

Effect of the addition of sweet red pepper (*Capsicum annuum*) oleoresin and yellow tree tomato (*Solanum betaceum* Cav.) juice on quality parameters and shelf life of margarines

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Research Article

Keywords: Antioxidant, Bioactive compounds, Color, Sensory analysis, Storage, Ultrasound

Posted Date: May 18th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1633702/v1>

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Abstract

The objective of this study was to evaluate different quality parameters and the oxidative stability of a vegetable margarine made with different concentrations of sweet red pepper (*Capsicum annuum*) oleoresin and yellow tree tomato (*Solanum betaceum* Cav.) juice using ultrasound technology. Oleoresin was added as an enriching agent in the fat phase at different concentrations: 100, 200 and 300 ppm, and tree tomato juice was added as an enriching agent in the aqueous phase at 5, 10 and 15%.

Physicochemical, microbiological, sensory and oxidative stability tests were carried out. The results showed that sweet red pepper oleoresin is a significant source of total carotenoids 1473 ± 10.24 mg/100 g, total phenolic compounds 1158 ± 8.65 mg GAE/100 g, flavonoids 814 ± 7.63 mg CE/100 g and ASTA color 765 ± 3.51 , additionally tree tomato juice is a source of vitamin C 22.34 ± 1.51 mg AA/100 g. The addition of these two natural extracts improved notably the antioxidant activity, peroxides index and p-anisidine during storage of the developed margarine formulations. The results of the physicochemical and microbiological tests showed that the vegetable margarine developed met the quality standards established for a commercial margarine. The qualification of the sensory analysis indicated that the evaluated attributes were acceptable in all the formulations, being the margarine elaborated with the addition of 200 ppm of sweet red pepper oleoresin and 10% of tree tomato juice, the one that presented the best sensory qualification.

1. Introduction

The development of new fatty foods that are safe and healthy is a need and current trend due to the global health and nutritional problems faced by the population due to the high consumption of processed foods such as margarines with low nutritional value and high use of synthetic additives. However, the new fatty products should not only respond to health and nutritional requirements, but also to the needs and gastronomic traditions of each country, historical approach, consumption habits, technological aspects, ease of preparation and acquisition, among others (Silva et al., 2021). Therefore, one of the trends and innovations in the formulation of new processed foods is the development of products enriched with bioactive compounds from the use of natural ingredients, such as natural extracts from fruits, vegetables, edible flowers, spices and promising aromatic herbs, including the use of their by-products that allow a beneficial contribution to the consumer and help prevent chronic diseases such as cancer, cardiovascular, respiratory and gastrointestinal diseases, among others (Yoo et al., 2008; Lopes et al., 2014; Ordoñez-Santos, Martínez-Girón, & Arias-Jaramillo, 2017; Panpipat et al., 2018; Mulík & Ozuna, 2020; Nor Adilah et al., 2020; Procopio et al., 2022).

Within processed foods are margarines, which are emulsions usually consisting of 80% fat phase and 20% aqueous phase with the addition of additives to extend their shelf life and improve their sensory attributes (Paduret, 2022). However, some synthetic additives such as colorants and antioxidants are currently used in the production of margarines, which may cause long-term health alterations (Azizkhani & Zandi, 2010). Due to the above, food science is in continuous search for natural ingredients, packaging and/or biomaterials that can be incorporated into processed foods, generating an increase in bioactive

compounds and allowing an increase in the shelf life of the food (Panpipat et al., 2018; Nor Adilah et al., 2020). One of these natural ingredients of aqueous nature corresponds to the juice from tree tomato (*Solanum betaceum* Cav.), which is a traditional Colombian drink that has a pleasant aroma and flavor, it is also economical and can be easily prepared at home or purchased in restaurants or market places, this ethnic drink is obtained by squeezing this delicious exotic fruit with yellow pulp and usually orange or red skin (epicarp), which is characterized by being a significant source of antioxidant compounds such as β -carotene 2.87 mg/L, α -carotene 1.80 mg/L, β -cryptoxanthin 2.20 mg/L, zeaxanthin 3.61 mg/L, lycopene 1.26 mg/L, Vitamin C 39.48 mg/100 mL, and provitamin A 406.01 μ g RAE/L. (Ordoñez-Santos & Martínez-Girón, 2020). Another natural ingredient of interest and of an oily nature consists of the oleoresin from red pepper (*Capsicum annuum*), which is obtained from the extraction process from the flour obtained from this fruit (Fernández-Trujillo, 2007). In Colombia, the sweet red pepper (*Capsicum annuum*) is one of the classic fruits and vegetables that can be easily and cheaply obtained in the different marketplaces, supermarkets and fruit and vegetable supply centers; it is generally used in different preparations and dishes of the typical Colombian gastronomy due to its pleasant aroma, sweet flavor and its striking colors ranging from green, red, yellow, orange and purple (Martínez-Girón & Ordoñez-Santos, 2015). Its fruit is a fleshy berry, and it is also a vegetable characterized by its high nutritional value being a source of iron 0.30–1.20 mg/100 g, zinc 0.20–0.70 mg/100 g, phosphorus 26–46 mg/100 g, potassium 211–340 mg/100 g, vitamin C (ascorbic acid) 127–300 mg/100 g, vitamin B6 0.17–0.29 mg/100 g, vitamin A 0.01–0.16 mg/100 g, vitamin E (α -tocopherol) 0.70–1.60 mg/100 g, β -carotene 0.50–1.64 mg/100 g, β -cryptoxanthin 0.50–1.20 mg/100 g, lutein + zeaxanthin 6–51 mg/100 g and phenolic compounds and derivatives 50–500 mg/100 g (Baenas et al., 2019). Another important characteristic of red pepper (*Capsicum annuum*) is the capsaicin and dihydrocapsaicin content and the level of pungency expressed in Scoville Heat Units (SHU). However, in the sweet red pepper used in this study this property is not taken into account, because its capsaicin and dihydrocapsaicin content is very low and it is classified as non-pungent (Othman et al., 2011). This characteristic is also common in the sweet yellow bell pepper (*Capsicum annuum*) from Colombia, unlike the green pepper which has a capsaicin content of 1.00 ± 0.90 μ g/g and Scoville heat units (SHU) of 15.830, however its levels of pungency are also classified as non-pungent. Within the group of peppers with spicy or pungency level, which are generally used in other countries such as Mexico, China and Spain, among others, is the green chili which has a capsaicin content of 138.50 ± 5.20 μ g/g, dihydrocapsaicin 146.40 ± 4.20 μ g/g Scoville heat units (SHU) of 2216.58 and levels of pungency (mildly pungent). The red chili, presents capsaicin content 309.30 ± 4.20 μ g/g, dihydrocapsaicin 238.20 ± 2.60 μ g/g, Scoville heat units (SHU) of 4949.08 and levels of pungency (moderately pungent). There is also hot chili that can present a capsaicin content of 4249 ± 190.30 μ g/g, dihydrocapsaicin 4482.20 ± 35.60 μ g/g, Scoville heat units (SHU) of 67984.60 and levels of pungency (highly pungent) according to what is reported by (Othman et al., 2011).

Oleoresin from red pepper (*Capsicum annuum*) is characterized by its intense red color and viscous texture, and it is used in different food applications mainly due to its high ASTA color value and presence of secondary metabolites with antioxidant power (Fernández-Trujillo, 2007). Oleoresin (*Capsicum annuum*) is a good source of unsaturated fatty acids such as omega-3 and omega-6, as well as bioactive

compounds such as carotenoid pigments and phenolic compounds (Melgar-Lalanne et al., 2017; Huang et al., 2022). However, to date there are no studies on the physicochemical, microbiological, sensory and antioxidant effects of the addition of this type of natural ingredient in the fat phase of margarines combined with the addition of tree tomato juice (*Solanum betaceum* Cav.) in the aqueous phase. A review of the literature shows that there are reports of other studies on the application of natural extracts from other sources in margarines, such as the application of rosemary extract (*Salvia rosmarinus*), basil (*Ocimum basilicum*), green onion (*Allium fistulosum*), lemon (*Citrus limon*), garlic (*Allium sativum*), thyme (*Thymus vulgaris*), marjoram (*Origanum majorana*), oregano (*Origanum vulgare*), nopal skin extract (*Opuntia ficus-indica*), red palm fruit extract (*Phoenix canariensis* L) and Gambier extract (*Uncaria gambir* Roxb.) according to the background information reported by (Azizkhani & Zandi, 2010; Lopes et al., 2014; Chougui et al., 2015; Djouab et al., 2017; Aini et al., 2020). In this order, studies on the enrichment of emulsions with natural extracts that present antioxidant activity is relevant, because it allows the development of new products and becomes an alternative to finding natural ingredients to replace synthetic additives which can generate toxicity problems and/or chronic diseases (Azizkhani & Zandi, 2010). Given the above, the objectives of this study focused on: (1) Performing the extraction and characterization of red pepper (*Capsicum annuum*) oleoresin produced from the skin and pulp of sweet red pepper fruits comparing different extraction methods; (2) Performing the characterization of tree Colombian tomato (*Solanum betaceum* Cav.), comparing different processing treatments; (3) developing new vegetable margarine formulations enriched with sweet red pepper oleoresin and addition of tree tomato juice (*Solanum betaceum* Cav.) as a potential natural ingredients source of bioactive compounds with colorant and antioxidant functionality; (4) evaluating the physicochemical, microbiological and sensory quality of the formulated margarines; (5) evaluating the oxidative stability of the margarines during storage.

