

# Ultrasound-assisted extraction of total carotenoids in papaya epicarp and its application in Frankfurt sausage

Extração assistida por ultrassom de carotenóides totais em epicarpo de mamão e sua aplicação em linguiça de Frankfurt

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#### ABSTRACT

The agro-industrial processing of fruits generates a significant volume of by-products, which can be valued as a source of natural ingredients in the food industry. The objective of this research was to optimize the ultrasound-assisted extraction (UAE) of total carotenoids in papaya epicarp and to use the extract as an ingredient during the storage of the Frankfurter sausage. The maximum assisted extraction of total carotenoids ( $66.03 \pm 0.60 \text{ mg}$  of  $\beta$ -carotene/100g) in the papaya epicarp by ultrasound is achieved when working at temperatures of 30 °C, for 60 min and a solid-liquid ratio 0.0064 g/ml oil. The temperature presented the highest effect of extraction of total carotenoids, followed by solid-liquid ratio, and time in the UAE. Frankfurt-type sausages made from carotenoid lipid extract show a significant reduction in nitrites (p <0.05), a significant increase in oxidation processes (p <0.05) and achieve the preservation of their characteristic colors during storage. The carotenoid extracts obtained from the papaya epicarp can be a natural coloring additive in the production of Frankfurt-type sausages since it allows the preservation of color during storage.

Index terms: Optimization; Box-Behnken; peroxide index; TBARS; CIE, \*\*\*\*

#### RESUMO

O processamento agroindustrial de frutas gera um volume significativo de subprodutos, que podem ser valorizados como fonte de ingredientes naturais na indústria alimentícia. O objetivo desta pesquisa foi otimizar a extração assistida por ultrassom (EAU) de carotenóides totais em epicarpo de mamão e utilizar o extrato como ingrediente durante o armazenamento da linguiça Frankfurter. A extração máxima assistida de carotenóides totais (66,03 ± 0,60 mg de β-caroteno/100g) no epicarpo de mamão por ultrassom é alcançada quando se trabalha a temperaturas de 30 °C, por 60 min e uma relação sólido-líquido de 0,0064 g/ml de óleo. A temperatura apresentou o maior efeito de extração de carotenóides totais, seguida da relação sólido-líquido e tempo nos UAE. As linguiças tipo Frankfurt elaboradas com extrato lipídico de carotenóide apresentam redução significativa de nitritos (p<0,05), aumento significativo dos processos de oxidação (p<0,05) e preservam suas cores características durante o armazenamento. Os extratos de carotenoides obtidos do epicarpo de mamão podem ser um aditivo corante natural na produção de linguiças tipo Frankfurt, pois permite a preservação da cor durante o armazenamento.

Termos para indexação: Otimização; Caixa-Behnken; índice de peróxido; TBARS; CIE<sub>1\*a\*b\*</sub>.

## INTRODUCTION

Papaya is a tropical fruit, and its production global reached 13.89 million tons in 2020, this fruit is valued for its nutritional properties, sensory characteristics and digestive effects (Food and Agriculture Organization Corporate Statistical Database - FAOSTAT 2022; Calvache et al., 2016). Due to its quality attributes, papaya is appreciated in the fruit industry where it is used in the preparation of beverages, chips, jams, sauces and pickles among others. These processes generate important byproducts such as the epicarp and seeds that represent approximately between 12 and 8.5%, respectively (Pathak; Mandavgane; Kulkarni 2019). The disposal of these papaya waste can generate additional costs for the fruit processing agro-industry; however, these matrices can be valued as additives in the industry since they are an important source of bioactive compounds. In the case of the papaya epicarp, it has been reported that it is a matrix that offers carotenoids, phenolic compounds, vitamins A, C, B and flavonoids (Calvache et al., 2016; Pathak; Mandavgane; Kulkarni 2019). In ripe papaya fruits, the main carotenoids identified are lycopene,  $\beta$ -cryptoxanthin and  $\beta$ -carotene, and there is evidence that these compounds

