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ORIGINAL ARTICLE

Polyphenol content, color and acceptability of carrot pickles added with yerba mate powder extract

Polifenóis, cor e aceitabilidade de conservas de cenoura adicionadas com extrato de pó de erva-mate

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Abstract

Yerba mate (*Ilex paraguariensis* Saint Hilaire) processing generates large amounts of powder that are not added to the final product. This powder has a similar composition to commercial yerba mate and it can be used to extract bioactive compounds. The work aims to prepare carrot pickles added with yerba mate powder extract to improve the Total Phenol Content (TPC) of the final product. The TPC and the color of the pickles were studied in the carrots and the liquid brine for a total of 120 days, by testing two storage temperatures (25 and 45 °C). The TPC was determined by the Folin-Ciocalteu method, and color parameters L*, a*, and b* were measured using a colorimeter. In addition, a sensory acceptability analysis was performed at the end of storage. Pickled carrots without extract addition showed significant losses of polyphenols during storage. The addition of increasing amounts of yerba mate powder extract significantly improved this parameter, thus achieving TPCs even higher than those of fresh carrots. The pasteurization, the addition of extract, and storage caused variations in the color of both fractions of the pickle, especially in the formulations with a higher proportion of yerba mate. However, the color changes were more important in the liquid brine, while in the carrots the variations were minimal. Sensory acceptability tests showed that the addition of extract did not influence the flavor of the pickles but caused changes in color acceptability. However, the product was accepted by consumers. The results showed that yerba mate powder extract can be added in small amounts into other foods for improving their TPC while causing minor modifications in color and sensory acceptability.

Keywords: *Daucus carota*; *Ilex paraguariensis*; Acidified vegetables; Storage; Bioactive compounds; Sensory evaluation.

Resumo

O processamento da erva-mate (*Ilex paraguariensis*) gera grandes quantidades de pó que não são adicionadas ao produto final. Este pó tem uma composição semelhante à da erva-mate comercial e pode ser utilizado para a extração de compostos bioativos. O objetivo deste trabalho foi preparar conservas de cenoura adicionadas com extrato de pó de erva-mate para melhorar o conteúdo de polifenóis totais do produto final. O conteúdo de polifenóis totais e a cor das conservas foram estudados nas cenouras e no líquido durante um total de 120 dias, testando duas temperaturas de armazenamento (25 e 45 °C). O conteúdo de polifenóis totais foi determinado pelo método de Folin-Ciocalteu e os parâmetros da cor L*, a* e b* foram medidos utilizando um colorímetro. Além disso, foi realizada uma análise de aceitabilidade sensorial no final do armazenamento. As cenouras em conserva sem adição de extrato mostraram perdas significativas de polifenóis durante o armazenamento. A adição de quantidades crescentes de extrato de erva-mate melhorou significativamente este parâmetro, atingindo conteúdos de polifenóis totais ainda mais elevados do que os das cenouras frescas. A pasteurização, a adição de extrato e a armazenagem causaram variações na cor de ambas as frações da conserva, especialmente nas formulações com uma maior proporção de erva-mate. As variações de cor foram mais importantes no líquido, enquanto que, nas cenouras, as variações foram mínimas. Os testes de aceitabilidade sensorial mostraram que a adição de extrato não influenciou o sabor das conservas, mas provocou alterações na aceitabilidade da cor. No entanto, o produto foi aceito pelos consumidores. Os resultados mostraram que o extrato de pó de erva-mate pode ser adicionado em pequenas quantidades a outros alimentos, melhorando o seu conteúdo de polifenóis totais e causando, ao mesmo tempo, pequenas modificações na cor e na aceitabilidade sensorial.

Palavras-chave: *Daucus carota*; *Ilex paraguariensis*; Vegetais acidificados; Armazenamento; Compostos bioativos; Avaliação sensorial.

1 Introduction

Vegetable-rich diets have been associated with a lower incidence of certain diseases such as diabetes, cancer, cardiovascular disease, and cognitive and neurological disorders. These health effects are related to the content of bioactive compounds, especially polyphenols present in the food (Lee et al., 2018; Moyo et al., 2020). Carrots (*Daucus carota* L.) are popular root vegetables, and they are well known as an important source of carotenoids, particularly β-carotene. These vegetables are also a good source of dietary fiber, minerals, and phenolic compounds, mainly chlorogenic acid (CGA) (Keser et al., 2020; Lee et al., 2018).