2. Materials And Methods

2.1 Obtaining and processing of sweet red pepper

The vegetal material used to obtain the oleoresin was sweet red pepper, Nataly variety, also known as Nathalie sweet red pepper. The red pepper fruits (*Capsicum annuum*) for commercial maturity were acquired in the local market of Palmira, Valle del Cauca, Colombia. Healthy, whole fruits weighing between 250–300 g were cleaned with water and disinfected with sodium hypochlorite at 150 ppm for 10 min. The plant material was removed from the stalk and seeds, cut into julienne strips and blanched at 90°C for 2 min for inactivation of the peroxidase enzyme according to preliminary studies (Martínez-Girón & Ordoñez-Santos, 2015). Subsequently, the pieces composed of red pepper epicarp and mesocarp were dried in a natural convection oven (Binder ED 53 UL) at a temperature of $58 \pm 2^\circ\text{C}$ for 24 h, until reaching a final moisture percentage of 8–10%; then the dried and conditioned samples were crushed in an electric grinder and taken to a sieving machine (Advantech DT-168) until obtaining the respective red pepper flour, also known as red pepper powder with a granulometry of approximately 250 μm .

2.2 Ultrasound-assisted formation of sweet red pepper oleoresin and comparison with other methods

The extraction of oleoresin was performed from the obtained flour by comparing three extraction methods consisting of the conventional maceration method (ME), Soxhlet extraction (SE) and ultrasound-assisted extraction (UAE). For conventional maceration extraction (ME) 1 gram of sample was diluted in 100 mL of ethanol and the resulting mixture was shaken in a heating plate at 40°C for 7 h. Soxhlet extraction (SE) was performed using food grade ethyl acetate as extraction solvent in a Soxhlet extractor at $75 \pm 5^\circ\text{C}$ for 2.5 h under continuous reflux. Ultrasound extraction was performed according to the method proposed by (Budiastra et al., 2020) with some modifications, the samples were diluted in ethanol in a ratio (1:4) and were taken to an Ultrasonic Cell Disruptor JY98-IIIDN equipment with a 15 mm probe, with an ultrasound frequency of 20 kHz and a power of 360 W with a pulse duration of 5 s on and 5 s off for 35 min. The ultrasound system was conditioned to a water ice bath in order to maintain the temperature below $40 \pm 5^\circ\text{C}$. In all cases the samples were filtered and the solvent was evaporated in a rotoevaporator (RE 100-Pro DLAB Scientific) at 60°C under vacuum and the recovered oily extract was taken to a natural convection oven (Binder ED 53 UL) at 40°C for 10 h to remove residual solvent. The red pepper oleoresins obtained by (ME, SE and UAE) were stored in sterile amber glass jars at refrigeration temperature ($4^\circ\text{C} \pm 2$) for subsequent analyses. For the calculation of the extraction yield percentage, the weight of the extract obtained was related to the weight of the initial red pepper sample.

2.3 Preparation of yellow tree tomato juice with ultrasound application and comparison with other treatments

Tree tomato juice (*Solanum betaceum* Cav.) yellow variety was made as this natural beverage is typically prepared in Colombia from whole, healthy, ripe fruits with orange skin and yellow pulp previously washed with water and disinfected with sodium hypochlorite 100 ppm for 10 min. The fruits were manually peeled and subsequently blanched at 85°C for 1 min, then immersed in a cold water bath to achieve the inactivation of the peroxidase enzyme according to preliminary studies developed (Ordoñez-Santos & Martínez-Girón, 2020). The undiluted tree tomato fruit juice was obtained and conditioned using a commercial blender for fruit processing, then filtered with a kitchen strainer to remove the seeds. The juice obtained was divided into three batches: the first batch corresponded to the control fruit juice without any fresh treatment, the second batch was subjected to pasteurization treatment (HP) at 80°C for 10 min, and the third batch was subjected to sonication treatment (US) at 240 W for 40 min at $30 \pm 2^\circ\text{C}$ in a Ultrasonic Cell Disruptor JY98-IIIDN, according to parameters evaluated in previous studies on fruit juice (Ordoñez-Santos, Martínez-Girón, & Arias-Jaramillo, 2017). The fruit juices obtained (control, HP and US) were stored in sterile amber glass jars at a refrigerated temperature of $4 \pm 2^\circ\text{C}$ for subsequent analysis without the addition of sugar or synthetic preservatives.

2.4 Characterization of sweet red pepper oleoresin and yellow tree tomato juice

2.4.1. Physicochemical parameters

The physicochemical properties evaluated in the samples were pH using a Metrohm 744 pH meter, soluble solids (°Brix) using a Boeco digital refractometer, Germany, reference 32395-EQ, % moisture using an AXIS infrared moisture balance reference ATS210, and density by pycnometer method.

2.4.2 ASTA color

The oleoresins obtained were subjected to ASTA color measurement as the main quality control variable in this type of product. Spectrophotometric measurements were carried out in a Thermo Spectronic Genesys 20 (USA), according to the specifications of ASTA method 20.1 clause C of the American Spice Trade Association, following the methodology reported in preliminary studies on sweet red pepper Nathalie variety from Colombia (Ordoñez-Santos & Martínez-Girón, 2015).

The calculation of color in ASTA units was determined by the Eq. (1).

$$ASTA_{color} = \frac{A_{460} \times F \times If}{W} \text{ (Ec. 1)}$$

Where A is the absorbance of the acetone extract at a wavelength of 460 nm, F is the ASTA color conversion factor (16.4 for *Capsicum* fruit and 164 for oleoresin), If is the instrumental correction factor, W is the weight of the sample in grams.

2.4.3 Superficial color CIELa*b*

The surface color of the samples was measured using a CR-400 Colorimeter (Konica Minolta, Tokyo Japan) in coordinates (L^* , a^* and b^*); using a D65 illuminant and a 2° observer, with calibration plate parameters $Y = 82.50$; $x = 0.3220$; $y = 0.3394$.

2.4.4 Total carotenoid content

The quantification of the total carotenoid content in the samples and extracts obtained was carried out by spectrophotometry in a Thermo Spectronic Genesys 20 (USA), using equations 2, 3 and 4 as reported by (Hornero-Méndez & Mínguez-Mosquera, 2001).

$$C. R = \frac{A_{508} \times 2144 - A_{472} \times 403.3}{270.9} \text{ (Ec. 2)}$$

$$C. Y = \frac{A_{472} \times 1724.3 - A_{508} \times 403.3}{270.9} \text{ (Ec. 3)}$$

$$C. T = CR + CY \text{ (Ec. 4)}$$

Where C.T is the total amount of carotenoids present, C.R corresponds to the fraction of red carotenoids, C.Y to the yellow carotenoids and A is the absorbance at a wavelength of 472 and 508 nm.

2.4.5 Total phenolic compounds

The total content of phenolic compounds in the samples and extracts obtained was determined according to the Folin-Ciocalteu reagent method, described by (Singleton, Orthofer, & Lamuela-Raventós, 1999). The obtained samples were mixed with 20 mL of ethanol and water, in a ratio (80:20%v/v), then filtered and a 10% solution of Folin-Ciocalteu was added. After 20 min of sonication, 1 mL of 20% sodium carbonate anhydride solution was added. The tubes were subjected to a controlled thermal bath at $40^{\circ}\text{C} \pm 5$ for 15 min, then allowed to cool in the dark for 45 min and absorbance readings were taken at 760 nm on a Thermo Spectronic Genesys 20 spectrophotometer (USA). The results were reported as mg gallic acid equivalents per 100 g (mg GAE/100g).

2.4.6 Total flavonoid content

Total flavonoid content was determined according to the method reported by (Yoo et al., 2008). 1 mL of each of the samples were mixed with catechin solutions and were volumetrically diluted with water to complete 5 mL. To the obtained mixtures, 0.3 mL of a 5% sodium nitrite solution was added at the beginning of the reaction. Subsequently, 0.6 mL of a 10% aluminum trichloride solution was added to each tube and continued mixing on a stirring plate. Then the samples were left at rest and a basic hydrolysis was carried out with the addition of a mixture of 1 M sodium hydroxide and water in a ratio (1:1). Finally, absorbance readings were taken at 510 nm in a Thermo Spectronic Genesys 20 spectrophotometer (USA). The results were reported as mg catechin equivalents per 100 g (mg CE/100g).

2.4.7 Vitamin C

Vitamin C content, was determined according to the titration method used by (Ordoñez-Santos, Martínez-Girón, & Arias-Jaramillo, 2017). The samples were gauged with distilled water to complete a volume of 100 mL and then mixed with a 20% glacial acetic acid solution. Then 10 mL of the mixture was taken and titrated with a solution of 2,6-dichloroindophenol (50 mg/100 g) against a control of a standard ascorbic acid solution. The results were reported as mg ascorbic acid equivalents per 100 g (mg AA/100g).

