An Acad Bras Cienc (2023) 95(4): e20191092 DOI 10.1590/0001-3765202320191092
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ECOSYSTEMS

Effect of hydrocolloid concentration in low-calorie orange jellies on preservation of bioactive compounds and antioxidant capacity

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Abstract: The purpose of this paper was to avaliate of the concentration of hydrocolloids (low methoxyl pectin [LMP], guar gum [GG], and carrageenan gum [CG]) in low-calorie orange jellies in order to maximize the amount of bioactive compounds and antioxidant capacity, and to study the influence on degradation these compounds. A mixture design with seven tests was used to analyze the total phenolic compounds, ascorbic acid (vitamin C) and antioxidant capacity (ABTS, DPPH and β-carotene/linoleic acid methods). The results were analyzed by response surface methodology and the Scott-Knott mean test at a significance level of 5% ($p \leq 0.05$). In general, the regions containing 0.5% GG and 0.5% GC had higher levels of the variables under study, and this combination preserved the bioactive compounds and antioxidant activity of jellies in relation to that of orange juice.

Key words: Health, degradation, mixture design, product.

INTRODUCTION

Citrus fruits are highly popular worldwide due to their high nutritional value, bioactive compounds (phenolics and ascorbic acid), antioxidant capacity, good taste, and widespread availability (Rasouli et al. 2019, Sun et al. 2019), where oranges are one of the most widely consumed citrus fruits (Escudero-López et al. 2015, Talens et al. 2017, Xia et al. 2019). Brazil is a major producer and processor of oranges (IBGE 2016, Albuquerque et al. 2019), mainly in the form of jellies (Lima et al. 2019).

Considering that jellies are an important alternative for the processing, utilization, and consumption of fruits, the market for fruit jellies has continued to expand due to the sensory acceptability, high value-added, and nutritional quality (Oliveira et al. 2016, Korus et al. 2017, Lima et al. 2019). However, there is a need for low-calorie products because of the increasing number of chronic non-communicable disease, such as diabetes and obesity (Horne et al. 2019). Therefore, low-calorie jellies represent a favorable and promising option for meeting the current needs of the population (Abolila et al. 2015).

In the preparation of low-calorie jellies, low-methoxyl pectin (LMP) is used, which forms a gel in the presence of divalent metal ions (usually calcium) and does not necessarily require the presence of sugars (Ngouémazong et al. 2012, Pereira et al. 2013, Lascol et al. 2016). However, this type of pectin does not impart the same rheological characteristics as that of the conventional product. Therefore, it is necessary to use other gelling agents such as carrageenan and guar gums (Moreira et al. 2011), as well as sweeteners and bulking agents (Pereira et al.

2017). Further, formation of the gel network is aided by the application of heat, but this may degrade the nutrients present in the fruits during processing, depending on the process severity (temperature and processing time) (Shinwari & Rao 2018). The loss of bioactive compounds during processing may be elevated or retarded by the product (jam/jelly) composition, including the presence of sugar, the type and concentration of pectin, the fruit and its cultivar, and pH (Beskow et al. 2015, Souza et al. 2015, Najafabadi et al. 2017, Shinwari & Rao 2018).

In this context, the aim of this study was evaluate the concentration of hydrocolloids in low-calorie orange jellies to obtain higher levels of bioactive compounds and enhance the antioxidant capacity, and to study the influence on degradation of these compounds.

MATERIALS AND METHODS

Processing of low-calorie orange jellies

Ripe oranges (Pera Rio cultivar) were obtained from a local market. The fruits were processed in the Sensory Analysis Laboratory in the Department of Food at the Federal University of Ouro Preto/MG. The fruits were washed under running water, sanitized in a 200 mg L^{-1} sodium hypochlorite solution for 15 min, selected, pulped in an electrical squeezer, and packed in low-density polyethylene bags and frozen at –18 °C. The following ingredients were used: sucrose (Alvinho, Governador Valadares, MG, Brazil), sucralose, acesulfame-k, polydextrose (Nutramax, Catanduva, SP, Brazil), potassium sorbate, low methoxyl pectin (LMP) (Rica Nata, Piracema, MG Brazil), guar gum (Pryme Foods, Sorocaba, SP, Brazil), and κ-carrageenan (Gastronomy Lab, Distrito Federal, DF, Brazil).