Under ordinary conditions of temperature and humidity, the shelf life of fresh carrots is no longer than a few days, as storage and handling cause loss of the carrots' quality (Papoutsis & Edelenbos, 2021; Ranjitha et al., 2017). Various techniques have been used to extend their shelf life, such as refrigeration and freezing, dehydration, coating, and pickling (Acosta et al., 2015; Behsnilian & Mayer-Miebach, 2017; Demiray & Tulek, 2017; Ranjitha et al., 2017). Pickling by direct acidification is generally carried out by adding an organic acid, which can be combined with pasteurization of the preserved food and the addition of other preservatives (Dupas de Matos et al., 2019). In this regard, the treatments that foods undergo during processing may modify their physical properties and chemical composition. In carrots, cooking causes changes in texture and color, as well as the loss of certain bioactive compounds (Guillén et al., 2017; Lee et al., 2018). Furthermore, vegetable pickling modifies their Total Phenolic Content (TPC) and thus the natural antioxidant properties of these foods (Sayın & Alkan, 2015). In this sense, the addition of phenolic compounds from external sources into processed foods could help to counteract the effects of processing on the content of these metabolites, improving the quality of the finished product. Previous studies have shown that the addition of antioxidant-rich ingredients to pickled vegetables improved the TPC of the food (Hassan & Sarfraz, 2018; Liao et al., 2017).

Yerba mate (*Ilex paraguariensis* Saint Hilaire) is a traditional crop in Argentina, Brazil, Paraguay, and Uruguay, which traditional use is the preparation of infusions and extracts (Cardozo Junior & Morand, 2016). Yerba mate extracts have a strong antioxidant activity that has been attributed to the polyphenolic compounds found in large amounts in yerba mate leaves (Mateos et al., 2018; Tonet et al., 2019). The bioactive compounds in yerba mate extracts have demonstrated several beneficial properties on human health, mainly attributed to their protective effect against oxidative stress (Riachi & De Maria, 2017). The industrial processing of yerba mate comprises the following stages: harvesting; roasting; drying; milling; aging; and blending (Rossi & Lozano, 2020). During grinding, large amounts of fine powder are generated, which are not added in their entirety to the final product because of its low particle size, mainly below 250 µm (Schneider Teixeira et al., 2016). Although it is composed of leaves and has a high content of polyphenols and minerals, this powder is a by-product of limited use (Schneider Teixeira et al., 2016; Vieira et al., 2008). Yerba mate powder has been used experimentally as an adsorbent material for the removal of heavy metals, as an inert material to trap a sweetener and minerals, and to develop yerba mate candies (Copello et al., 2013; Schmalko et al., 2012; Scipioni et al., 2010; Vieira et al., 2008). Given the known benefits of yerba mate, the powder generated in its processing could have several applications in the development of new products as a low-cost source of polyphenolic compounds (Vieira et al., 2008).

Yerba mate extracts have been added to other foods such as white chocolate or carrot and orange juices to improve the polyphenol content of the finished products (Ferrario et al., 2018; Zanchett et al., 2016). In all cases, the addition of extract caused a significant increase in the polyphenol content and antioxidant capacity of the food. The extract has also been used for technological purposes, as an antimicrobial in fish patties, or to develop coatings for post-harvest conservation of peaches (Sapelli et al., 2020; Tonet et al., 2019). However, to the best of our knowledge, there are no studies in the literature on the incorporation of yerba mate powder extracts into other foods. In this sense, the addition of the extract to carrot pickles could contribute to improving the polyphenol content of the finished product. Therefore, the work aimed to elaborate carrot pickles added with different amounts of yerba mate powder extract and to study the influence of storage at two different temperatures on the TPC, color parameters, and sensory acceptability of the food product.

2 Material and methods

2.1 Yerba mate powder extract

The yerba mate powder was provided by a yerba mate industry located in southern Misiones province, in Argentina. The fraction of the powder that passed through the 40-mesh screen was used, which corresponds to a particle size of less than 425 μm. Solid-liquid extraction was performed using water as a solvent with a solid:liquid ratio of 1:10 in weight; at a temperature of 80 °C for 30 minutes (FAC, model Balus X2, Buenos Aires, Argentina), with constant stirring (Gerke et al., 2018). The extract was separated by centrifugation (Rolco, model 2036, Buenos Aires, Argentina) and further filtration with Whatman No. 3 filter paper. The soluble solids concentration in the extract was determined by mass loss in an oven at 103 ± 2 °C until reaching constant weight (Argentine Standardization and Certification Institute, 1995).

2.2 Carrot pickles preparation

The preparation of the pickles was carried out following a formulation obtained from preliminary tests. The carrots were purchased from a supermarket located in the city of Posadas (Misiones), washed with tap water, peeled, and sliced into uniform slices of 5 mm thickness. Both ends were discarded. Carrot slices with diameters between 20 and 40 mm were used. The pickling brine was prepared by dissolving 1.42 g of salt and 3.10 g of sugar in 100 mL of a solution prepared by mixing one part of distilled water and two parts of white vinegar. For the addition of the extract, a fraction of water in the brine was replaced by yerba mate extract in proportions of 5, 10, and 15% v/v (designated as 5%YM, 10%YM, and 15%YM). Preliminary trials indicated that using higher concentrations of the extract resulted in pickles with unpleasant colorations and off-flavors. Additionally, a control pickle without extract addition was prepared (0%YM).

