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In vitro bioaccessibility of antioxidant compounds from structured fruits developed with gellan gum and agar[1]

Bioacessibilidade *in vitro* de compostos antioxidantes de frutas estruturadas desenvolvidas com goma gelana e ágar

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ABSTRACT - This study aims to evaluate the bioaccessibility of antioxidant compounds of structured fruits. Samples were prepared with 50% of each pulp (mango/caja, mango/cashew apple and mango/acerola), agar and gellan gum (low acyl-LA and high acyl-HA) in LA:HA ratios of: 100:0, 75:25 and 50:50, in a concentration of 0.75%. There was a reduction in the antioxidant compounds contents after *in vitro* digestion. The bioaccessible ascorbic acid levels ranged from 15.10% (LA100/HA0 mango/acerola) to 71.18% (LA50/HA50 mango/cashew apple); Total Extractable Polyphenols (TEP) ranged from 24.58% (mango/caja pulp) to 75.50% (LA75/HA25 mango/acerola); antioxidant activity ranged from 21.10% (LA75/HA25 mango/caja) to 51.05% (LA75/HA25 mango/acerola). Mango/acerola ascorbic acid bioaccessibility was lower and the mango/cashew apple HA gellan gum sample antioxidant activity was higher than pulp, probably due to temperature increasing at processing. It was concluded that the agar and gellan gum (HA and LA) hydrocolloids were able to contain these compounds in the production process of the structured and during digestion, which proves the similarity of structured fruits with fresh pulps.

Key words: *In vitro* digestion. Antioxidants. Hydrocolloids.

RESUMO - Este estudo tem como objetivo avaliar a bioacessibilidade de compostos antioxidantes de frutas estruturadas. As amostras foram preparadas com 50% de cada polpa (manga/cajá, manga/caju e manga/acerola), ágar e goma de gel (baixo acil-LA e alto acil-HA) em relações LA:HA de: 100:0, 75:25 e 50:50, em uma concentração de 0,75%. Houve redução no conteúdo de compostos antioxidantes após a digestão *in vitro*. Os níveis de ácido ascórbico bioacessível variaram de 15,10% (LA100/HA0 manga/acerola) a 71,18% (LA50/HA50 manga/caju); os Polifenóis Extraíveis Totais (PET) variaram de 24,58% (manga/polpa de cajá) a 75,50% (LA75/HA25 manga/acerola); a atividade antioxidante variou de 21,10% (LA75/HA25 manga/cajá) a 51,05% (LA75/HA25 manga/acerola). A bioacessibilidade de ácido ascórbico da manga/acerola estruturada foi menor e a atividade antioxidante da amostra goma gelana HA manga/caju foi maior em relação à polpa, possível resultado da elevação de temperatura aplicada no processamento. Concluiu-se que os hidrocoloides ágar e goma gelana (HA e LA) foram capazes de reter esses compostos no processo de produção do estruturado e durante a digestão, o que comprova a semelhança das frutas estruturadas com polpas frescas.

Palavras-chave: Digestão *in vitro*. Antioxidantes. Hidrocolóides.

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INTRODUCTION

Structured fruits are food products obtained from a mixture of fruit pulps and hydrocolloids, aiming to increase the period of consumption of fruits and reproduce fruit succulence. These products may grant consumption of exotic fruits in non-traditional regions or increase the consumption availability of seasonal fruits, preserving the nutritional and sensorial traits of fresh fruits and pulps (PARN *et al*., 2015). The hydrocolloids participate in the macroscopic structure of food, exerting considerable influence on their sensory and nutritional qualities (DICKINSON, 2011). Agar and gellan gum may be used to structure pulps due to their properties related to moisture decreasing, gelification, and encapsulation ability (COSTA *et al*., 2020).