The low-calorie orange jellies with different formulations were processed in open stainless-steel pots according to the methodology proposed by Souza et al. (2012), with modifications. The mixture of orange juice (60%), sucrose (20%), and polydextrose (18.925%) was heated to 30 °Brix. The hydrocolloids (low methoxyl pectin, guar gum, and carrageenan gum) used in the formulation were dissolved in 5 mL of water and immediately added to the mixture containing a total of 1% gelling agents, according to the experimental design (Table I). The total quantity of hydrocolloids was defined by the previous tests. The cooking process was continued until a total soluble solids content of 60 °Brix was obtained. The sweeteners were added in the proportions prescribed by Souza et al. (2013) (0.01875% acesulfame-k and 0.00625% sucralose). Potassium sorbate (0.05%) was added at the end of the cooking process (diluted 1:1 in water at room temperature) to prevent degradation at the high processing temperature (above 85 °C). The low-calorie orange jellies were placed in previously sterilized glass jars, where filling was performed at a high temperature (85 °C). The containers were closed, cooled to room temperature, and stored in an incubator chamber at 25 °C for later analysis.

Determination of ascorbic acid (vitamin C)

Standard AOAC methodology (1984), modified by Benassi & Antunes (1998), was used for the determination of the ascorbic acid content.

Each sample studied (1 g) were diluted in 100 mL of 2% oxalic acid solution and a 25 mL aliquot was then titrated with 0.025% 2,6-dichlorophenolindophenol (DCFI) solution until a pink coloration was obtained; the solution was previously standardized with L-ascorbic acid solution. The procedure was performed in triplicate. The results are expressed as milligrams of the ascorbic acid per gram of fresh weight (mg/100 g f.w.).

Table I. Level and composition of hydrocolloids in formulation of jelly.

Formulations	Variables			Low methoxyl pectin (LMP) (%)	Guar gum (GG) (%)	Carrageenan gum (CG) (%)
	X_1	X_2	X_3			
F1	100	0	0	1	0	0
F2	0	100	0	0	1	0
F3	0	0	100	0	0	1
F4	50	50	0	0.5	0.5	0
F5	50	0	50	0.5	0	0.5
F6	0	50	50	0	0.5	0.5
F7	33	33	34	0.33	0.33	0.34

Obtaining extracts of samples for analysis of phenolic compounds and antioxidant capacity

The procedure for obtaining the extracts of orange jellies and orange juices was adapted from Larrauri et al. (1997). The sample (10 g) was weighed, and 40 mL of methanol/water solution (50:50 v/v) was then added. This solution was stirred (200 rpm) at room temperature for 60 min and allowed to stand in a cool (8 °C) environment for 30 min. The supernatant was then filtered, recovered, and transferred to a 100 mL flask. An acetone/water (40 mL; 70:30 v/v) mixture was then added to the residue while stirring (200 rpm) at room temperature for 60 min, and the mixture was allowed to stand in a cool (8 °C) environment for 30 min. After this time, the supernatant was transferred to a volumetric flask containing the first supernatant and the volume was made up to 100 mL with distilled water. This procedure was performed under light, and the extract was stored at –18 °C for a week.

Total phenolic compounds

The methodology adapted from Folin-Ciocalteu (Waterhouse 2002) was used for the determination of the total phenolic compounds in the orange juice and jellies. The extract (0.5 mL) was pipetted and transferred to test-tubes containing 2.5 mL of 10% (v/v) Folin-Ciocalteu reagent and 2.0 mL of 4% (w/v) sodium carbonate solution. The contents of the tubes were homogenized and then held for 120 min under light, and the absorbance was then determined at 750 nm. Absolute ethanol was used as the blank.

The total phenolic content was determined by interpolating the absorbance of the sample against the calibration curve constructed with gallic acid standards (5, 10, 15, 20, 30, and 40 μg mL^{-1}). The results were expressed as milligrams of gallic acid equivalent (GAE)/g f.w.

Antioxidant capacity

The antioxidant capacity was determined using the ABTS (2,2'-azino-bis-(3 ethylbenzenothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), and -carotene/linoleic acid methods. The method of Re et al. (1999) was used for the ABTS assay, with minor modifications. The ABTS radical cation ($ABTS^{+}$) was generated by the reaction of 5 mL of aqueous ABTS solution (7 mM) with 88 μL of 140 mM (2.45 mM final concentration) potassium persulfate. The mixture was kept in the dark for 16 h before use and then diluted with ethanol to obtain an absorbance of 0.7±0.05 units at 734 nm using a spectrophotometer. The jelly and orange juice

extract (30 µL), or a reference substance (Trolox - 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), were allowed to react with 3 mL of the resulting blue–green ABTS radical solution in the dark. The decrease in the absorbance at 734 nm was measured after 6 min. Ethanolic solutions of known Trolox concentrations were used for calibration. The results were expressed as micromoles of Trolox equivalents (TEs) per gram of fresh weight (µmol of TEs/g f.w.).