Carrot pickles were elaborated by placing 70 g of carrot slices in sterilized glass jars (180 mL capacity) and adding 85 mL of pickling brine. The jars were purchased from a retail store in the city of Posadas (Misiones). Jars were hermetically sealed and pasteurized in a boiling water bath for 20 minutes. After pasteurization, the pickle jars were placed in a water bath at 10ºC to stop the heat treatment. 30 jars of each tested extract concentration were prepared, from 0%YM (control) to 15%YM. Additional pickle jars were prepared for the sensory analysis.

2.3 Storage and sample collection

After the heat treatment, the pickles were separated into two batches. One batch was stored at 25 °C and the other one at 45 °C to simulate the thermal range that could occur during storage along the supply chain of these products. From each batch of pickles, three units of each tested formulation were separated at 0, 15, 30, 60, and 120 days of storage. Sampling was carried out following a geometric progression, considering that during the first days of storage the changes are more pronounced, while in the following days the changes are less significant. Sampling corresponding to 0 days was carried out right after pasteurization when the pickle jars reached room temperature. From each unit, an aliquot of brine was removed and put in an airtight container, and the carrot slices were set in polyethylene bags. Both fractions were stored at –18 °C until use.

2.4 Total phenol content analysis

The TPC was quantified in fresh carrots, pickled carrots, pickling brine, and yerba mate extract. The extraction of phenolic compounds from carrots was performed on a portion of the unfrozen and milled sample. A solvent mixture of methanol:water 80:20 was used for the extraction, in a solid-liquid ratio of 1:10 (Keser et al., 2020). Previous tests with different concentrations of aqueous methanol indicated that this solvent concentration is optimal for the extraction of TPC of carrots (data not shown). The extraction was performed at 70 °C for 30 minutes. After concluding the first extraction, the liquid was separated, and a second extraction was performed on the solid under the same conditions. Both extracts were mixed and filtered, and the TPC was determined on an aliquot of the filtrate, using the Folin-Ciocalteu technique. Briefly, 0.4 mL of diluted sample was added to 2 mL of 10% Folin-Ciocalteu reagent. The mixture was allowed to react for 5 minutes and 1.6 mL of 10% w/v Na_2CO_3 was added. After 60 minutes, the absorbance at 760 nm was measured on a UV-Visible spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). The TPC analysis in the yerba mate extract and the pickling brine was performed using a similar methodology. Gallic Acid (GA) was used as a standard in a concentration range of 20 to 100 µg/mL. The moisture content was determined to express the TPC in carrots on a Dry Weight (DW) basis, by keeping a portion of milled solid sample in an oven at 105 °C until constant weight was reached. The moisture content was calculated by mass loss (Camorani et al., 2015).

2.5 Color parameters

The color of fresh carrots, pickled carrots, and pickling brines was analyzed with a colorimeter (Mini EZScan 4500L, HunterLab, Reston, VA, USA). Color parameters L* (luminosity from 0 = black to 100 = white), a* (negative values of a* indicate an increase in green color and positive values of a* indicate an increase in red color), and b* (negative values of b* indicate an increase in blue color and positive values of b* indicate an increase in yellow color) were measured both on carrot slices and a fixed volume of the pickling brine of each pickle formulation. The carrot slices were randomly chosen from each jar.

2.6 Sensory analysis

An acceptability test was carried out with volunteers who regularly consume pickled vegetables. The sensory panel was constituted by 30 panelists of both genders, between 20 and 55 years old. Panelists were trained in the procedure of the test. Samples used for the test were carrot pickles from both batches, stored for 120 days. Panelists evaluated a total of eight carrot slices and eight closed pickle jars, using a seven-point hedonic scale ("I extremely dislike it", "I dislike it", "I slightly dislike it", "I do not like it nor dislike it", "I slightly like it", "I like it" and "I extremely like it"). The order of presentation of the samples was balanced in all cases. To guarantee the panelists' safety, the pH value of each carrot pickle was measured before the sensory test, assuring it was below 4.6 (Acosta et al., 2015).

Panelists received a tray with four carrot slices coded with randomly chosen three-digit numbers, along with a worksheet to write down their perceptions. They were instructed to record their opinion on the color of the carrot slices first, without having tasted them. Then the panelists tasted the samples, recording their perceptions of the flavor of the samples. Afterward, they were asked to assess the global acceptability of the samples, based on the observation of the pickle jars corresponding to the previous slices. Panelists did not receive information on which jar corresponded to each slice. After evaluating the first four samples, panelists took a break and continued the evaluation of the next four samples.

2.7 Statistical analysis

Statistical analysis of data was performed with the STATGRAPHICS Centurion XV (v15.2.06) program. Data were analyzed using Analysis of Variance (ANOVA) and Tukey HSD test was used to calculate mean differences. Data from the sensory evaluation was transformed into a 1-7 scale for statistical analysis, where 1 corresponds to "I extremely dislike it" and 7 to "I extremely like it". Differences were considered significant for $p < 0.05$.