Gellan gum is a product of the fermentation of monosaccharides, such as glucose, by a bacterium called *Sphingomonas elodea* (WUSTENBERG, 2015). It is found as high acyl (HA), showing acyl groups in its structure, as well as low acyl (LA), which in turn does not have the acyl groups. The presence of acyl groups in gellan gum structure result in soft, elastic, transparent and flexible gels without significant thermal hysteresis, while its absence forming hard, brittle and non-elastic gels besides of exhibiting enough thermal hysteresis (IMESON, 2010). Some applications of HA and LA gelan gum are edible coatings, dairy and vegetable beverage stabilizers, microencapsulation of probiotics, and structuring of fruit pulps (COSTA *et al*., 2020; DANALACHE *et al*., 2016; KIANI; MOUSAVI; MOUSAVI, 2010; SHERAFATI *et al*., 2013; XU *et al*., 2019).

Agar is a structural polysaccharide obtained from the cell wall of red algae (Rhodophyceae), is thermo-reversible, and gels at temperatures of 30 to 40 °C. It may form strong gels subject to high syneresis due to the intense aggregation of double helices (STEPHEN; PHILLIPS; WILLIAMS, 2006). These hydrocolloids have excellent entrapment properties of substances, such as bioactive compounds, avoiding their degradation (COSTA *et al*., 2017). Agar has been used in the encapsulation of substances, stabilization of beverages, edible coatings, structuring of various foods such as fruit pulps and pasta (COSTA *et al*., 2020; KAVOOSI *et al*., 2018; PADALINO *et al*., 2013; ZHAO *et al*., 2018; ZIEDAN *et al*., 2018).

Bioactive compounds such as phenolic compounds, ascorbic acid, carotene, among others, and the antioxidant activity of foods are widely studied and discussed (KIM *et al*., 2018; LIU *et al*., 2020; PEANPARKDEE; PATRAWART; IWAMOTO, 2020). However, few studies address the effects of gastrointestinal digestion on these components. The antioxidant content of food does not represent the amount absorbed by the body, as these compounds are subjected to different chemical, physical, biochemical and gut microbiota conditions during their passage through the gastrointestinal tract, which can alter their properties and biological activity (MOSELE *et al*., 2016). Therefore, it is of great importance to perform *in vitro* digestion assays as a way of simulating the physiological conditions which occur during human gastrointestinal digestion from the mouth to the intestine, and thus evaluate the bioaccessibility of the antioxidant compounds present in foods (LAFARGA *et al*., 2019; LIMA *et al*., 2017).

However, no studies to date have shown the influence of agar and gellan (high and low acylation) hydrocolloids on the bioaccessibility of bioactive compounds in fruit pulps. Thus, the objective of this study was to evaluate the *in vitro* bioaccessibility of the antioxidant capacity of structured mixed tropical fruits containing LA and HA gellan gum, as well as agar.

MATERIAL AND METHODS

Chemicals and reagents

The $ABTS^+$ radical (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), Folin-Ciocalteau reagent, gallic acid, ascorbic acid, 2,2'-bipyridyl, ferric trichloride, trichloroacetic acid, phosphoric acid, pancreatin, pepsin, and bile salts were purchased from Sigma Aldrich (Saint Louis, USA). LA gellan gum (Kelcogel® F, Atlanta, USA) and HA gellan gum (Kelcogel® LT, Atlanta, USA) were purchased from CP Kelco (Wilmington, USA). Agar was obtained from Sosa® (Barcelona, Spain).

Mixed structured fruits elaboration

The mixed structured fruits were prepared using frozen mango, caja, cashew apple, and acerola pulps supplied by a fruit pulp processing company located in the city of Fortaleza-CE, Brazil (latitude: 0° 45' 47" S, longitude: 380 31' 23" W). Based on a sensory test of ordering and preference performed by Leal *et al*. (2016), two different pulps in the proportion of 50% of each were used for preparing the mixed pulps (control samples) resulting in the following combinations: mango with caja, mango with cashew apple, and mango with acerola.