The DPPH-free radical scavenging capacity was estimated using the method reported by Brand-Williams et al. (1995). The DPPH solution (600 µM) was diluted with ethanol to obtain an absorbance of 0.7±0.02 units at 517 nm. Orange juice and jelly extracts (0.1 mL) were allowed to react with 3.9 mL of DPPH radical solution for 30 min in the dark, and the decrease in the absorbance of the resulting solution was monitored. The absorbance of the reaction mixture was measured at 517 nm. The results were expressed as EC_{50} (gram of fresh mass per gram of DPPH). EC_{50} expresses the minimum concentration of antioxidant required to reduce the initial DPPH concentration by 50%.

The antioxidant activity was also determined by the β-carotene/linoleic acid method, following the procedure reported by Marco (1968), with a few modifications. Briefly, an aliquot (50 µL) of the β-carotene chloroform solution (20 mg mL^{-1}) was added to a flask containing 40 µL of linoleic acid, 1.0 mL of chloroform, and 530 µL of Tween 40 and then mixed. The chloroform was evaporated using an oxygenator. After the evaporation process, oxygenated distilled water (approximately 100 mL) was added to obtain an absorbance of 0.65±0.5 units at 470 nm. An aliquot (0.4 mL) of Trolox solution (200 mg L^{-1}) or diluted jelly and orange juice extracts (200 mg L^{-1}) was added to 5 mL of the β-carotene solution and incubated in a water-bath at 40 °C. The measurements were performed after 2 min and 120 min at an absorbance of 470 nm using a spectrophotometer. The antioxidant activity was calculated as the percent protection relative to the control (% Protection = 100 -% Oxidation).

Experimental design and statistical analysis

In this study, a mixture design (Cornell 1983) was used to evaluate the effects the concentrations of LMP (X1), guar gum (X2), and carrageenan gum (X3), as indicators of the antioxidant capacity and bioactive compounds in the low-calorie orange jellies. The design and experimental levels for the three factors are presented in Table I.

Statistical analyses were based on the predicted model. The general model of the regression function was adjusted to the values of the variable answers. Those values have linear and non-linear terms according to Equation 1.

$$Y_1=B_1X_1+B_2X_2+B_3X_3+B_{12}X_1X_2+B_{13}X_1X_3+B_{23}X_2X_3 \quad \text{Eq. 1}$$

To evaluate the data adjustment, the analysis of variance (ANOVA) and the coefficient of determination (R^2) for each parameter were determined and analyzed by using Statistica software 8.0 (2007).

In addition, degradation of the components of the orange juice due to jelly processing was assessed according to the methodology proposed by Souza et al. (2015). The amount of each bioactive compound and the antioxidant activity of the jelly were corrected by considering the proportion of orange juice added (60%) and the concentration factor due to the evaporation process – the average jelly yield (75%).

Proportion of orange juice needed to produce 100 g of jelly equivalent to proportion of orange juice added×(100 g of jelly/jelly yield)

Considering these factors, the corrected value for each bioactive compound and the antioxidant activity after processing the orange juice to form the jelly were determined by dividing the respective initial value for the jelly

by a factor of 0.80 (equivalent 60% of juice/((100 g of jelly) x 0,75 (jelly yield)). Thereafter, the degradation percentage of each bioactive compound and antioxidant capacity were calculated according to Equation 2 (in module).

Degradation (%) = (amount present in orange juice – amount present after jelly processing) × 100/amount present in orange juice Eq. 2

The Scott–Knott mean test was used to verify if there was a difference between the samples at a significance level of 5% ($p \leq 0.05$) by using the Sisvar software (Ferreira 2014).

RESULTS AND DISCUSSION

Effect of the concentration of hydrocolloids to maximize bioactive compounds and antioxidant capacity

The bioactive compounds and antioxidant activity data were subjected to response surface methodology analysis using response surface regression (RSREG), and a predicted equation was developed for each attribute (Table II). A complete quadratic model was used to fit the dependent variables. All models presented R^2 values greater than 0.7 and significant ($p \leq 0.05$) regressions, indicating that they were suitable for the predictions (Henika 1982, Mehmood et al. 2018, Mehmood et al. 2019). Figure 1a to e shows the contour curves.