3 Results and discussion

3.1 Total phenol content (TPC)

3.1.1 Carrot pickles without yerba mate extract

The TPC in fresh carrot was 1335 ± 32 $\mu g_{GA}/g_{DW}$ (equivalent to 19.17 ± 0.45 mg_{GA}/100 g fresh weight). Other authors found values in the order of those found in this research, also using the Folin-Ciocalteu technique (Buratti et al., 2020; Han et al., 2017; Sayın & Alkan, 2015). However, significantly different values have also been reported. Guillén et al. (2017) reported 63.18 $mg_{GA}/100g_{DW}$ using the same technique, approximately half of the amount found in this research. On the other hand, Keser et al. (2020) reported a total amount of phenolic compounds of 100.90 mg/g fresh weight, determined by Liquid Chromatography-Mass Spectrometry (LC-MS/MS). The variation in the reported values can be attributed to differences between carrot varieties, the amount of analyte recovered with each extraction technique, as well as the analytical technique applied.

After pasteurization, the TPC in carrots decreased by 37%, to 843 ± 38 $\mu g_{GA}/g_{DW}$. This reduction could be attributed to the thermal degradation of the phenolic molecules and/or the migration of these compounds into the liquid medium (Buratti et al., 2020; Guillén et al., 2017). In fact, the TPC in the pickling brine after pasteurization was 81 ± 3 μg_{GA}/mL, while these compounds were not detected in the liquid before the heat treatment. The migration of phenolic compounds from the pickled food to the liquid medium has been previously reported (Chung et al., 2017). The TPC per jar (that is, in 70 g fresh weight of carrot slices plus 85 mL of pickling brine) increased from 13.5 ± 0.3 mg_{GA}/jar before pasteurization to 17.4 ± 0.7 mg_{GA}/jar after the treatment, indicating a greater number of reactive groups against the Folin-Ciocalteu reagent. This could be related both to cell wall degradation and to the increase of free phenolic groups as a consequence of hydrolysis reactions of glycosylated phenolic moieties or phenolic bounds to cell wall components, reactions favored by heating and acidic conditions (Lee et al., 2018; Liu et al., 2019). These results suggested that carrot phenolic compounds are rather stable to the heat treatment and were not degraded in pasteurization, but their migration from the carrot slices to the brine caused a decrease in the TPC of the fraction intended for consumption.

During storage at 25 °C (Figure 1A), the TPC in carrots without yerba mate extract decreased by 50.5% after 15 days of storage, reaching a value of 417 ± 17 $\mu g_{GA}/g_{DW}$, that remained without statistically significant variations ($p > 0.05$) until the end of storage. A 35% decrease in the brine TPC was observed between 0 and 30 days, from 81 ± 3 to 52 ± 2 μg_{GA}/mL. The decrease found in the TPC of both fractions during storage could be attributed to the oxidation of phenolic compounds by a non-enzymatic route and to reactions between other food molecules, both of which are known to generate colored compounds (Lund, 2021; Zhang et al., 2015).

On the other hand, pickles without yerba mate extract stored at 45 °C exhibited an initial decrease in the TPC after 15 days of storage, both in carrot slices and pickling brine, showing similar values to those found at 25ºC. After that time, an increase in this parameter was observed (Figure 1B). This growth could be explained by the combined effect of the acidic medium and higher temperature on the degradation of the cell wall of carrots, promoting both the migration of phenolic compounds into the pickling brine during storage and their extraction for the quantitative analysis (Lee et al., 2018). Chung et al. (2017) studied soybean pickles. The authors found an initial decrease in polyphenol content, which gradually increased during storage, indicating that the variations could be associated with textural changes in the food. Sayın & Alkan (2015) studied carrot pickles, finding an initial decrease in polyphenols at 15 days, and subsequent increases up to 60 days. They attributed this to the hydrolysis of glycosylated polyphenols at low pH.

The decrease in the TPC in carrots slices and the brine during storage at 25 °C indicated that these compounds were susceptible to degradation. After 120 days of storage at 25 °C, the TPC in pickled carrots was only 25.2% of the TPC in fresh carrots. These results illustrated the importance of adding polyphenols from external sources into pickled vegetables to increase the content of these compounds in the finished product.

3.1.2 Carrot pickles with yerba mate extract

The soluble solids concentration in the extract was 2.74 ± 0.10 g/100 mL, and the TPC was 5950 ± 260 μg_{GA}/mL (equivalent to 6.23 g_{AG}/100 g_{DW}). Holowaty et al. (2016) reported TPC values in yerba mate between 9.14 and 9.51 g_{AG}/100 g_{DW}. However, the authors performed the extraction using 70% methanol as a solvent. Rocha Saraiva et al. (2019) reported 9.926 g_{GA}/100 g_{DW} using 100% methanol. The extraction yield found in this work was slightly lower than reported in the literature, which depends on the origin of the samples and the extraction technique (Gullón et al., 2018; Zielinski et al., 2020). The solvent used to obtain the extract in this paper was suitable for incorporation into other foods.