2022 | Lavras | Editora UFLA | www.editora.ufla.br | www.scielo.br/cagro All the contents of this journal, except where otherwise noted, is licensed under a Creative Commons Attribution BY. reduce the incidence of degenerative diseases (Sancho; Yahia; González-Aguilar, 2011). Different studies have reported the extraction of carotenoid compounds combining the ultrasound process with vegetable oil as a solvent (Ordóñez-Santos; Pinzón-Zarate; González-Salcedo, 2015; Goula et al., 2017; Ordóñez-Santos; Girón; Rodríguez-Rodríguez 2019; Chutia; Mahanta 2021; Lara-Abia; Welti-Chanes; Cano, 2022). These researchers highlight the extraction efficiency, reducing solvent consumption, less extraction times and less energy consumption compared to conventional methods. Ultrasound is a mechanical process that allows the increase in mass transfer during extraction by interacting with the solvent and the plant matrix due to the periodic collapse of the cavitation bubble (Deng et al., 2022). The authors reported that the violent collapse of bubbles generates cavitation that causes many secondary phenomena, such as shock waves, radicals (such as H2O2 and OH-), local high temperature (~5000 °C) and pressure (50-2000 atm). In addition, Yolmeh and Jafari (2017) show the need to apply the Response Surface Methodology (RSM), since it allows studying the effects of independent variables and their interactions in the extraction of carotenoid compounds. However, the response to the extraction of carotenoid compounds depends on the plant matrix, as reported by Murador, Da Cunha and De Rosso (2014). These authors reported that the type of plant matrix influences the cellular structure of carotenoids since these pigments are linked to different proteins that form lipid droplets or crystalline structures that allow the release or retention of carotenoids. Therefore, other studies are necessary to know the effect of carotenoid extraction factors in the extraction of carotenoids by ultrasound in other fruit by-products, this information is of great interest for the agro-industrial sector. In addition, consumer interest in purchasing healthy foods has encouraged the offer of products with natural ingredients rich in bioactive compounds in the food processing industry (Ordóñez-Santos; Esparza-Estrada; Vanegas-Mahecha, 2021). This type of product not only has a positive impact on food quality but can also benefit consumer health (Aziz; Karboune, 2018). In this sense, several studies have evaluated fruit by-products as sources of natural antioxidants during the refrigerated storage of meat products: De Almeida et al. (2015) worked with jaboticaba epicarp extract in bologna-type sausages; Basanta et al. (2018) investigated the cherry epicarp in chicken patisserie; and Cilli et al. (2020) evaluated the grape epicarp in salmon burgers. However, there are no studies using lipid extract of pigments obtained from fruit by-products in meat products. The aim of the present study

was to optimize the UAE of total carotenoids in the papaya epicarp and to use the extract as an ingredient during the storage of the Frankfurter sausage.

### **MATERIAL AND METHODS**

#### Obtaining lipid extract of papaya by UAE

Papaya epicarp flour (PEF) was obtained according to Velasco-Arango et al. (2021). The samples were lyophilized in a Labconco FreeZone lyophilizer with a blocking tray dryer using a vacuum pressure of 0.120 mbar, and an operating temperature of -50 °C. The lyophilized sample was reduced to a particle size of up to 0.074 mm, in an IKA Labortchnik blade grinder, this flour was stored in glass vials under refrigeration conditions at a temperature of 4 °C. The concentration of total carotenoids in the flour (mg of  $\beta$ -carotene / 100 g of dry sample) was determined following the methodology described by Ordóñez-Santos, Pinzón-Zarate, and González-Salcedo (2015), using an ultrasound, ultrasonic cleaner HB-S49DHT (China), operating at 42 kHz and input power of 240 W. The total carotenoid content in the study samples was calculated using the molar extinction coefficient of 7.1 \*  $10 \land 4 \land M \land (-1)$  $cm^{(-1)}$  by measuring the absorbance of the lipid extract at 464 nm in a spectrophotometer (Jenway 6320D, USA), using sunflower oil as blank. The optimization of the UAE of total carotenoids in papaya epicarp was carried out by applying response surface methodology combined with a Box-Behnken design with three factors and three levels, with fifteen experimental runs, three repetitions included in the central point. The independent variables of this study selected were temperature (X1, ° C), extraction time (X2, min) and solid-liquid ratio (X3, g / ml). Table 1 shows the coded factors the central points of each factor and their extreme values (-1, +1).

**Table 1:** Independent variables and their levels used in a Box-Behnken design.

Factor	-1	0	+1
Temperature (°C)	30	40	50
Time (min)	60	70	80
solid-liquid ratio (g/mL)	0.0032	0.0048	0.0060

# Evaluation of the lipidic extract in Frankfurt sausage during storage

The previously optimized lipid extract of papaya epicarp was used as an ingredient in Frankfurt sausages.