In general, the lowest quantity of bioactive compounds (total phenolics, ascorbic acid) and lowest antioxidant capacity (antioxidant capacity DPPH, antioxidant capacity, and β-carotene) were obtained in the region with 0.5% LMP and 0.5% GG (Figure 1a to e). Guar gum does not form a gel because the galactose residues present in its structure make it difficult for the chains to approach each other, thus preventing strong cohesion (Cubero et al. 2002). However, combining GG with gelling agents (locust bean gum and κ-carrageenan gum) promotes a synergistic effect depending on the concentration (Arda et al. 2009). In addition, according to Lima et al. (2019), the interaction of LMP and GG (in the concentrations of the present study) makes the orange jelly less viscous and more fluid, thus hindering the retention of water molecules. This may have contributed to the lower level of bioactive compounds and antioxidant activity (Souza et al. 2010, Borah et al. 2019).

Furthermore, higher values for the total phenolics, antioxidant capacity DPPH, antioxidant capacity β-carotene, and ascorbic acid were obtained in regions containing 0.5% CG and 0.5% GG, with the exception of the antioxidant capacity ABTS, which was higher

Table II. Predicted model of bioactive compounds and antioxidant activity data of low-calorie orange jellies.

	Predicted model	R^2 value
Total phenolics (mg GAEs/g f.w.)	$Y=0.08X_1 + 0.07X_2 + 0.08X_3 - 0.03X_1X_2 + 0.05X_1X_3 + 0.08X_2X_3$	0.84
Antioxidant capacity – ABTS (µmol/g f.w.)	$Y = 6.05X_1 + 1.06X_2 + 0.71X_3 - 12.08X_1X_2 + 0.15X_1X_3 - 1.51X_2X_3$	0.99
Antioxidant capacity – DPPH (EC_{50} – g f.w./g DPPH)	$Y = 67006.40X_1 + 51244.10X_2 + 64202.20X_3 + 264742.10X_1X_2 - 72740.70X_1X_3 - 42202.90X_2X_3$	0.72
Antioxidant capacity – β-carotene (% protection)	$Y = 46.37X_1 + 68.54X_2 + 48.52X_3 - 88.60X_1X_2 + 6.38X_1X_3 - 12.93X_2X_3$	0.83
Ascorbic acid (mg/100 g f.w.)	$Y = 317.58X_1 + 297.38X_2 + 226.71X_3 - 455.10X_1X_2 + 16.38X_1X_3 + 273.17X_2X_3$	0.98

X_1: Low methoxyl pectin (LMP); X_2: Guar gum (GG); X_3: Carrageenan gum (CG).

for jellies tending to 1% LMP. As previously mentioned, GG does not form gels, but it potentiates the action of gelling agents (Sharma et al. 2018), and because the gels formed by these two hydrocolloids (CG and GG) and LMP have better consistency (Lima et al. 2019), this effect may have contributed to the higher values of the variables under study.

Thus, to obtain low-calorie orange jellies with higher levels of bioactive compounds and antioxidant capacity, around 0.5% GG should be used in combination with 0.5% CG.

Influence of the concentration of hydrocolloids in degradation of bioactive compounds and antioxidant capacity

Table III shows the percentage degradation of the bioactive compounds and the decay of the antioxidant capacity of low-calorie orange jellies in relation to orange juice.

Loss of the bioactive compounds and the antioxidant capacity did not occur in a similar way (Table III).

The percentage degradation of the total phenolic compounds reached 55.92% in formulation F4 (0.5% LMP and 0.5% GG), which