2.5 Preparation of margarine enriched with sweet red pepper oleoresin and yellow tree tomato juice obtained by ultrasound

The vegetable margarine was produced manually at laboratory scale. A total of three formulations F1, F2 and F3 were made, compared with a control that consisted of a margarine formulation without the addition of red pepper oleoresin in the fat phase or tree tomato juice in the aqueous phase (Table 1). The ingredients of the aqueous phase were taken to a heating plate with stirring and dissolved at $75 \pm 5^{\circ}\text{C}$ for 15 min, subsequently the ingredients of the fatty phase were stirred at $55 \pm 5^{\circ}\text{C}$ for 15 min. The emulsion (fat phase + aqueous phase) was formed using Ultraturrax T18 IKA equipment at a stirring speed of 18000 rpm for 3 min. The resulting homogeneous mixture was placed in a freezer (Thermo Scientific Ref. MR25PA-GAEE-TS) at -20°C for 12 h in order to achieve crystallization of the fatty acids, then the resulting margarine samples were stored at 4°C in the absence of light. The base composition of the vegetable margarine was a mixture of palm oil with refined sunflower oil as the fat phase and water as the aqueous

phase. The additives used were: soy lecithin (emulsifier), glyceryl monostearate (emulsifier), sodium chloride (salt), citric acid (synergistic agent), EDTA (sequestrant), potassium sorbate (preservative), yellow tree tomato juice (solvent with natural fruit flavor with coloring and antioxidant power) and sweet red pepper oleoresin (natural colorant and antioxidant). The graphic scheme of the developed margarines is presented in (Fig. 1). Sweet red pepper oleoresin obtained by UAE as well as tree tomato juice subjected to US were selected because they presented the highest concentration of bioactive compounds compared to the other methods applied. Sweet red pepper oleoresin (SRPO) was added at concentrations of 100, 200 and 300 ppm in the fat phase of the emulsion and tree tomato juice (TTJ) was added at 5, 10 and 15% in the aqueous phase. The addition percentages of oleoresin and tree tomato juice were obtained from preliminary tests, where it was taken into account that the concentration of the natural ingredients added in all formulations should not exceed the final percentage of acidity (% oleic acid) allowed in margarines, which should be less than 0.30% according to (NTC 250, 2022).

Table 1
Margarine formulations

Phase	Ingredients	Control	F1	F2	F3
Fat	Extract oleoresin (ppm)	0	100	200	300
	Palm oil (%)	50	45	40	35
	Sunflower oil (%)	30	35	40	45
	Soy lecithin (%)	0.80	0.80	0.80	0.80
	Glyceryl monosterate (%)	0.20	0.20	0.20	0.20
Aqueous	Water (%)	18.50	13.49	8.48	3.47
	Tree tomato juice (%)	0	5	10	15
	Salt (%)	0.37	0.37	0.37	0.37
	Cítric acid (%)	0.07	0.07	0.07	0.07
	EDTA (%)	0.01	0.01	0.01	0.01
	Potassium sorbate (%)	0.05	0.05	0.05	0.05

2.6 Physicochemical and microbiological quality parameters of the margarine formulations

The physicochemical quality parameters evaluated in each of the margarine formulations were the % of acidity in terms of free fatty acids (Ca 5a-40 method), refractive index (Cc 7–25 method), saponification value (Cd 3–25 method), iodine index (Cd 1–25 method), according to the protocols established by (AOCS, 2005). Melting point by capillary method according to the procedure reported by (Lounis et al., 2018), salt content using Mohr's method according to (AOAC, 1990). The solid fat content (SFC) in the

formulated margarines was measured using a Bruker Minispec NMR equipment Model No.120 (Rheinstetten, Germany). The samples were deposited in NMR tubes and melted at $65^{\circ}\text{C} \pm 5$ for 10 min. They were then conditioned at 0°C in the NMR equipment for 90 min and finally deposited in a controlled thermal bath at temperatures of 10, 20, 30 and 40°C for 30 min. The results were reported for each temperature by direct measurement from the equipment software. The spreadability was calculated as the spreading distance (cm) or length extension presented by the margarine when applied and spread over the bread according to the method used by (Aini et al., 2020). The microbiological quality parameters in the samples consisted of the evaluation of total coliform count (MPN/g), *Escherichia coli* count (MPN/g), yeast count (CFU/g) and *Staphylococcus aureus* (CFU/g) according to the protocols established for microbiological analysis in food for human consumption (NTC 4899, 2015; INVIMA, 1998).

2.7 Sensory quality parameters

Sensory attributes of the margarines were conducted to determine the degree of product satisfaction in terms of color, aroma, flavor, palatability and overall acceptability. An untrained sensory panel of 50 male and female volunteers ranging in age from 20 to 55 years in good physical and mental health were selected for the study. The margarine samples were coded with two-digit numbers and two different letters to avoid bias and the panelists were given informed consent for voluntary participation. The code of ethics for sensory analysis was applied according to the protocol established by (IFST, 2015) as a guide and guidelines for ethics and good practices for sensory analysis of food. People with allergies or any type of food intolerance were not taken into account in the sensory analysis. For the evaluation of sensory attributes, a 9-point hedonic scale was used, where 1 refers to the lowest rating and 9 to the highest rating, with the following descriptions: (I disliked extremely = 1; I did not like it much = 2; I did not like moderately = 3; I did not like it lightly = 4; It's indifferent to me (I did not like it, it did not displease me) = 5; I liked it slightly = 6; I like it moderately = 7; I liked it a lot = 8; I liked it extremely = 9).

2.8 Oxidative stability during storage

The margarines were stored at 4°C for 180 days, taking into account that this is the average shelf life of a commercial margarine. During this time, the peroxide index (Cd 8b-90 method), p-anisidine (Cd 18-90 method) was evaluated every 30 days according to the protocols established by the (AOCS, 2005). The antioxidant activity was evaluated according to the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical inhibition method. The samples were left in darkness at 25°C for 60 minutes and proceeded to absorbance readings at 515 nm in a Thermo Spectronic Genesys 20 (USA) equipment according to the procedure reported by (Chougui et al., 2015) in margarines.

2.9 Statistical analysis

The results were reported as the average value $\pm$ SD. The experimental design used in this study was randomized blocks, the treatments were control formulation, 100, 200 and 300 ppm of margarine formulations with addition of SRPO (sweet red pepper oleoresin) and addition of tree tomato juice (TTJ) at 5, 10 and 15%, with five replicates per treatment. The data was analyzed with a one-way ANOVA and

differences between means were compared using Tukey's test ($p < 0.05$). All statistical calculations were performed using SPSS 18 for Windows.

3. Results And Discussion

3.1 Characterization of sweet red pepper oleoresin and yellow tree tomato juice with ultrasound application and comparison with other methods

Table 2 shows the characterization parameters evaluated in sweet red pepper oleoresin (SRPO) comparing three extraction methods. As can be seen for SRPO, pH, soluble solids ($^{\circ}\text{Brix}$), % moisture and density did not show significant differences. The low values obtained for pH, soluble solids and moisture in the oleoresin are positive in that they prevent the development of deterioration reactions and growth of microorganisms during the shelf life of the oleoresin. Unlike the previous parameters, ASTA color, concentration of bioactive compounds (carotenoids, phenolics, flavonoids, vitamin C), CIEL a^*b^* surface color and extraction yield percentage did show significant differences ($p < 0.05$) between the extraction methods used. Other authors have also compared different extraction methods and found that the use of emerging technologies such as ultrasound (US) is appropriate in the extraction of oleoresins, because it is an environmentally friendly technique, uses low solvent, short times and low temperatures that do not affect thermolabile compounds even though lower yields are obtained with respect to conventional methods such as maceration and Soxhlet, which are easier techniques and use lower cost equipment, but require high volumes of organic solvents, long extraction times and high temperatures (Procopio et al., 2022). In this sense (Budiastra et al., 2020) carried out the extraction of pepper oleoresin comparing the extraction method by maceration (ME) for 7 hours with the extraction method by ultrasound (UAE) for 75 min, finding that ultrasound allowed obtaining pepper oleoresin in a shorter time without affecting bioactive compounds of interest including some volatile substances. (Huang et al., 2022) performed the extraction of oleoresin from red pepper (*Capsicum frutescens* var) comparing the method of extraction by maceration (ME), ultrasound-assisted extraction (UAE) and accelerated solvent extraction (ASE), finding that UAE and ASE allowed an increase in ASTA color and in the concentration of carotenoid compounds and capsaicinoids. ASTA color is one of the most important parameters in red pepper oleoresin, the value obtained in this study shows that the extract obtained by UAE presents a high coloring power quality. ASTA color is also associated with the value of total carotenoid content which was similar to that reported by (Huang et al., 2022) who found an ASTA color of 750 and a total carotenoid value of 13600 $\mu\text{g/g}$ in red pepper oleoresin (*Capsicum frutescens* var) and was lower than the value reported by (Ferraz et al., 2021) who found a total carotenoid content of 89.03 mg/g in oleoresin obtained from red pepper flour. The results obtained for the concentration of carotenoid pigments also coincide with the values found for CIEL a^*b^* surface color. The luminosity parameter L^* represents the amount of light reflected by the oleoresin, this parameter determines that values close to 100 represent a wide light reflection (translucent) and values close to 0 represent a low light reflection (opaque). According to the results, the luminosity L^* differs according to the extraction method used, as well as the color parameter a^* (green to red) and the color parameter b^* (yellow to blue), which according to the results indicate that red coloration is the predominant color of red pepper oleoresin. These results are similar to those reported by