In the production of Frankfurt sausage, pork meat (pH 6.3) and commercial quality dorsal pork fat were used, both acquired in a supermarket in the city of Palmira, Valle del Cauca, Colombia. The other inputs and additives used in the formulation of the sausage were purchased in supermarkets in the same city. Formulation and elaboration process of the sausage follows Pinzón-Zárate, Hleap-Zapata and Ordoñez-Santos (2015) as showed in Table 2. The stability of the sausage with lipid extract of papaya epicarp vacuum packed at 0 and 30 days of storage under refrigeration conditions (6  $\pm$  2 °C) was evaluated by analyzing the variables of residual nitrite response (mg / Kg), peroxide index (meq O2 / Kg sample), p-anisidine value (µmol / µg), TBARS (mg MDA / kg) and surface color (L\* C\* h<sup>o</sup> y  $\Delta E$ ). All measurements were made by triplicate, and it presented as an average value  $\pm$  standard deviation. The determination of residual nitrites was carried out according to the methodology proposed by Zahran and Kassem (2011). The peroxide index was established according to the (American Oil Chemists' Society - AOCS, 2003) method Cd 8-53, which measures the initial oxidation state or the formation of primary lipid oxidation products of the analyzed samples. The p-anisidine value was quantified according to the AOCS (2017) Cd 18-90 method, which measures the oxidative state of lipids in relation to the quality of their flavor.

The determination of lipid oxidation on the evaluation days was carried out by analyzing the reactive substances to 2-thiobarbiturate - TBARS (Basanta et al., 2018). In the samples the color coordinates were determined at five different points using a colorimeter (Konica Minolta Meter CR-100, Osaka, Japan). Three cylindrical samples of 5 cm in length were taken by making a longitudinal cut to analyze their internal color. Color measurements were expressed in terms of L\*, a\* and b\* tristimulus coordinates, and the hue or hue angle (h°) was calculated, as well as the chromaticity or saturation index (C) using the following Equations 1 to 2, respectively:

$$h^{\circ} = \arctan \frac{b^*}{a^*} \tag{1}$$

$$C^{\circ} = (a^{*2} + b^{*2})^{1/2}$$
(2)

Total color difference ( $\Delta E$ ) was calculated by the following Equation 3:

$$\Delta E = \left[ \left( \Delta L \right)^2 + \left( \Delta a \right)^2 + \left( \Delta b \right)^2 \right]^{1/2}$$
(3)

Ingredient	Percentage of inclusion in the formulation (%)			
Pork meat (pH 6.3)		65		
Pork back fat	16			
Wheat flour	5			
lce	10			
Supplies and additives	4			
Total	100			
Supplies and Additives	%	Weight in relation to the meat mass (g/kg)		
Salt	0.846	7.0		
Sugar	0.242	2.0		
Garlic	0.459	3.8		
Onion powder	0.060	0.5		
Pepper	0.060	0.5		
Phosphates	0.484	4.0		
Sausage seasoning	1.208	10.0		
Monosodium glutamate	0.121	1.0		
Ascorbic acid	0.121	1.0		
Nitral	0.300	2.48		
Papaya epicarp lipid extract	0.098	0.83		
Total	4.000	33.1		

Table 2: Formulation used for Frankfurt sausage

elaboration

#### **Statistical analysis**

The optimization of the UAE of total carotenoids in the papaya epicarp was performed using the response surface methodology with a three-factor Box-Behnken design, and three levels with fifteen experimental runs, including three repetitions in central point. The independent study variables selected were temperature (X1, °C), extraction time (X2, min) and solid-liquid ratio (X3, g/ml). A polynomial Equation 4 of second order including all interaction terms was used to estimate carotenoid pigment concentrations:

$$Y = \beta_0 + \sum_{i}^{n} \beta_i X_i + \sum_{i}^{n} \sum_{j}^{n} \beta_{ij} X_i X_j + \sum_{i}^{n} \beta_{ii} X_i^2$$
(4)

Where, Y is a response variable,  $\beta_0$  represent intercept model coefficient,  $\beta_i$ ,  $\beta_{ii}$  and  $\beta_{ii}$ , represent lineal, quadratic and second order coefficient terms, respectively, X and Xare independent variables  $(i \neq j)$ , and k is the number of variables. The effect of the factor on the response variables was identified with an ANOVA test (p <0.05) and the fit of the model was evaluated with the coefficient of determination ( $\mathbb{R}^2$  and  $\mathbb{R}$ adj), lack of adjustment (FIT) and % CV. The T-Student test was used to validate the optimization model between theoretical and experimental values. In the storage experiment, a T-Student was applied to identify significant differences (p <0.05) during the storage time. In the respective statistical analysis, the statistical software Design Expert 11 and SPSS Statics 19 were used.