The structured mixed fruits were elaborated by adding agar hydrocolloids, LA and HA gellan gum in the LA/HA ratios of LA100/HA0, LA75/HA25 and LA50/HA50 (w/w), using the previously prepared mixed pulps, maintaining a total hydrocolloid concentration of 0.75% relative to the weight of the pulp. The mixtures were then homogenized and heated (88 °C/60 s) in a food processor (Termomix, model SPM-018, brand Yammi) and subsequently poured into rectangular silicone molds (width x height x length = 27 x 10 x 50 mm) and refrigerated at 5 °C for 12 h until the analysis, according to the methodology described by Costa *et al*. (2020). The mixed pulp and structured mixed fruit samples were prepared in three replicates.

In vitro gastrointestinal digestion (IGD)

The procedures for performing the digestion as well as the preparation of simulated gastric and intestinal fluids followed the methodology described by Miller *et al*. (1981) and Lima *et al*. (2014). This method consists of two sequential states: (i) gastric (pH 2.0, pepsin) and (ii) intestinal (pH 7.5, pancreatin and bile). The dialyzed fluids obtained from the simulated gastrointestinal digestion were used to perform the analysis.

Ascorbic acid content

The determination of the ascorbic acid content of the samples (mixed pulps, structured fruits and gastric and intestinal fluids) was performed through the titrimetric and spectrophotometric methods.

The titrimetric method followed the methodology based on reducing the indicator 2.6-dichlorophenolindofenol to 0.02% (DFI) in solution containing the sample diluted in 50 mL of 0.5% oxalic acid (INSTITUTO ADOLFO LUTZ, 2008), with the ascorbic acid content being expressed as mg ascorbic acid per 100 g of sample.

In turn, the spectrophotometric method was based on the iron reduction potential according to Chen and Wang (2002). The reading was carried out at 525 nm. Thus, a standard curve of ascorbic acid (0 to 10 μmoL) was used to quantify the ascorbic acid and the results were expressed as mg ascorbic acid per 100 g of sample.

The bioaccessible percentage of ascorbic acid was calculated according to Briones-Labarca *et al*. (2011) (Eq. 1).

$$\text{Bioaccessible\%} = 100 \text{ x } (\text{A/B}) \qquad (1)$$

Where A - ascorbic acid content of the dialysate (mg/100 g), B - ascorbic acid content of the sample (mg/100 g).

Total extractable polyphenol (TEP) determination

Extracts for total extractable polyphenol (TEP) content and total antioxidant activity (TAA) assays were prepared from mixed pulps and structured fruits, according to Larrauri, Rupérez and Saura-Calixto (1997), with adaptations. The samples were homogenized with 50% ethanol solution and kept at rest for 1 h in the absence of light, then centrifuged and the supernatant collected. The precipitate was then homogenized with 70% acetone solution and subjected to the same conditions as above. After centrifugation, the supernatant was collected and added to that obtained in the first step of the process.

The TEP were determined by the Folin-Ciocalteu method (OBANDA; OWUOR, 1997) by reading the extracts and dialysed in a spectrophotometer at 700 nm. The results were expressed as mg of gallic acid equivalent (GAE)/100 g of sample.

The bioaccessible percentage was calculated according to Briones-Labarca *et al*. (2011) (Eq. 2):

$$\text{Bioaccessible\%} = 100 \text{ x } (\text{A/B}) \qquad (2)$$

Where: A is the phenolic compound content of the dialysate (mg GAE/100 g), B is the total phenolic compound content of the sample, structured fruit or mix pulp (mg GAE/100 g).

Total antioxidant activity (TAA)

The determination of TAA by the $ABTS^+$ method was performed by reading the dialysates and extracts (as described above) in a spectrophotometer at 734 nm, according to the methodology described by Re *et al*. (1999) and adapted by Rufino *et al*. (2010). The results were expressed in the μM Trolox/g sample. The bioaccessible percentage was calculated according to Briones-Labarca *et al*. (2011) (Eq. 3):

$$\text{Bioaccessible\%} = 100 \text{ x } (\text{A/B}) \qquad (3)$$

Where: A corresponds to the antioxidant activity of the dialysate (μM/g), B the total antioxidant activity of the sample (μM/g).