(Ferraz et al., 2021) who found values of L^* 3.08, a^* 15.51 and b^* 4.32 in oleoresin obtained from red pepper flour. With regard to the total content of phenolic compounds, flavonoids and vitamin C, which are related to the antioxidant power of oleoresin, as well as carotenoid pigments, it was observed that UAE presented an increase in its concentration compared to the conventional methods of ME and SE. These results are consistent to those reported by (Sricharoen et al., 2017) who found a favorable effect on bioactive compounds present in different species of red pepper oleoresins (*Capsicum annuum*, *Capsicum chinense*, *Capsicum frutescens*, *Capsicum frutescens* + *Capsicum chinense*) obtained by ultrasound extraction, recording values of total phenolic compounds between 6392–12115 mg/kg, carotenoids in terms of β -carotene between 99.47–350.12 mg/kg, flavonoids between 7333–23110 mg/kg and antioxidant activity (DPPH) between 2304–6071 expressed as milligrams of catechin equivalent per kilogram. Another important factor in the comparison study was the percentage yield obtained in each of the extraction methods, although the yield obtained by ultrasound-assisted extraction (UAE) was lower than that of the conventional methods of maceration (ME) and Soxhlet (SE), the value obtained is adequate for the production of oleoresins, which is similar to the one reported by (Huang et al., 2022) who obtained a yield of 18.70% by the maceration method (ME), 16.50% using ultrasound-assisted extraction (UAE), and 15% by solvent-assisted extraction (ASE). Other authors such as (Sricharoen et al., 2017) have evaluated the oleoresin content of red pepper from different *Capsicum* species obtained by ultrasound-assisted extraction, finding values ranging from 19.23–45.13 g/100 g. Finally, regarding the composition of capsaicinoids, in this study no values were reported for capsaicin content, dihydrocapsaicin and pungency level in terms of Scoville heat units (1 mg/kg = 16.10 SHU) to the sweet red pepper oleoresin obtained, because in preliminary studies they were not detected, which coincides with what was reported by (Othman et al., 2011) who reported that the sweet red pepper evaluated presented 0 pungency level in Scoville heat units (SHU). In Table 3, the characterization parameters evaluated in tree tomato juice (TTJ) with application of two treatments and its comparison with the fresh control sample are presented. As can be observed for TTJ, as in the case of red pepper oleoresin, pH, soluble solids ($^{\circ}$ Brix), % moisture and density did not show significant differences, while, concentration of bioactive compounds (carotenoids, phenolics, flavonoids, vitamin C) and surface color CIEL a^*b^* , did show significant differences ($p < 0.05$) according to the treatment applied. The results obtained from the fresh treatment are similar to those reported by (Ordoñez-Santos & Martínez-Girón, 2020) who found a pH value of 3.59, $^{\circ}$ Brix 4.73, carotenoid pigments 11.74 mg/L and vitamin C 39.48 mg/100 mL in tree tomato juice diluted in water. Regarding the tree tomato juice evaluated in this study, it can be observed in Table 3 that the sonication treatment applied had a positive effect on the bioactive compounds of interest in the juice when compared to the pasteurized juice (HP) and the fresh control sample. A literature review did not find any studies on the effect of ultrasound application on Colombian tree tomato juice without water addition; however, studies reported on other fruit juices show that the application of ultrasound (US) generates an increase in the concentration of bioactive compounds, mainly in carotenoid pigments and phenolic compounds. In this sense (Ordoñez-Santos, Martínez-Girón, & Arias-Jaramillo, 2017) found that the application of ultrasound treatment 240 W/40 min, increased the content of phenolic compounds, carotenoid pigments without affecting the surface color of yellow cape gooseberry juice (*Physalis peruviana* L.) compared to fresh and pasteurized juice, the vitamin C content presented a gradual decrease with pasteurization and ultrasound

treatment as well as the results obtained in this study. (Nadeem et al., 2018) found that the application of ultrasound 525 W/15 min on a mixture of carrot (*Daucus carota*) and grape (*Vitis vinifera*) juice notably increased phenolic and flavonoid compounds finding maximum values of 286 mg GAE/100 mL and 196 EC/100 mL respectively. Another study by (Suo et al., 2022) reported that the application of ultrasound to yellow squash juice (*Cucurbita moschata* D.) at 400 W/10 min, did not affect the color index and improved the concentration of carotenoid pigments, presenting a positive effect in 12 days of storage at 4°C. The above results related to the increase in the concentration of bioactive compounds is due to the mechanical rupture of the tissues caused by the cavitation effect generated by the sonication waves, which allows the release of the secondary metabolites of interest found in the fruit juice. This fact is of great importance, because it allows obtaining a natural beverage source of bioactive compounds, such as the one developed in this study.

Table 2
Characterization of sweet red pepper oleoresin

Parameters	Methods		
	ME	SE	UAE
pH	4.06 ± 0.16 ^a	4.01 ± 0.13 ^a	4.04 ± 0.12 ^a
Soluble solids (°Brix)	7.14 ± 0.03 ^a	7.13 ± 0.04 ^a	7.18 ± 0.03 ^a
Humidity (%)	10.12 ± 0.91 ^a	9.87 ± 0.85 ^a	10.08 ± 0.89 ^a
Density (g/mL)	0.98 ± 0.01 ^a	0.98 ± 0.02 ^a	0.97 ± 0.02 ^a
ASTA color	574 ± 3.82 ^c	619 ± 4.56 ^b	765 ± 3.51 ^a
Total carotenoids (mg/100 g)	1362 ± 9.46 ^b	1106 ± 11.67 ^c	1473 ± 10.24 ^a
Total phenolic compounds (mg GAE/100 g)	956 ± 9.22 ^b	876 ± 10.47 ^c	1158 ± 8.65 ^a
Total flavonoid content (mg CE/100 g)	810 ± 7.90 ^a	737 ± 8.49 ^b	814 ± 7.63 ^a
Vitamin C (mg AA/100 g)	179 ± 4.55 ^a	108 ± 5.56 ^c	136 ± 5.08 ^b
<i>L</i>	4.28 ± 0.15 ^a	3.23 ± 0.14 ^b	4.22 ± 0.17 ^a
<i>a</i> *	15.76 ± 0.87 ^b	16.21 ± 1.15 ^a	15.45 ± 1.06 ^b
<i>b</i> *	4.11 ± 0.56 ^c	5.08 ± 0.76 ^{ab}	5.86 ± 0.68 ^a
% Yield	19.29 ± 1.33 ^b	22.34 ± 1.25 ^a	17.02 ± 1.24 ^{bc}
Means in a row with different superscript letters were significantly different (p < 0.05)			

Table 3
Characterization of tree tomato juice

Parameters	Treatments		
	Control	HP	US
pH	3.39 ± 0.02 ^a	3.40 ± 0.02 ^a	3.41 ± 0.03 ^a
Soluble solids (°Brix)	4.27 ± 0.03 ^a	4.30 ± 0.02 ^a	4.32 ± 0.02 ^a
Humidity (%)	87.43 ± 0.47 ^a	87.56 ± 0.51 ^a	87.79 ± 0.54 ^a
Density (g/mL)	0.92 ± 0.01 ^a	0.93 ± 0.01 ^a	0.93 ± 0.01 ^a
Total carotenoids (mg/100 g)	4.19 ± 0.05 ^b	3.54 ± 0.63 ^c	5.37 ± 0.44 ^a
Total phenolic compounds (mg GAE/100 g)	83.65 ± 6.78 ^b	78.71 ± 4.62 ^c	124.68 ± 3.45 ^a
Total flavonoid content (mg CE/100 g)	54.78 ± 3.21 ^b	48.27 ± 2.94 ^c	73.39 ± 2.46 ^a
Vitamin C (mg AA/100 g)	28.52 ± 2.33 ^a	22.45 ± 1.76 ^b	22.34 ± 1.51 ^b
<i>L</i> [*]	39.46 ± 0.67 ^c	41.48 ± 0.73 ^b	47.48 ± 0.48 ^a
<i>a</i> [*]	2.42 ± 0.04 ^a	1.89 ± 0.05 ^b	2.51 ± 0.03 ^a
<i>b</i> [*]	18.76 ± 2.45 ^b	18.44 ± 1.80 ^b	20.21 ± 1.56 ^a
Means in a row with different superscript letters were significantly different (p < 0.05)			

3.2 Physicochemical, microbiological and sensory quality of enriched margarine

Table 4 shows the physicochemical quality parameters evaluated in the different margarine formulations (control, F1, F2 and F3). All the parameters evaluated showed significant differences (p < 0.05) between the formulations. As can be seen in Table 4, margarines enriched with SRPO and TTJ presented lower values of pH, moisture, melting point, fat solids and higher values of acidity, salt content, spreading length, refractive index, iodine index, saponification, carotenoids, phenolic compounds, flavonoids, vitamin C and surface color CIEL **a*b**. The results obtained from the basic physicochemical analysis tests are within the range of commercial margarines, which generally have a pH range of 4-5.5, acidity < 0.30%, moisture 18–20%, salt content 0.10–0.40% and melting point 32–39°C, although this value may be lower depending on the fatty solids content. The results obtained in this study are similar to those reported by (Chougui et al., 2015) who formulated a margarine with addition of 100 ppm vitamin E and 50 ppm walnut skin extract finding pH values of 4 to 4.50, moisture 17.11 to 20.95%, salt content 0.22 to 0.34% and boiling point 32.95 to 33.70°C.