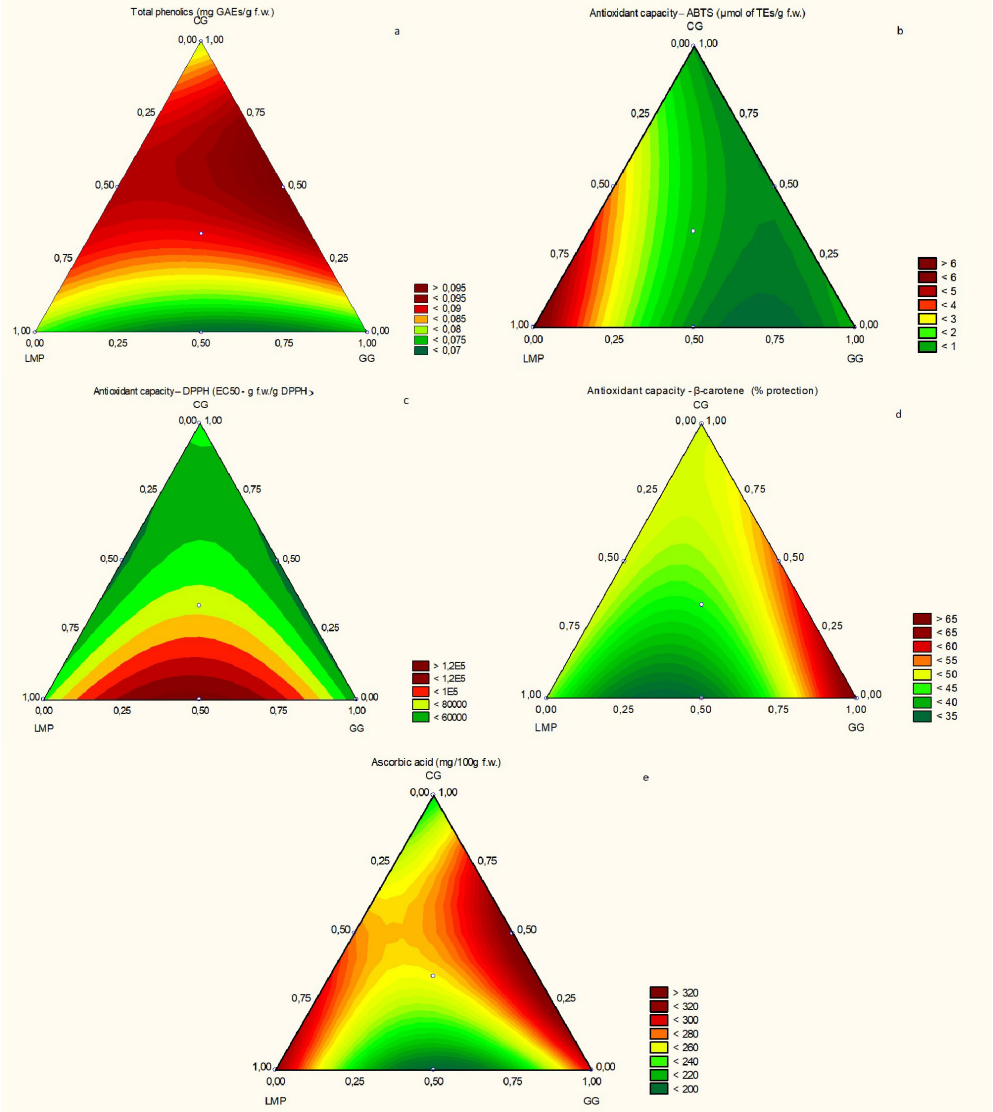

Figure 1. Contour plot for total phenolics (mg GAEs/g f.w.) (a); antioxidant capacity – ABTS (µmol/g f.w.) (b); antioxidant capacity – DPPH (EC50 – g f.w./g DPPH) (c); antioxidant capacity – β-carotene (% protection) (d), and ascorbic acid (mg/100 g f.w.) (e). LMP: low methoxyl pectin; GG: guar gum; CG: carrageenan gum.

is the highest value ($p \leq 0.05$) (Table III). The lowest level of degradation of this compound (around 39%) was obtained with formulations F6 (0.5% GG and 0.5% CG) and F7 (0.33% LMP, 0.33% GG, and 0.33% CG) ($p > 0.05$). Souza et al. (2015) also observed ~50% loss of phenolic compounds in blackberry jellies, and Savikin et al. (2009) observed less than 50% loss in berry jelly. Degradation of phenolic compounds may occur due to non-enzymatic reactions that occur when cell structures are disrupted and at high cooking temperatures combined with exposure to oxygen (Souza et al. 2015).