#### **RESULTS AND DISCUSSION**

# Optimization of the UAE of total carotenoid pigments in papaya epicarp flours

The mean values of the concentration of total carotenoids extracted from the papaya epicarp during the ultrasound process vary between 34.66 and 66.00 mg of  $\beta$ -carotene /100 g (Table 3). Our results exceed the papaya epicarp values reported by Calvache et al. (2016) (12.50 mg  $\beta$ -carotene /100g). The authors performed the extraction of carotenoids using conventional solid-liquid extraction combined with agitation and centrifugation, the extraction solvents were methanol, hexane, and acetone. These obvious differences may be associated with genetic factors and ripening stages in study fruits, or differences in extraction methods.

In the UAE, the factor with the greatest effect on the extraction of carotenoids was temperature, followed by solid-liquid ratio, and time (Table 4). The interactions, temperature-time, temperature-ratio, and the quadratic effect on the independent variables have a significant effect on the extraction of total carotenoids (Table 4). The parameter lack fit of adjustment was not significant (p>0.05), the determination coefficients in the regression model R2 = 0.9815, and R2adj = 0.9577, (Table 4), show that the model manages to predict the variations within the system, and the experimental values show a good fit with the following regression model:

Figure 1 shows the effect of the factors on the concentration of total carotenoids present in the papaya epicarp during the UAE. In Figure 1a, it is observed that time and temperature influence the extraction of total carotenoids, and the concentration of carotenoids increases significantly by increasing these two factors. Our results are consistent with those obtained in other studies, where they applied the extraction of carotenoids by ultrasound using vegetable oils as extraction solvent (Ordóñez-Santos; Pinzón-Zarate; González-Salcedo 2015, Kunthakudee et

al., 2020). Li et al. (2015) report that the use of ultrasound allows breaking the cell walls of the matrix to promote the release of bioactive compounds, and Yolmeh, Najafi and Farhoosh (2014) indicates that temperature reduces the viscosity and increases the solubility of the pigment, facilitating the diffusion of carotenoids to sunflower oil. In Figure 1b, it is observed how the solid-liquid ratio and the extraction temperature significantly influence the extraction, and the increase of the two independent variables significantly increases the concentration of total carotenoids. The impact of the solid-liquid relationship on the extraction of carotenoids during the ultrasound process has also been reported in other investigations (Song et al., 2018, Silva et al., 2019; Kunthakudee et al., 2020).

The mass transfer of the carotenoid pigments from papaya epicarp matrix to solvent was mainly due to the existence of a concentration gradient as commented by Tao and Sun (2015). In Figure 1c, the effect of the extraction time and the solid-liquid ratio in the extraction of total carotenoids in the study samples was validated. The optimization parameters of 60 minutes of extraction, a temperature of 30 °C and a solid-liquid ratio of 0.0064 g/ml, allow obtaining a maximum extraction of 66.45 mg  $\beta$ -carotene / 100 g in the papaya epicarp. When validating the optimal extraction conditions experimentally, a value of 66.03  $\pm$  0.60 mg  $\beta$ -carotene/100 g is obtained, this does not present significant differences (p> 0.05) with the theoretical value obtained in the present investigation. Likewise, these results surpass the conventional maceration method (52.64  $\pm$  1.57 mg  $\beta$ -carotene/100 g), showing that the UAE method is efficient for the extraction of the carotenoid pigments present in the papaya epicarp. Other investigations also reported that the UAE exceeds the conventional methods of extraction of carotenoids in vegetable by-products, Ordoñez-Santos, Pinzón-Zarate and González-Salcedo (2015) obtained 163.47>123.40 mg/100 g dried peel. Ordonez-Santos et al. (2019) reported 151.50>113.40 mg/100 g dried peel, and Ordoñez-Santos, Esparza-Estrada and Vanegas-Mahecha (2021) obtained 140.70 >106.24 /100 g dried peel. The explanation of the higher yields in the UAE was commented by Ordoñez-Santos Esparza-Estrada and Vanegas-Mahecha (2021), the authors reported that it is due to the physical phenomenon of cavitation generated during the ultrasound process, cavitation creates microbubbles in the liquid phase, and the increase in pressure and temperature allows the collapse of the bubbles, triggering violent shocks that facilitate the mechanical fracture of the plant matrix, achieving an increase in the coefficient of mass transfer from the medium to the solvent.