Table 4
Physicochemical quality parameters of developed margarines

Parameters	Control	F1	F2	F3
pH	5.32 ± 0.21 ^a	4.73 ± 0.18 ^b	4.51 ± 0.20 ^b	4.12 ± 0.14 ^c
Humidity (%)	20.13 ± 0.48 ^a	18.07 ± 0.45 ^b	16.54 ± 0.39 ^{bc}	15.91 ± 0.28 ^c
Acidity (%)	0.15 ± 0.02 ^c	0.20 ± 0.16 ^b	0.26 ± 0.08 ^a	0.29 ± 0.05 ^a
NaCl Content (%)	0.21 ± 0.01 ^c	0.28 ± 0.02 ^b	0.29 ± 0.02 ^b	0.37 ± 0.03 ^a
Melting point (°C)	35.47 ± 0.47 ^a	32.56 ± 0.17 ^b	32.44 ± 0.11 ^b	32.27 ± 0.22 ^{bc}
Spreadability (cm)	5.24 ± 0.76 ^d	7.26 ± 0.55 ^c	8.02 ± 0.61 ^b	9.25 ± 0.59 ^a
Refractive index	1.411 ± 0.03 ^b	1.423 ± 0.01 ^b	1.456 ± 0.02 ^a	1.472 ± 0.02 ^a
Iodine index (g I ₂ /100 g)	52.24 ± 1.23 ^d	58.09 ± 1.28 ^c	63.15 ± 1.06 ^b	73.76 ± 1.25 ^a
Saponification index (mg KOH/g)	164 ± 3.74 ^b	169 ± 3.11 ^b	184 ± 2.76 ^a	186 ± 2.58 ^a
% SFC 10°C	46.56 ± 0.23 ^a	42.27 ± 0.18 ^b	40.54 ± 0.17 ^{bc}	39.56 ± 0.32 ^{bc}
% SFC 20°C	22.54 ± 0.19 ^a	19.23 ± 0.16 ^b	18.54 ± 0.20 ^b	17.61 ± 0.14 ^c
% SFC 30°C	11.69 ± 0.27 ^a	10.33 ± 0.11 ^b	9.23 ± 0.12 ^c	6.28 ± 0.25 ^d
% SFC 40°C	4.73 ± 0.07 ^a	3.46 ± 0.05 ^b	2.42 ± 0.03 ^c	1.77 ± 0.01 ^d
Total carotenoids (mg/100g)	0.08 ± 0.01 ^d	1.64 ± 0.02 ^c	2.37 ± 0.04 ^b	3.52 ± 0.03 ^a
Total phenolic compounds (mg GAE/100g)	1.58 ± 0.13 ^d	9.78 ± 0.74 ^c	14.34 ± 0.55 ^b	37.23 ± 0.93 ^a
Total flavonoid content (mg CE/100g)	2.04 ± 0.05 ^d	5.23 ± 0.18 ^c	9.17 ± 0.22 ^b	17.41 ± 0.33 ^a

Means in a row with different superscript letters were significantly different (p < 0.05)

Parameters	Control	F1	F2	F3
Vitamine C (mg AA/100g)	0.78 ± 0.01 ^d	3.57 ± 0.06 ^c	10.46 ± 0.15 ^b	15.43 ± 0.23 ^a
<i>L</i>	70.15 ± 1.77 ^d	78.34 ± 2.09 ^c	82.26 ± 2.21 ^b	88.91 ± 3.31 ^a
<i>a</i> *	- 6.11 ± 0.16 ^d	-2.93 ± 0.05 ^c	0.83 ± 0.07 ^b	1.64 ± 0.08 ^a
<i>b</i> *	11.46 ± 1.04 ^d	23.45 ± 1.13 ^c	31.07 ± 1.16 ^b	39.17 ± 2.04 ^a
Means in a row with different superscript letters were significantly different (p < 0.05)				

In relation to spreadability, which consists of the spreadability of the margarine, the results coincide with those reported by (Aini et al., 2020) who found values of 7.25 to 13 cm in margarines enriched with Gambier extract, which indicates that the margarines developed have an easy spreadability in bread, crackers or other baked products. Another variable evaluated consisted of the melting point, which is a very important quality parameter, as well as the fatty solids content because they are related to the ease of melting of the margarine in the mouth, the texture sensation on the palate due to the crystallization of fatty acids and the degree of exudation of the margarine depending on the storage temperature. Fat solids varied with increasing temperature in all cases, the values obtained for fat solids at 40°C were below 5 to 6%, which indicates that the F2 and F3 enriched margarines present a creamy and spreadable consistency with low hardness, due to the reduction of palm oil and the addition of natural ingredients. Similar results were reported by (Nadeem et al., 2017) who evaluated different margarines enriched with chia oil, finding fat solids content of 42.36, 16.58, 2.19 and 1.52% at temperatures of 10, 20, 30 and 37°C respectively. On the other hand (Esmaeilifard et al., 2016) evaluated different commercial margarines finding melting points between 32 to 40.95°C and fatty solids content of 43%, 19.17%, 4.06% and 0.83% at temperatures of 10, 20, 30 and 35.50°C respectively. In turn (Lounis et al., 2018) reported fat solids values of 17.10%, 6.70%, 0.50% at temperatures of 20, 30 and 40°C and a melting point of 35.90 in different commercial margarines used in baking. Other parameters of interest corresponded to the evaluation of the refractive index which is related to the purity of the used oils and unsaponifiable fractions, the iodine index which is associated with the content of polyunsaturated fatty acids, the higher the number of insaturations the higher its value increases and the saponification index which is related to the amount of hydroxide necessary to neutralize the free fatty acids present in the samples. The addition of SRPO and TTJ in the formulations developed produced an increase in these variables; however, the results are desirable for vegetable margarines. Authors such as (Lounis et al., 2018) found iodine index values in ranges from 41.71 to 88.99 g I₂/100 g in different margarine formulations. Additionally (Martínez-Girón et al., 2016) found refractive index values of 1.412–1.462, saponification index 173–198 mg KOH/g, iodine index 48–56 g I₂/100 g in industrial margarine made from palm oil and soybean oil.

In relation to the bioactive compounds evaluated, commercial margarines are generally not a significant source of these compounds, which is why the margarines developed in this study are an alternative for enrichment using natural ingredients. The content of carotenoids, phenolic compounds, flavonoids and vitamin C increased in all cases as the addition of sweet red pepper oleoresin and tree tomato juice increased in each of the formulations as expected due to the concentration of these compounds found in both extracts (Tables 2 and 3), which indicates that they are natural ingredients that allow for the enrichment of fatty products such as margarines. A review of the literature did not find any studies on the evaluation of the content of bioactive compounds for margarines formulated with SRPO and TTJ, which indicates that this study is a pioneer in the evaluation of the functionality of the combination of these two natural extracts. Another parameter evaluated was surface color, which also represents an important quality parameter in margarines, due to its direct relationship with the visual impact generated by an attractive color to the consumer. The results obtained were consistent because the F1, F2 and F3 enriched margarines presented higher values of brightness and color b^* as expected due to the addition of SRPO and TTJ. The results obtained for lightness and color b^* are similar to those reported by (Djouab et al., 2017) who evaluated margarines enriched with red skin extract of (*Phoenix canariensis* L) reporting values of lightness L^*79 , color $a^*4.90$, color b^*32 . The results obtained are also similar to those found in studies where they have evaluated the color of commercial margarines. For example: (Nor Adilah et al., 2020) obtained a lightness L^*72 , color $a^*-2.80$, color $b^*20.30$ in commercial margarines stored at 4°C. Other authors such as (Paduret, 2022) evaluated the color of commercial margarines used by manufacturers of bakery products reporting values of $L^*95.41$, color $a^*-6.46$, color $b^*22.22$. Finally (Esmaeilifard et al., 2016) evaluated the color in commercial margarines during storage for six weeks reporting values of $L^*65.30$, color $a^*26.79$, color $b^*108.55$. According to these results, it can be evidenced that the margarines developed in this study presented favorable results of surface color, due to the fact that despite not having addition of synthetic colorants as in the case of commercial margarines, values of brightness, color a^* and b^* were reached with adequate levels, indicating that the added extracts corresponding to sweet red pepper oleoresin and yellow tree tomato juice are an alternative as a source of natural color.

Table 5 shows the microbiological quality parameters evaluated in the different margarine formulations (control, F1, F2 and F3), showing that good results were obtained in all formulations, indicating an acceptable quality suitable for human consumption. The results did not show significant differences according to the type of formulation developed, including the control formulation. These results can be attributed to the good hygienic practices (GHP) and good manufacturing practices (GMP) that were taken into account during the processing of the margarines. In turn, it has been reported that red pepper oleoresin presents antimicrobial properties (Baenas et al., 2019) which represents a positive effect in the fortified margarines. On the other hand, another influencing factor is the quality of the ingredients or raw materials used, generally the aqueous phase of the margarine is more prone to present microbiological risk than the fatty phase, because this is the phase that confers the highest free water content to the fatty product, for the above, in all formulations drinking water of quality suitable for human consumption with acceptable microbiological parameters was used, additionally the tree tomato juice was subjected to

ultrasound treatment (US). Another factor that was taken into account in this study was that whey was not used as milk flavoring in the formulations, because milk can increase the microbiological risk in margarines, mainly because it can be a medium for the development of bacteria, molds and yeasts that can generate secondary compounds that alter the characteristic odor and flavor of the margarine. A total of four of the main altering microorganisms that can affect the microbiological quality of a fatty product were evaluated. According to the microbiological quality results (Table 5), *Escherichia coli* was absent, yeasts, *Staphylococcus aureus* and total coliforms were below 10 colony forming units (CFU/g). The above results indicate that the formulations were developed with the expected quality criteria according to the margarine standard NTC 250 (2022). Similar results were reported by (Chougui et al., 2015) who found the absence of fecal coliforms and yeasts, *staphylococcus aureus* < 10 CFU/g in margarines made with prickly pear or prickly pear (*Opuntia ficus-indica*) skin extract.

