is not affected during its useful life.

Conclusion

The added concentration of extract and the extraction conditions to each fermented drink do not affect the intensity of the flavor. Likewise, the antioxidant activity against the DPPH radical demonstrated a synergistic effect between the ginger extract and the fermented dairy beverage produced. On the other hand, to neutralize the ABTS radical, the fermented dairy drink with the extract did not have the same effect; this is because the antioxidant activity of the fermented drinks is independent of the addition of the extract. Likewise, the antioxidant activity by the FRAP method demonstrates that the extract concentration does not influence the final antioxidant activity of the yogurt. However, fermentation is a part of the process that was shown to influence bioactivity directly, and this had to do with the concentration of phenols in yogurt, which is affected by the presence of ginger extract since the concentration is increased only in non-fermented drinks. Even so, ginger and the peptides derived from fermentation have an entirely synergistic effect because yogurt's antioxidant activity does not decrease.

On the contrary, it is increased with the presence of ginger extract. A dairy drink added with ginger is an alternative to having ingredients with antioxidant capacity that benefit health. Above all, as in this study, the presence of ginger in yogurt is an alternative for the technological diversification of a well-known product.

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