The addition of the extract to the pickles induced a significant increase in the TPC both in the pickling brine and the carrot slices, under all experimental conditions. This could demonstrate the migration of the phenolic compounds from the pickling brine, where the extract was added, towards the carrot slices, which was the portion intended for consumption. The polyphenol content of yerba mate determined by Folin-Ciocalteu method has shown a good correlation with antioxidant capacity measurements, indicating that these compounds are responsible for the antioxidant effect of yerba mate, in particular, chlorogenic acid (Mateos et al., 2018). Figure 1 shows the effect of adding different concentrations of the extract on the TPC in pickled carrots slices, and Table 1 shows the values of TPC in the pickling brine at the beginning and the end of storage (0 and 120 days).

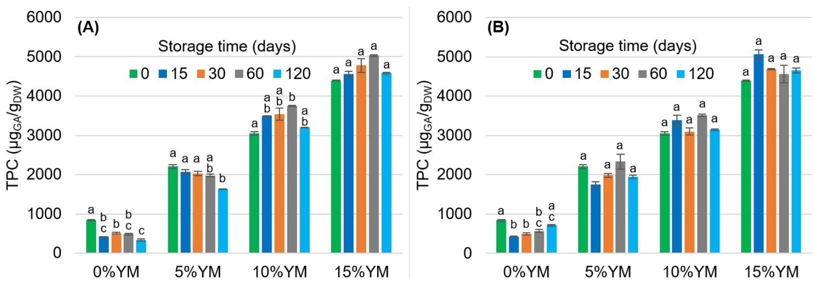

Figure 1. TPC in pickled carrots stored at (A) 25 °C and (B) 45 °C. Different letters indicate significant differences within a yerba mate extract concentration (%YM) at each temperature.

Table 1. TPC in pickling brines at 0 and 120 days.

Formulations	TPC (μg_{GA}/mL)		
	0 days	120 days	
		25 °C	45 °C
0%YM	81 ± 3^{aA}	52 ± 1^{aB}	120 ± 5^{aC}
5%YM	229 ± 18^{bAB}	188 ± 1^{bA}	252 ± 8^{bB}
10%YM	365 ± 26^{cA}	259 ± 36^{bA}	340 ± 4^{cA}
15%YM	509 ± 45^{dA}	363 ± 1^{cB}	342 ± 8^{cB}

YM: Yerba Mate. Different lowercase letters indicate significant differences within a column, and different capital letters indicate significant differences within a row ($p < 0.05$). Results shown as mean (n = 3) ± standard deviation.

Carrot slices with 5%YM stored at 25 °C showed a progressive decrease of 25.9% in the TPC, from the beginning until 120 days of storage, reaching a value of 1636 ± 21 $\mu g_{GA}/g_{DW}$. This amount is in the order of the TPC found for fresh carrots. Although these decreases in the TPC are statistically significant, they are considerably lower than the losses found in pickled carrots without extract addition (25.9% and 50.5%, respectively). In the same formulation kept at 45 °C, storage time did not have a significant influence on the TPC in carrot slices ($p > 0.05$). During storage at both temperatures, there were similar tendencies in the brines of this formulation, compared to those detailed for samples without extract addition. On the other hand, in pickles with 10 and 15%YM, storage time did not significantly influence the TPC in carrot slices at any temperature. In the pickling brines of the formulation with 15%YM, gradual decreases in the TPC were observed up to 120 days (Table 1), attributable to the oxidation of phenolic compounds in the liquid.

These results suggested that phenolic compounds from the yerba mate extract were more stable in the brine acidic medium than the natural phenolic compounds from carrots. In all cases, the influence of the yerba mate extract addition to pickled carrots on the TPC was more important than the influence of temperature and storage time on the same parameter. The addition of yerba mate extracts to other foods has shown to increase their polyphenol content and antioxidant capacity. Rocha Saraiva et al. (2019) found that milk fortification with 0.5% yerba mate (and higher) increased the TPC and antioxidant capacity of fresh cheeses. They also found that both parameters decreased during the storage of the cheeses, attributing this to the formation of polyphenol-protein complexes. The addition of yerba mate extract also improved the TPC and antioxidant capacity of fermented milk and white chocolate (Rodrigues Ramos et al., 2017; Zanchett et al., 2016). Furthermore, the supplementation of vegetable pickles with other herbs has also been studied. Hassan & Sarfraz (2018) found that the addition of herbs and spices in the formulation of pickles produced significant increases in the TPC and antioxidant capacity of the food.

3.2 Color parameters

3.2.1 Pickling brine

Replacing a fraction of water in the pickling brine with yerba mate extract produced modifications in the color of the carrot pickles. Table 2 shows the values of parameters L*, a*, and b* for the pickling brine at 0, 30, and 120 days of storage at 25 °C and 45 °C.

Table 2. Color parameters for pickling brines at 0, 30, and 120 days of storage at 25 and 45 °C.