Table 5

Microbiological quality parameters in developed margarines

Parameters	Control	F1	F2	F3
Total coliforms (MPN/g)	< 10	< 10	< 10	< 10
<i>Escherichia coli</i> (MPN/g)	Ausente	Ausente	Ausente	Ausente
Yeasts (CFU/g)	< 10	< 10	< 10	< 10
<i>Staphylococcus aureus</i> (CFU/g)	< 10	< 10	< 9	< 9

Table 6 shows the results of the sensory quality evaluation of the margarines developed (control, F1, F2 and F3). The panelists stated in the sensory evaluation by means of a hedonic scale that the F2 formulation with the addition of oleoresin at a concentration of 200 ppm and 10% of tree tomato juice was the one that presented the best attributes of color, aroma, flavor, palatability and general acceptance. The analysis of sensory properties in fatty products such as margarines is very relevant because it allows knowing the degree of acceptability that the product will have and is related to the preference at the time of consumption and the frequency of use at home, restaurants, market places, among others. The scores for each of the sensory attribute values evaluated in the margarines are shown in Table 6. The decrease in the percentage of palm oil and the addition of sweet red pepper oleoresin in the fat phase and tree tomato juice in the aqueous phase, significantly affected the sensory attributes when compared to the control margarine ($p < 0.05$). Among the formulations developed, F2 and F3 presented the best color, odor, flavor, palatability and overall acceptability. These results are consistent with the data obtained from the physicochemical quality parameters of the enriched margarines, which presented greater spreadability due to the increase of sunflower oil in the fat phase, which reduces to a certain extent the hardness conferred by palm oil. The melting point obtained in the margarines is also a parameter related to the sensory attribute, due to the relation it has with the softness of the margarine and the melting sensation on the palate, which is lower than the normal temperature of the human body that generally ranges between 37°C. This quality parameter, besides being related to the palatability of margarine, is also related to the fatty solids content, which allows the product to remain solid at room temperature without

melting easily. In this order of ideas, the results obtained show that the addition of sweet red pepper oleoresin and tree tomato juice, together with the increase of sunflower oil in margarines, can provide good organoleptic properties to consumers.

Table 6
Sensory attributes of developed margarines

Sensory attributes	Control	F1	F2	F3
Color	3.22 ± 0.21 ^d	7.13 ± 0.36 ^b	8.78 ± 0.45 ^a	6.15 ± 0.30 ^c
Aroma	2.28 ± 0.89 ^d	5.67 ± 0.26 ^c	8.63 ± 0.32 ^a	7.11 ± 0.14 ^b
Taste	4.32 ± 0.64 ^c	6.13 ± 0.47 ^{bc}	8.12 ± 0.41 ^a	6.89 ± 0.20 ^b
Palatibility	6.07 ± 0.33 ^d	7.34 ± 0.35 ^c	8.75 ± 0.29 ^a	8.02 ± 0.18 ^b
General Acceptance	4.56 ± 0.15 ^c	6.45 ± 0.44 ^c	8.83 ± 0.41 ^a	7.01 ± 0.49 ^b
Means in a row with different superscript letters were significantly different (p < 0.05)				

It is noteworthy that no sensory evaluation studies of margarines with the addition of sweet red pepper oleoresin and tomato juice were found, however, other studies have evaluated the sensory attributes of margarines with the addition of natural extracts, in this sense (Aini et al., 2020) formulated margarines with the addition of Gambier extract (*Uncaria gambir* Roxb.) in concentrations of 150 to 600 ppm, finding scores of 3.16 to 3.80 in sensory attributes on a scale of 1 to 5 points. Other authors have evaluated the sensory attributes of fatty products with the addition of unconventional oils and/or fats. In that sense (Nadeem et al., 2017) made a margarine with 15% addition of chia oil finding that color, flavor and texture attributes were not affected during 90 days of storage. On the other hand (de Souza Mesquita et al., 2020) made a mayonnaise with sunflower oil enriched with pigments from the peach palm fruit (*Bactris gasipaes*), finding that the attributes of color, aroma, taste, texture, and general acceptance presented values between 8.24 to 8.56 on a 9-point hedonic scale. (Shin et al., 2021) made a formulation of margarine with the addition of 80% duck fat as a substitute for soybean oil, finding average values of 6 points for the sensory attributes of appearance, flavor, taste, spreadability, and general acceptability on a 9-point hedonic scale. According to the above, the chemical nature of the fat phase and the aqueous phase and the percentages applied in the formulations influenced the organoleptic characteristics of the margarines, which were designed in such a way that in addition to containing nutrients and bioactive compounds, they would generate a preference of consumer liking. The results obtained from the sensory evaluation in this study are relevant because one of the disadvantages of margarines enriched with natural extracts is that they usually present undesirable flavors for consumers (Nor Adilah et al., 2020).

3.3 Oxidative stability during storage of fortified margarine

Figure 2 shows the change in the peroxide index during storage of the margarines developed. It can be observed that the margarine formulations with the addition of 200–300 ppm of SRPO and 10–15% of

tree tomato juice presented a good behavior in relation to the peroxide index, which was lower than 5 meq O₂/kg, allowing greater oxidative stability in the product. This value is within the range recommended by (NTC 250, 2022), which establishes that the ideal peroxide index for a margarine is 1 meq O₂/kg inside the factory and 5 meq O₂/kg outside the factory. This behavior may be related to the antioxidant effect of sweet red pepper oleoresin and tree tomato juice, due to the presence of bioactive compounds, mainly carotenoid pigments and the total content of phenolic compounds. The same effect was presented in (Fig. 3, 4) where in all cases good performance is observed in terms of antioxidant activity and p-anisidine in formulations with addition of SRPO and TTJ. The peroxide index was taken into account in this stability study because it is associated with the formation of primary oxidation products such as hydroperoxides that can be formed in the fatty product. The results obtained in this study agree with what was reported by (Djouab et al., 2017) who reported that the addition of natural extract of red palm fruit (*Phoenix canariensis* L) presents a positive effect on the peroxides index, registering values of 1.30 to 3.20 meq O₂/kg in the storage of margarines for 20 days at 5°C. In another study (Esmaeilifard et al., 2016) they found peroxides values of 4.16 meq O₂/kg in bakery margarines stored for 6 weeks at 5°C. On the other hand (Zaeromali et al., 2014) evaluated the peroxides index in industrial margarine 90 days after its preparation at 25°C finding a value of 2.47 meq O₂/kg, this low value is possibly due to the addition of synthetic additives and addition of vitamins that the formulation of this industrial margarine contained. In turn, the p-anisidine value was evaluated because it is associated with secondary oxidation products, such as the formation of ketones and aldehydes during margarine storage. The F2 and F3 enriched margarines presented higher p-anisidine values (Fig. 3), possibly due to the amount of polyunsaturated fatty acids in sunflower oil and SRPO that make margarines more susceptible to secondary oxidation, however the values obtained are low and adequate for fatty products without addition of synthetic antioxidants. When comparing these results with other studies, it is observed that the p-anisidine value varies according to the formulation, temperature and storage time, for example (Azizkhani & Zandi, 2010) evaluated the p-anisidine value in margarines enriched with rosemary extract (*Salvia rosmarinus*), obtaining a value of 5.50 during 25 days of storage at 60°C. In another study (Esmaeilifard et al., 2016) found p-anisidine values from 3 to 5.80 in baking margarines stored for 6 weeks at 5°C. On the other hand (Panpipat et al., 2018) evaluated margarines enriched with different β -sitosterol esters obtaining p-anisidine values between 20 to 70 during 20 days of storage at temperatures of 25 and 55°C. It is noteworthy that although red pepper oleoresin is a source of polyunsaturated fatty acids (Fernandez-Trujillo, 2007), it did not negatively influence the oxidation resistance related to the degree of auto-oxidation that the fatty acids in the margarine formulations may present during storage (Zaeromali et al., 2014). Studies conducted with other types of extracts have found that the addition of oleoresins and/or vegetable skin extract improve antioxidant activity in fatty foods influencing their stability (Chougui et al., 2015; Procopio et al., 2022). On the other hand (Lopes et al., 2014) evaluated the antioxidant activity as the percentage of DPPH radical inhibition in samples of margarines enriched with extracts of mixtures of traditional species such as: basil (*Ocimum basilicum*), green onion (*Allium fistulosum*), lemon (*Citrus limon*), garlic (*Allium sativum*), thyme (*Thymus vulgaris*), marjoram (*Origanum majorana*), oregano (*Origanum vulgare*), finding values ranging from 66.05 to 83.12% DPPH in freshly

prepared margarines without evaluating the change of antioxidant activity during storage time. According to the results obtained in this study, it is evident that formulation F3 followed by F2 with the addition of sweet red pepper oleoresin SRPO and tomato tree juice TTJ, successfully reduces lipid oxidation during storage of the formulated margarines, and consequently helps the stability of the fatty product during the shelf life of an industrial margarine, which is approximately 180 days. The above is a consequence of the concentration of bioactive compounds present in the SRPO and TTJ such as: total carotenoids, total phenolic compounds, flavonoids and vitamin C or ascorbic acid (Tables 2 and 3) which were favored by the effect of ultrasound applied on the samples, similar results were found by (Sricharoen et al., 2017) who evaluated bioactive compounds in oleoresins of different species of peppers (*Capsicum*) obtained by ultrasound. The bioactive compounds present in the evaluated extracts play an important role as natural antioxidants, synergistic agents and preservatives when applied in margarine formulations, because they allowed controlling primary and secondary oxidation products during storage thanks to their ability to react directly with free radicals, such as hydroperoxides, and convert them into non-reactive oxygen species (Yoo et al., 2008), which is evidenced by the results obtained from the antioxidant activity during storage (Fig. 4).