The ABTS method indicated higher degradation of the antioxidant activity (about 95%) for formulations F4 (0.5% LMP and 0.5% GG) and F6 (0.5% GG and 0.5% CG), with no significant difference at $p > 0.05$. The DPPH method indicated the highest degradation in the antioxidant activity for formulation F7 (0.33% LMP, 0.33% GG, and 0.33% CG) ($p \leq 0.05$). The β-carotene/linoleic acid method indicated the highest degradation for formulations F3 (1% CG) and F4 (0.5% LMP and 0.5% GG). The differences in the analytical results for degradation of the antioxidant capacity may be due to the methods of analysis, because the $ABTS^{\cdot+}$ method is limiting due to differences in the incubation time and the low selectivity of the radical in the reaction with hydrogen donor atoms, and the results may thus be divergent from those of other methodologies (Campos & Lissi 1997). The methodology based on the percentage lipid peroxidation is more sensitive to lipophilic antioxidant compounds, a fact that may restrict its use. Thus, this may be the cause of the difference in the results compared to those of the DPPH methodology (Brand-Williams et al. 1995). Koleva et al. (2002) compared three methodologies (DPPH, β-carotene/linoleic acid, and gas chromatography) for evaluating the antioxidant activity of extracts from different plants. The different methods produced divergent results in the determination of the antioxidant activity of the extracts due to differences in the polarity of the samples, where the DPPH method was sensitive regardless of the polarity.

However, formulations F3 (1% CG), F4 (0.5% LMP and 0.5% GG), and F7 (0.33% LMP, 0.33% GG, and 0.33% CG) suffered the highest losses of vitamin C. Vitamin C is very sensitive and several factors can trigger its degradation, including the

Table III. Average bioactive and the standard deviation compound degradation and antioxidant activity decay of orange juice due to processing in the form of jelly.

Formulation	Total phenolics (mg GAEs/g f.w.)	Antioxidant capacity – ABTS (μmol/g f.w.)	Antioxidant capacity – DPPH (EC_{50} – g f.w./g DPPH)	Antioxidant capacity – β-carotene (% protection)	Ascorbic acid (mg/100 g f.w.)
F1	45.58±6.16^{c}	21.50±2.06^{f}	44.92±0.68^{d}	26.14±3.22^{b}	33.73 ±6.04^{b}
F2	51.00±3.77^{b}	85.91±0.07^{c}	14.84±0.29^{f}	25.36±5.06^{b}	37.97±8.86^{b}
F3	47.76±0.00^{b}	90.29±0.07^{b}	39.06±0.32^{e}	35.33±3.22^{a}	52.77±7.44^{a}
F4	55.92±3.27^{a}	95.06±0.07^{a}	73.15±0.35^{c}	40.61±7.13^{a}	58.22±13.73^{a}
F5	42.86±3.27^{c}	57.89±1.17^{e}	77.54±0.23^{b}	12.57±4.37^{c}	40.88±10.24^{b}
F6	39.59±6.25^{d}	95.44±0.07^{a}	78.01±0.17^{b}	8.12±4.37^{c}	29.54±10.07^{b}
F7	36.32±3.26^{d}	81.22±0.20^{d}	95.33±1.39^{a}	9.35±0.54^{c}	49.59±15.14^{a}

Mean values with common letters in the same column indicate that there is no significant difference among samples ($p \leq 0.05$) from Scott-Knott's mean test.

presence of oxygen, light, and high temperatures (Zerdin et al. 2003, Roidoung et al. 2017).

The highest losses of bioactive compounds and antioxidant capacity occurred in the formulations containing GG alone (F2), in binary combinations of GG and LMP (F4), and in ternary combinations of LMP, GG, and CG (F7). As previously stated, GG does not form a gel and the gel formed by LMP and GG is more fluid, failing to trap water (Lima et al. 2019), which may have led to less protection of the compounds under study.

CONCLUSION

The low-calorie orange jelly formulations showed differences in relation to the bioactive compounds and the antioxidant activity. The mixture design technique was useful for evaluated effect the hydrocolloid concentration in these jellies in order to obtain higher levels of these compounds. In general, to obtain higher contents of the variables under study, it is necessary to use 0.5% guar gum and 0.5% gum carrageenan in the preparation of the jellies. In addition, this combination preserved the bioactive compounds and antioxidant activity of the jellies in relation to that of orange juice, except for the antioxidant capacity ABTS.

Acknowledgments

The authors thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG-APQ 02047-14) and Universidade Federal de Ouro Preto (UFOP) for financial support, and the authors are grateful for the donation of sweeteners from Nutramax®.

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How to cite

LIMA MB, SANTOS HV, BARBOSA JC, PENNA LO & PEREIRA PAP. 2023. Effect of hydrocolloid concentration in low-calorie orange jellies on preservation of bioactive compounds and antioxidant capacity. An Acad Bras Cienc 95: e20191092. DOI 10.1590/0001-3765202320191092.

Manuscript received on September 11, 2019; accepted for publication on January 7, 2020

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