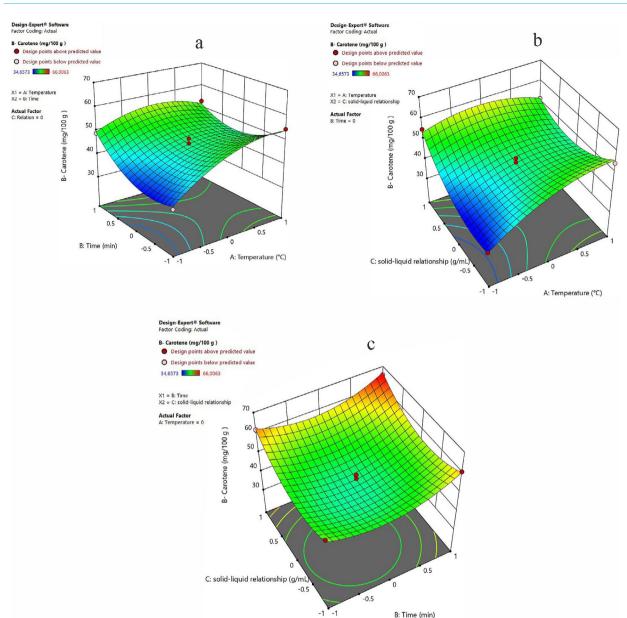
Treatment	A: Temperature (°C)	B: Time (min)	C: solid-liquid ratio (g/ mL)	Total carotenoids β-carotene (mg/100g)
3	-1	0	1	34.66 ± 0.14
12	-1	-1	0	35.50 ± 0.24
6	0	0	0	44.27 ± 1.01
7	0	0	0	45.68 ± 0.98
10	0	0	0	46.16 ± 0.76
13	0	0	0	46.63 ± 0.45
17	0	0	0	48.68 ± 0.63
8	-1	1	0	48.89 ± 0.24
1	1	0	1	51.47 ± 0.18
4	1	1	0	52.83 ± 0.24
11	1	0	-1	54.35 ± 0.47
15	1	-1	0	54.51 ± 1.03
2	-1	0	1	54.90 ± 0.49
16	0	-1	-1	55.61 ± 0.36
14	0	1	-1	60.57 ± 0.27
5	0	-1	1	61.71 ± 0.45
9	0	1	1	66.00 ± 1.17

Table 3: Box-Behnken design r	matrix and response	values for total carotenoids.
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Table 4: Analysis of variance (ANOVA) for the fitted quadratic polynomial model for extraction.

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Source	Sum of squares	df	Mean squares	F-value	p-value	Singnificance <sup>a</sup>
Modele	1106.41	9	122.93	41.22	< 0.0001	***
A - Temperature	192.20	1	192.20	64.45	< 0.0001	***
B - Time	54.94	1	54.94	18.42	0.0036	**
C – Solid-liquid ratio	104.45	1	104.45	35.03	0.0006	**
AB	56.78	1	56.78	19.04	0.0033	**
AC	133.61	1	133.61	44.80	0.0003	**
BC	0.1121	1	0.1121	0.0376	0.8518	NS
A <sup>2</sup>	115.70	1	115.70	38.80	0.0004	**
B <sup>2</sup>	199.77	1	199.77	66.99	< 0.0001	***
C <sup>2</sup>	256.42	1	256.42	85.98	< 0.0001	***
Residual	20.88	7	2.98			
Lack of fit	10.58	3	3.53	1.37	0.3722	NS
Pure error	10.29	4	2.57			
Total color	1127.28	16				

 $r^2$  = 0.9815;  $r^2$  adj = 0.9577; <sup>a</sup> \*Significative (p < 0.05); \*\* Extremely significant (p < 0.01). NS = Non significative.