Finally, the results are relevant because when oxidation products were controlled, there were no undesirable odors and flavors, and there was no oxidative rancidity in storage of the margarines formulated (F2 and F3) with the addition of SRPO and TTJ at 4°C. Additionally, this is a pioneering study for the evaluation of quality parameters, since there are few studies where margarines enriched with the addition of natural extracts in both components of their formulation (fat phase and aqueous phase) are developed and the effect of their addition on oxidative stability during 180 days of storage is presented.

4. Conclusions

Sweet red pepper oleoresin (SRPO) and tree tomato juice (TTJ) are an important source of total carotenoids, total phenolic compounds, flavonoids, vitamin C and ASTA color. The addition of SRPO in the fat phase and TTJ in the aqueous phase increased the concentration of bioactive compounds in the margarine formulations, improving the oxidative stability of this product during storage. The evaluations of physicochemical and microbiological quality and oxidative stability showed that margarines formulated with the addition of oleoresin up to a level of 300 ppm and 15% addition of tree tomato juice presented favorable results, with the formulation with the addition of 200 ppm of SRPO and 10% of TTJ being the best sensorially qualified. Therefore, sweet pepper oleoresin (lipidic) and tree tomato juice (aqueous) are two natural ingredients suitable for the preparation of emulsions with application as colorant and antioxidant when applied in the fat phase and aqueous phase, respectively, in enriched and functional foods.

Declarations

Acknowledgements

The author thanks Fleischmann Food S.A for the training as a laboratory analyst at the margarine and bakery plant. Also to # 909 Minciencias-Colombia convocatory and the Universidad Nacional de Colombia sede Palmira and Universidad del Valle-Sede Palmira.

Availability of data and material: All data generated or analyzed during this study are included in this article.

Conflicting interests/Competing interests: The author declare that there is no any conflict of interests.

Author contribution: Jader Martínez-Girón: Conceptualization, Methodology, Resources, Formal analysis, Validation, Writing - Original Draft, Review & Editing.