Formulations	0 days	30 days		120 days	
		25 °C	45 °C	25 °C	45 °C
			L*		
0%YM	68.05 ± 0.50^{aA}	68.37 ± 1.24^{aA}	67.17 ± 0.85^{aA}	$67.63 \pm 1{,}32^{aA}$	57.01 ± 0.38^{aB}
5%YM	67.52 ± 0.29^{aA}	66.23 ± 0.84^{bA}	64.46 ± 1.00^{bB}	$67.52 \pm 0{,}41^{aA}$	58.26 ± 0.71^{aC}
10%YM	64.85 ± 0.99^{bAB}	66.18 ± 0.71^{bA}	63.50 ± 0.94^{bcB}	64.61 ± 1.23^{bAB}	51.49 ± 0.51^{bC}
15%YM	64.56 ± 1.00^{bA}	64.51 ± 0.72^{bA}	62.26 ± 1.10^{cB}	66.23 ± 0.09^{abA}	42.46 ± 0.56^{cC}
			a*		
0%YM	-2.09 ± 0.13^{aA}	-2.24 ± 0.08^{abAB}	-2.30 ± 0.05^{aB}	$-2.13 \pm 0{,}03^{aA}$	-0.19 ± 0.02^{aC}
5%YM	-2.57 ± 0.01^{bA}	-2.37 ± 0.14^{aA}	-2.02 ± 0.08^{bB}	$-2.07 \pm 0{,}04^{aB}$	-0.57 ± 0.01^{bC}
10%YM	-2.64 ± 0.07^{bA}	-2.36 ± 0.06^{aB}	-1.85 ± 0.08^{bC}	$-2.27 \pm 0{,}05^{bB}$	0.94 ± 0.04^{cD}
15%YM	-2.58 ± 0.07^{bA}	-2.15 ± 0.04^{bB}	-1.62 ± 0.12^{cC}	$-2.42 \pm 0{,}03^{cD}$	2.58 ± 0.02^{dE}
			b*		
0%YM	5.88 ± 0.23^{aA}	6.02 ± 0.26^{aA}	10.68 ± 0.84^{aB}	6.14 ± 0.10^{aA}	24.27 ± 0.06^{aC}
5%YM	9.11 ± 0.07^{bA}	9.70 ± 0.07^{bA}	14.43 ± 0.27^{bB}	11.01 ± 0.55^{bC}	24.25 ± 0.22^{aD}
10%YM	12.08 ± 0.41^{cAB}	12.45 ± 0.67^{cA}	16.66 ± 0.84^{cC}	11.21 ± 0.17^{bB}	26.90 ± 0.20^{bD}
15%YM	14.19 ± 0.58^{dA}	14.71 ± 0.31^{dA}	19.14 ± 0.41^{dB}	13.92 ± 0.71^{cA}	26.77 ± 0.20^{bC}

YM: Yerba Mate. Different lowercase letters indicate significant differences within a column for each parameter, and different capital letters indicate significant differences within a row ($p < 0.05$). Results shown as mean (n = 3) ± standard deviation.

The addition of extract caused a small decrease of parameter L* in the pickling brine, from the carrot pickles formulated with 10%YM. This loss of luminosity was accentuated during storage at 45 °C, especially at growing yerba mate extract concentrations. On the other hand, the addition of extract produced an increase of parameter b* at the beginning of storage (0 days), proportional to the amount of extract added, while a* decreased. Parameters a* and b* showed minimal variations during storage at 25 °C; while in pickles stored at 45 °C the values of both color parameters significantly increased, indicating a shift in the color of the pickling brine towards red and yellow, respectively. In all formulations, color modifications were more important in the batch stored at 45 °C.

The addition of yerba mate extracts to other foods produced color changes. Garcia Coró et al. (2020) found that the addition of yerba mate to jerked beef produced a decrease in a* and an increase in b* at the beginning of storage, showing a similar trend to that reported in this work. The three color parameters showed modifications during jerked beef storage. Meanwhile, Ferrario et al. (2018) added yerba mate extracts to carrot and orange juices. They found that the addition of the extract did not change the parameter L* but decreased both a* and b*. The main pigments found in yerba mate extracts are chlorophylls and their derivatives generated in roasting and drying operations of the industrial processing of this crop. These derivatives include pheophytins and pheophorbides, among others, and are characterized by the conversion of the bright green color of chlorophylls to olive-brown shades (Holowaty et al., 2016). Additionally, there are other pigments in yerba mate extracts: carotenoids (such as lutein) and golden yellow resinous substances (Silveira et al., 2016; Nabechima et al., 2014). A complex mixture of these pigments explains the greenish-brown color that the yerba mate extract provides to carrot pickles when added to the pickling brine. Meanwhile, color changes observed during the storage of pickled carrots could be attributed to the oxidation of polyphenols and polymerization of the oxidized products, previously discussed.

3.2.2 Carrot slices

Table 3 shows the 0, 30, and 120-days values of parameters L*, a*, and b* for raw and pickled carrots without the addition of yerba mate extract, at both tested storage temperatures. The values of the color parameters found for raw carrot are in the order of those reported by other researchers (Guillén et al., 2017; Keser et al., 2020).