**Figure 1**: Effect of the time, temperature, and solid-liquid relationship on the extraction of total carotenoids in the papaya epicarp. Response surface (time – temperature) for PEF (A), solid-liquid relationship – temperature (B), and solid-liquid relationship - time (C).

# Evaluation of the lipidic extract in Frankfurt sausage during storage

Schaefer et al. (2022) reported that lipid oxidation is one of the limiting factors for the storage of meat products, these reactions generate changes in unpleasant taste, odor, and color, in addition to the formation of toxic compounds and the reduction of nutritional value. The oxidation of lipids is generated by the chain reaction of metal ions, reactive oxygen, and nitrogen species (Manessis et al., 2020). The authors commented that in the first two stages (initiation and propagation), mainly hydroxyperoxides are produced, and their degradation generates the final phase of oxidation, obtaining final products such as aldehydes, ketones, alcohols, and carbonyl compounds. The higher the concentration of these products, the higher levels of oxidation reach the lipids in the sample. Table 5 lists the response variables evaluated during the storage of the Frankfurt sausage processed with the lipid extract of the papaya epicarp as a natural additive, obtained during the optimization stage, and the meat product can be seen in Figure 2. Table 5 show that residual nitrite was significantly reduced during storage time. This reduction may be due to the interaction between Mb and nitrite transformed to NO, that is, there is an oxidation reaction and a consumption of NO2- generating NO which is regulated in the active site of Mb giving rise to the pink color in the sausages forming NOMb.

**Table 5**: Physicochemical properties assessed inFrankfurt sausages produced with lipidic extract frompapaya epicarp.

Parameter	Storag	T Ctudoot		
raiaineter	0	30	T-Student	
Residual nitrite (mg/Kg)	41.35 ± 0.18ª	21.66 ± 0.21 <sup>b</sup>	***	
Peroxide index (meq O2/Kg sample)	23.50 ± 0.97ª	29.78 ± 0.38 <sup>b</sup>	**	
P-anisidine (µmol/µg)	25.18 ± 0.61ª	28.57 ± 0.46 <sup>b</sup>	**	
TBARS (mg MDA/kg)	0.41 ± 0.03ª	0.51 ± 0.03 <sup>b</sup>	***	
L*	$73.37 \pm 0.29^{a}$	73.09 ± 0.67ª	NS	
C*	11.82 ± 0.11 <sup>a</sup>	12.77 ± 0.38 <sup>b</sup>	**	
h°	44.33 ± 0.55 <sup>a</sup>	48.89 ± 0.87 <sup>b</sup>	**	
ΔE		1.51 ± 0.28		

(\*) significant p <0.05; (\*\*) significant p <0.01; (\*\*\*) significant p <0.001; (NS) There are no significant differences (p <0.05)  $^{a-b}$  Means within columns with different letters are significantly different (p <0.05); (--) Does not apply.

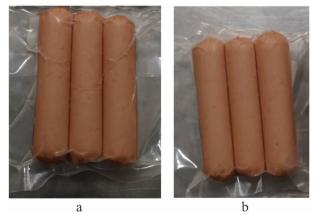


Figure 2: Frankfurt sausages evaluated during 0 (a) and 30 days (b).