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Figures

Margarine enriched with SRPO and TTJ

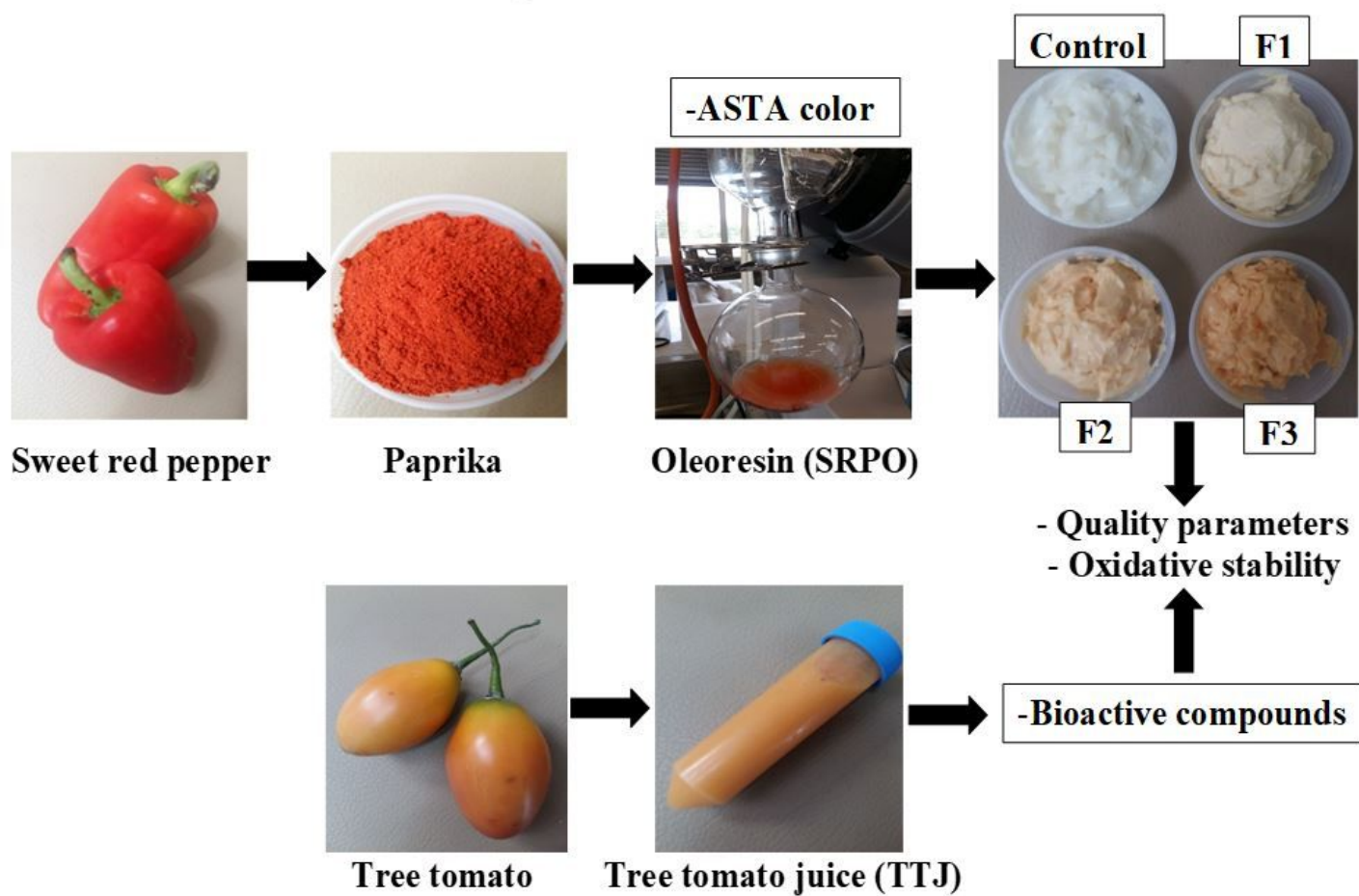


Figure 1

Graphic scheme of the developed margarines

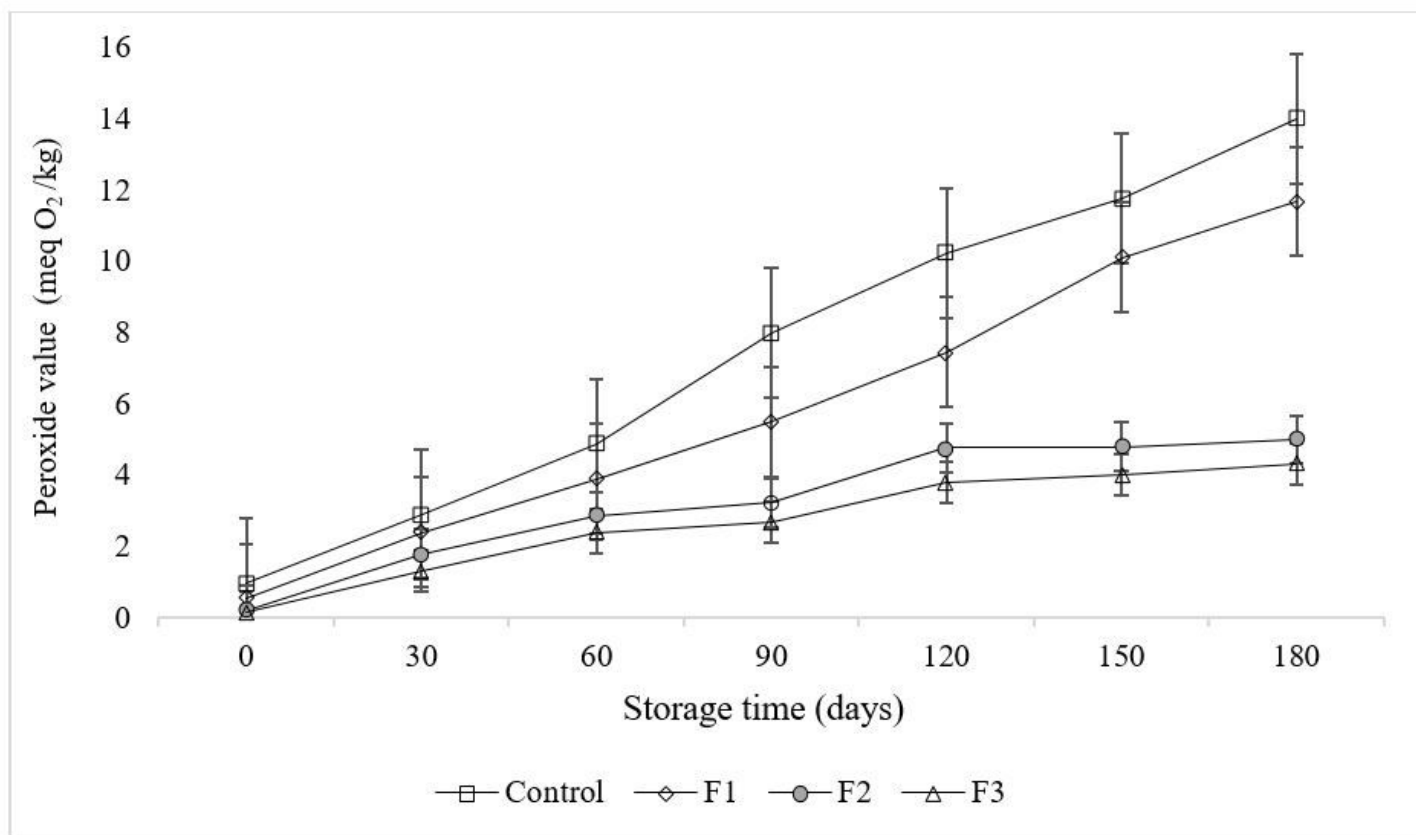


Figure 2

Change of peroxides index in margarine storage

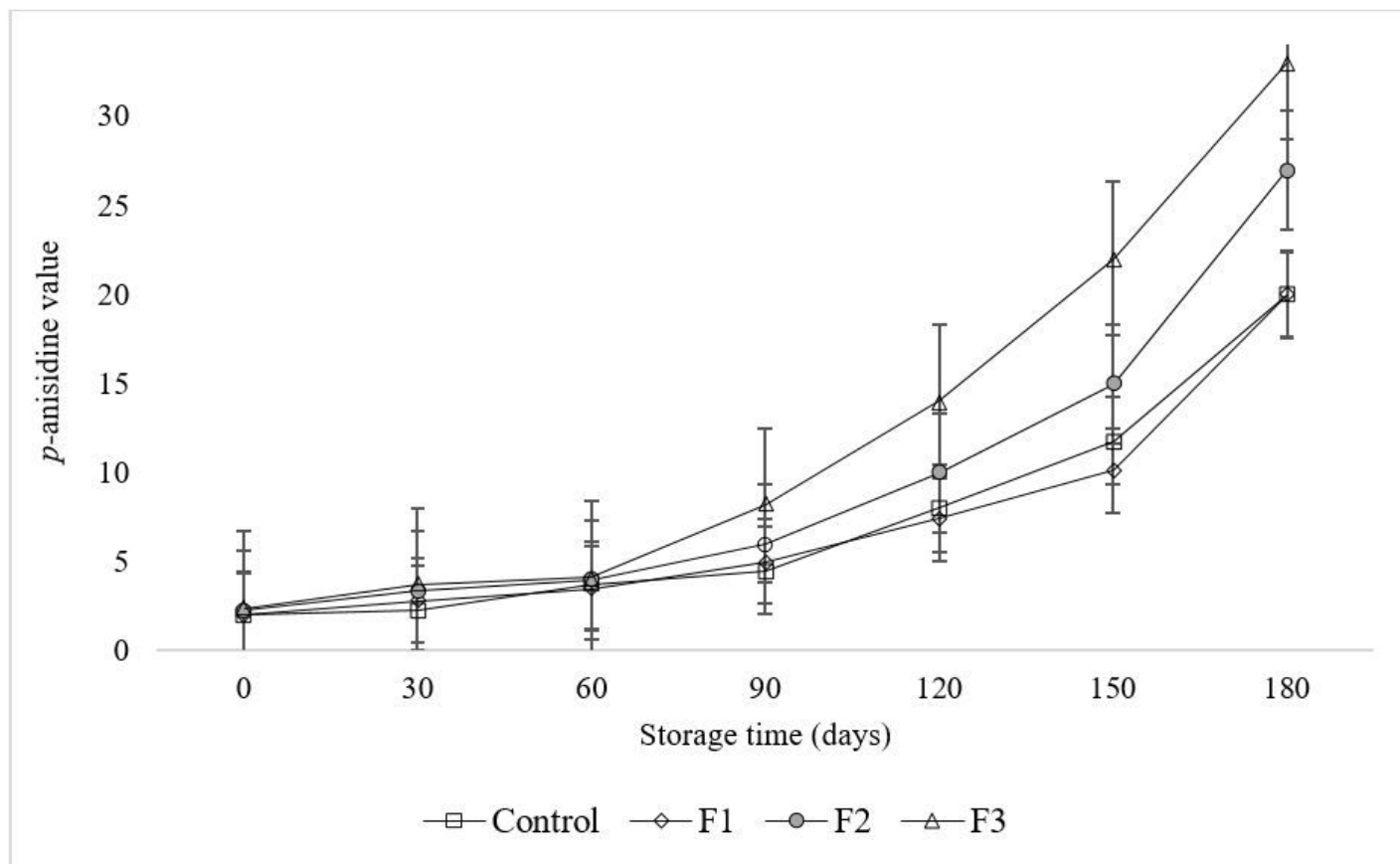


Figure 3

Change of p-anisidine value in storage of margarines

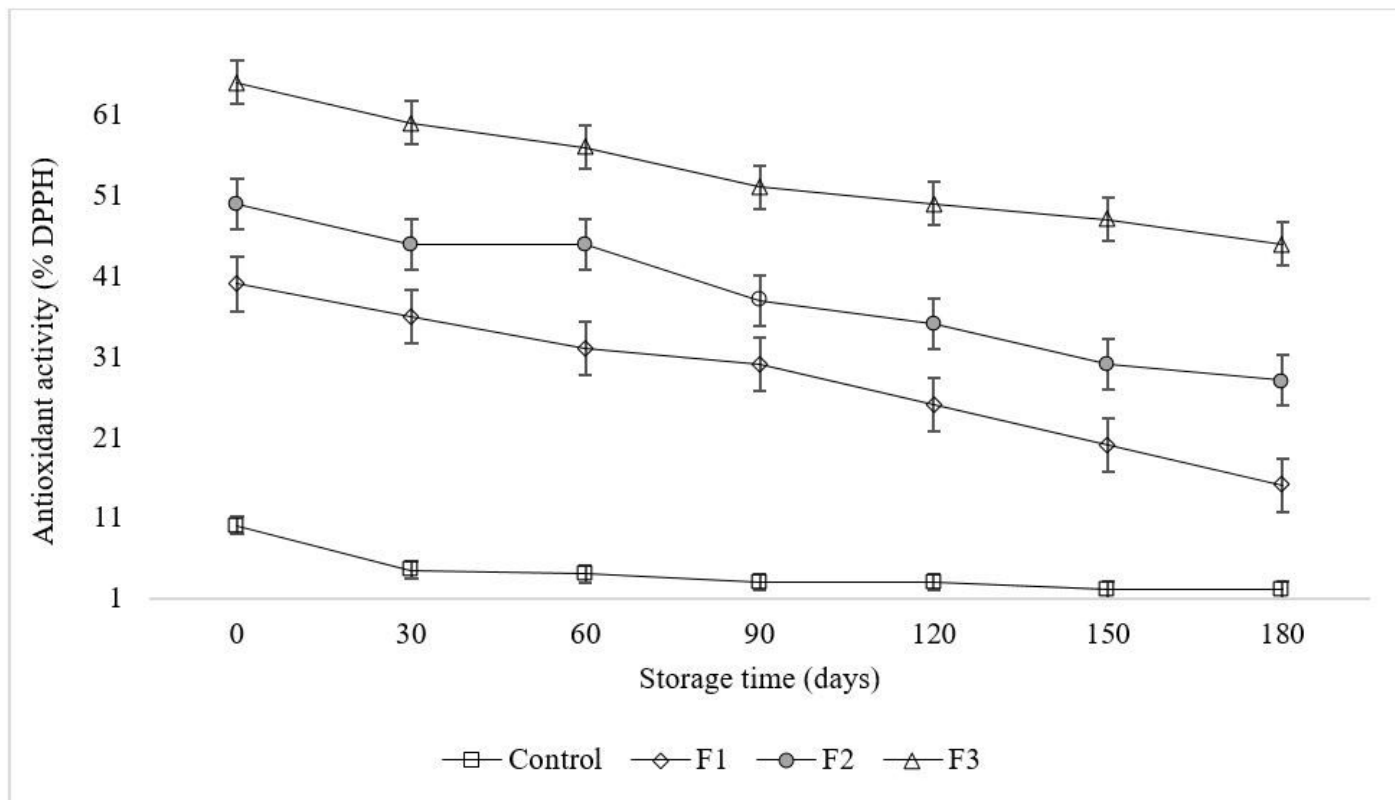


Figure 4

Change in antioxidant activity during storage of margarine