Table 3. Color parameters for raw carrot and pickled carrots without yerba mate extract addition.

Time (days)	L*		a*		b*	
	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C
Raw	42.63 ± 1.30^{a}		29.28 ± 3.63^{ab}		40.68 ± 5.27^{a}	
0	49.39 ± 0.32^{b}		32.59 ± 2.73^{a}		48.13 ± 2.55^{b}	
30	48.60 ± 0.55^{bA}	43.94 ± 3.02^{aB}	32.40 ± 1.56^{aA}	27.89 ± 0.90^{bB}	43.94 ± 3.02^{abA}	45.04 ± 0.98^{abA}
120	48.13 ± 0.53^{bA}	44.61 ± 0.43^{aB}	31.08 ± 1.77^{aA}	28.51 ± 2.12^{abB}	41.67 ± 2.19^{aA}	44.76 ± 3.24^{abA}

Different lowercase letters indicate significant differences within a column, and different capital letters indicate significant differences within a row for each parameter ($p < 0.05$). Results shown as mean (n = 3) ± standard deviation (n = 9 for raw carrots).

Parameters L* and b* increased after the heat treatment, indicating an increase in carrot luminosity and yellowness, respectively. Parameter a* did not show significant differences before and after pasteurization. Different effects of heat treatments on the color of carrots have been reported in the literature. Guillén et al. (2017) reported decreases in L* and a* after boiling carrots, while cooking at a temperature lower than 100 °C only lowered parameter L*. Camorani et al. (2015) reported decreases in all three color parameters after boiling frozen carrots. Lee et al. (2018) found decreases in L* and b*, as well as an increase in a*. In all cases, color changes after heat treatments were attributed to structural modifications or losses in carrot carotenoids. Guillén et al. (2017) reported that all cooking methods they studied produced losses of carotenoids.

Storage of pickled carrots without the addition of extract caused minimal changes in color parameters at both tested temperatures. In all cases, the 120-day values were similar to those found in raw carrots and carrots after pasteurization. This suggests that carrot pigments are relatively stable under the studied conditions. This fact could be related to the removal of air from the medium, achieved by pasteurization, which could help prevent the oxidation of carotenoids (Soto et al., 2020).

The 0, 30, and 120-day values of color parameters found for pickled carrots with extract addition are shown in Table 4. The addition of different concentrations of yerba mate extract did not significantly change the values of L*, a*, and b* at the beginning of storage, compared to the pickled carrot slices without extract addition. However, the luminosity of carrots decreased during the storage of all formulations. Parameters a* and b* showed similar behavior, indicating an increase in greenness and blueness, respectively. For all three parameters, changes during storage were more important in the batch stored at 45 °C. Ning et al. (2017) found a decrease in L*, a*, and b* parameters in the addition of green tea powder to whole wheat bread, attributed to the chlorophyll content and the formation of colored polyphenol and chlorophyll derivatives. Moreover, Shokery et al. (2017) reported that the addition of green tea or moringa to yogurt lead to a decrease in L* and an increase in b*, so that the functional foods were darker and greener. The addition of polyphenol-rich extracts to other foods produces various color changes in the processed food, usually associated with changes on luminosity. This parameter should be studied for each case.

Table 4. Color parameters for pickled carrot slices with yerba mate extract.

Time (days)	L*		a*		b*	
	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C
5%YM						
0	49.79 ± 0.76^{a}		33.95 ± 3.76^{a}		49.66 ± 5.10^{a}	
30	48.29 ± 1.57^{abA}	43.43 ± 2.11^{bB}	31.51 ± 1.85^{aA}	27.72 ± 1.84^{bB}	43.43 ± 2.11^{bA}	41.01 ± 2.46^{bA}
120	47.26 ± 0.54^{bA}	43.89 ± 0.77^{bB}	30.46 ± 1.41^{aA}	24.53 ± 1.41^{bB}	42.08 ± 3.61^{bA}	36.52 ± 2.17^{bB}
10%YM						
0	50.58 ± 1.89^{a}		33.96 ± 2.96^{a}		49.74 ± 3.63^{a}	
30	47.18 ± 1.13^{bA}	43.42 ± 2.33^{bB}	29.81 ± 2.20^{bA}	26.01 ± 1.70^{bB}	43.42 ± 2.33^{bA}	38.45 ± 1.89^{bB}
120	44.73 ± 1.30^{cA}	43.43 ± 0.29^{bB}	30.20 ± 0.48^{bA}	25.76 ± 1.19^{bB}	41.65 ± 1.91^{bA}	39.60 ± 2.11^{bA}
15%YM						
0	49.32 ± 0.72^{a}		33.65 ± 2.90^{a}		49.76 ± 2.49^{a}	
30	47.48 ± 0.96^{bA}	44.23 ± 0.33^{bB}	31.15 ± 1.43^{abA}	26.55 ± 1.43^{bB}	43.88 ± 1.28^{bA}	39.51 ± 2.34^{bB}
120	45.74 ± 0.96^{cA}	42.38 ± 0.57^{cB}	29.91 ± 1.30^{bA}	24.49 ± 0.82^{bB}	42.52 ± 1.86^{bA}	36.32 ± 1.72^{bB}

YM: Yerba Mate. Different lowercase letters indicate significant differences within a column for each formulation, and different capital letters indicate significant differences within a row for each parameter ($p < 0.05$). Results shown as mean (n = 3) ± standard deviation.