As can be seen in Table 5, the increase in the storage time of the sausages, significantly increased the peroxide index values, p-anisidine value, total volatile bases and TBARS. These results agree with those presented by Bian et al. (2019) who register an increase of TBARS in sausage type products and values of volatile basis during storage. The work presented by Santos et al. (2020) also reports an increase in the peroxide index in stored emulsified chicken patties after 30 days of storage. These changes are probably associated with the action of lipases (basically lipoxygenases) and microbial activity, which act on small amounts of polyunsaturated fatty acids (Pateiro et al., 2015). Other researchers studied the effect of natural antioxidants during storage of sausages, Manzoor, Ahmad and Yousuf (2022), reported a reduction in the value of TBARS in chicken sausages formulated with mango epicarp extract after 15 days of storage. Schaefer et al. (2022) recorded a reduction of TBARS in sausages produced with dehydrated fruit from the Hovenia dulcis plant after 28 days of storage, the authors stated that the presence of tannins in the samples possibly delayed the lipid oxidation reactions. The presence of double bonds in fats favors their oxidation, leading to the formation of peroxides, aldehydes, ketones and alcohols, among other substances (Shahidi; Rubin; D'Souza, 1986). The oxidation of lipids becomes a determining factor since it intervenes in the formation of more than 80% of the volatile compounds of the flavor (Zhao et al., 2020). Normal oxidation leads to the formation of an approving taste, while over-oxidation leads to the development of unpleasant flavors and aromas, such as rancidity. For this reason, the regulation of the level of lipid oxidation is fundamental in the elaboration of processed meat products, and in storage, where the greatest changes are produced, linked to the transformation of intramuscular phospholipids, which undergo important alterations (Gong et al., 2017). Preliminary experiments showed that the sausages formulated with 25% lipid extract of papaya epicarp and 75% nitrites presented the lowest values of  $\Delta E$  (0.98 ± 0.20), showing that there are no differences in the perception of color against to the control (nitrite = 3.30 g / Kg). Pathare, Opara and Al-Said (2013) indicated that values of  $\Delta E < 1.5$  guarantee that there are no differences in color perception in two study samples. Table 5 shows the mean values of the physicochemical variables evaluated in the sausages after 30 days of storage. The color parameters  $C^*$ , h° and  $\Delta E$  change with storage time, on the contrary, the color attribute L\* was not statistically affected after the evaluated period (Table 5). The value of  $\Delta E = 1.51 \pm 0.28$  after storage shows that there are differences in color perception with respect to day zero,

as reported by the authors Pathare, Opara and Al-Said (2013) who indicate that values of  $\Delta E > 1.5$  shows differences in color perception in two study samples. Rubio et al. (2008) also report that the L\* coordinate was not significantly affected after 210 days of storage of fermented pork sausages. In another work developed by Dong et al. (2020) report that the surface color evaluated in vacuum-packed red Harbin-type sausages did not show significant differences during 12 days of storage at room temperature. (Dong et al., 2020). Hamzaoui et al. (2020) noted a slight decrease in luminosity (L\*) in beef sausages enriched with polysaccharides obtained from green algae, the authors attribute it to browning reactions. Contrary to what was obtained in this research, Buriti, Cardarelli and Saad (2008) showed an increase in luminosity (L\*) during 21 days of storage, the authors affirm that this increase was due to the coagulation of the protein, which leads to the release of water towards the surface and therefore promotes greater scattering of light. Regarding the increase in C\*, ho and  $\Delta E$  during the storage of the sausages (Table 5), these changes may be associated with enzymatic reactions, loss of moisture or the isomerization of carotenoids. Similar results were observed in the investigations carried out by Šojić et al. (2017), the authors affirm that the bioactive compounds (terpenes, phenolic compounds, carotenoids, etc.) of the vegetable extracts used in the preparation of the sausages act as promoters of the reaction between the nitrite present and the myoglobin of the muscle, promoting the formation of the pink pigment nitrosyl hemo chrome, which modifies the color coordinates, the saturation index and the hue angle. In the development of meat products, the presence of nitrites plays an important role, which basically consists of two actions: prolonging the useful life of the products, through the work they exert on microorganisms, and improving certain sensory characteristics such as they are color, aroma and flavor, in addition to contributing to retard lipid oxidation.

# CONCLUSIONS

The maximum extraction of total carotenoids (of  $66.03 \pm 0.60 \text{ mg }\beta$ -carotene / 100g) in the epicarp of papaya assisted by ultrasound is achieved when working at a temperature of 30 °C, a time of 60 min and a solid-liquid ratio 0.0064 g/ml oil. Lipid oxidation of sausages increased during storage, a situation that could generate changes in the aroma and flavor of this meat product. The carotenoid extracts obtained from the papaya epicarp could be a natural coloring additive in the production of frankfurters since it allows to preserve the color during storage.

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### **AUTHORS CONTRIBUTIONS**

Conceptual Idea: Ordoñez-Santos L.E.; Methodology design: Ordoñez-Santos L.E., Hleap-Zapata J.I.; Data collection: Velasco-Arango V.A.; Data análisis and interpretation: Ordoñez-Santos L.E., Velasco-Arango V.A., Hleap-Zapata J.I.; Writing and editing: Ordoñez-Santos L.E., Velasco-Arango V.A., Hleap-Zapata J.I.

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