The color parameters showed more important variations in the brine than in the carrot slices. Furthermore, as mentioned, the carrot pigments seem to be rather stable in the acidic environment of the pickle. These results suggested that the main cause of darkening and color changes in carrot pickles with yerba mate extract was the oxidation of polyphenolic compounds in the pickling brine. This hypothesis was consistent with the results found for the decrease in the TPC in the pickling brine during storage.

3.3 Sensory analysis

The addition of unconventional ingredients to other foods may cause a lower sensory acceptability of the finished product, especially in terms of color and overall acceptability (Gramza-Michałowska et al., 2016). The results of the sensory analysis are shown in Table 5. Storage temperature did not influence the global acceptability of carrot pickles, while the amount of extract added caused a significant decrease in the scores assigned to this attribute. Similarly, growing extract concentrations resulted in a decrease in color acceptability in pickles stored at 45 °C. Samples stored at 25 °C did not show significant differences in this parameter. These results agree with the differences found in the instrumental determination of color. Concerning the flavor, panelists did not find significant differences in any of the tested extract concentrations. This demonstrates that the amounts of yerba mate extract added did not provide the characteristic bitterness and distinctive flavor of yerba mate to the pickles (Ferrario et al., 2018). The bitter taste of foods added with yerba mate extracts has been attributed to the polyphenol and flavonoid content of the extract (Faion et al., 2015). Zanchett et al. (2016) reported that higher concentrations of yerba mate reduced the acceptability of white chocolate added with the extract, affecting both flavor and acceptability of the samples. Rodrigues Ramos et al. (2017) reported a good acceptability of a fermented milk added with yerba mate and sweet potato.

Table 5. Flavor, color, and global acceptability of pickled carrots.

Formulations	Global acceptability		Flavor		Color	
	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C
0%YM	5.84 ± 1.40^{aA}	6.00 ± 1.05^{aA}	4.83 ± 1.56^{aA}	5.14 ± 1.56^{aA}	5.38 ± 1.27^{aA}	6.30 ± 0.87^{aB}
5%YM	4.89 ± 1.33^{bcA}	5.23 ± 1.56^{aA}	5.37 ± 1.44^{aA}	4.93 ± 1.44^{aA}	5.39 ± 1.22^{aA}	3.50 ± 1.60^{bB}
10%YM	5.62 ± 1.05^{abA}	5.08 ± 1.25^{abA}	5.58 ± 1.05^{aA}	5.00 ± 1.34^{aA}	5.38 ± 1.46^{aA}	4.93 ± 1.27^{cA}
15%YM	4.16 ± 1.46^{cA}	4.19 ±1.43^{bA}	4.94 ± 1.53^{aA}	4.53 ± 1.54^{aA}	4.91 ± 1.52^{aA}	4.01 ± 1.88^{bcA}

YM: Yerba Mate. Different lowercase letters indicate significant differences within a column, and different capital letters indicate significant differences within a row for each attribute ($p < 0.05$). Results shown as mean (n = 30) ± standard deviation.

These results indicated that the addition of concentrations up to 10% of yerba mate extract did not affect the acceptability of pickled carrots stored at 25 °C, in comparison to the control pickles without extract addition. In pickles stored at 45 °C, panelists find differences in the color of the product from the formulation with 5%YM, causing a clear decrease in acceptability. In pickles with up to 10%YM stored at 25 °C, the average scores assigned by panelists corresponded to the "I slightly like it" and "I like it" tags, indicating that the product was accepted by consumers.

4 Conclusions

Both the heat treatment and storage time produced significant modifications of the phenolic contents in pickled carrots stored at 25 and 45 °C. Because of this, it is advantageous to add concentrated extracts of these antioxidant compounds obtained from external sources. The addition of yerba mate extract to carrot pickles caused significant increases in the TPC of the food product. The extract also produced changes in color parameters both in carrots slices and pickling brine. Storage temperature had a strong influence on the quality of pickled carrots, affecting the studied physicochemical parameters and the sensory acceptability of this food. This variable should be measured and controlled during the storage of pickled vegetables, especially if it develops over a long time. It is concluded that the replacement of 5% of the water in the pickling brine with yerba mate extract was adequate since it allowed to achieve a TPC similar to the one in fresh carrots, even after 120 days of storage: while accomplishing minor modifications in color parameters and without jeopardizing the acceptability of the food.

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