Statistical analysis

The study was conducted in a completely randomized design with three replicates of each experiment. The results obtained from the analysis of mixed pulp and structured fruits were submitted to analysis of variance (ANOVA) and compared using the Tukey and Dunnet test at the level of 5% probability. To determine whether the bioactive compounds (ascorbic acid and TEP) of the structured and mixed pulp samples contributed to the antioxidant capacity, Pearson’s correlation coefficients were calculated at 5% probability using the Student’s t test for all variables. All data were analyzed using the XLSTAT program version 0.7.

RESULTS AND DISCUSSION

Bioaccessibility of ascorbic acid content

The acid ascorbic results, by the titration method, before and after the IGD of the pulps and structured fruits samples, as well as the bioaccessibility percentages, are shown in Table 1.

Table 1 - Bioaccessibility of ascorbic acid content by the titration method in mixed fruit pulps and structured mixed fruits with different hydrocolloids

Sample	Ascorbic acid (mg/100 g)		Bioaccessibility (%)
	Native	Bioaccessible	
Mango with caja			
Mixed pulp	19.62 ± 0.01 A	13.06 ± 3.22 B	66.61 ± 16.46
Agar	22.42 ± 0.02* aA	13.08 ± 3.24 aB	58.35 ± 14.54 a
LA100/HA0	19.61 ± 0.01 bA	13.07 ± 3.26 aB	66.64 ± 16.58 a
LA75/HA25	22.42 ± 0.02* aA	14.93 ± 3.21 aB	66.58 ± 14.33 a
LA50/HA50	19.62 ± 0.02 bA	13.96 ± 3.87 aB	71.18 ± 19.85 a
Mango with cashew apple			
Mixed pulp	92.51 ± 0.05 A	35.60 ± 1.55 B	38.49 ± 1.66
Agar	105.80 ± 1.81* aA	37.37 ± 0.02 aB	35.33 ± 0.59 a
LA100/HA0	100.76 ± 1.83* bA	39.18 ± 3.11 aB	38.88 ± 2.79 a
LA75/HA25	104.82 ± 0.06* abA	37.36 ± 0.04 aB	35.64 ± 0.05 a
LA50/HA50	105.79 ± 1.85* aA	32.06 ± 0.03 aB	30.31 ± 0.56* a
Mango with acerola			
Mixed pulp	536.74 ± 0.59 A	101.74 ± 0.23 B	18.96 ± 0.03
Agar	533.20 ± 8.94 bA	88.45 ± 4.67* aB	16.58 ± 0.70* a
LA100/HA0	569.71 ± 9.29* aA	86.00 ± 0.21* aB	15.10 ± 0.24* a
LA75/HA25	539.85 ± 1.57 bA	91.43 ± 4.45* aB	16.94 ± 0.85* a
LA50/HA50	538.92 ± 2.57 bA	86.10 ± 0.17* aB	15.93 ± 0.01* a

Means followed by at least one different lower case letter in the same column, in the same flavor, and averages followed by at least one different capital letter on the same line differ significantly at the 5% probability level by the Tukey test ($p \leq 0.05$). * Averages in the same column, in the same flavor, present a significant difference in comparison to the respective mixed pulp at the 5% probability level by the Dunnett test ($p \leq 0.05$)

Before *in vitro* gastrointestinal digestion (IGD), the ascorbic acid content of the structured fruits of the three pulps remained statistically equal to or even higher than the mixed pulps by the titration method (Table 1). Mango with cashew apple formulation stood out due to ascorbic acid contents of 100.76 to 105.80 mg/100 g, being superior to that of the mango with cashew apple mixed pulp (92.51 mg/100 g). It is suggested that the hydrocolloids used may have protected the ascorbic acid content, preventing its degradation. Studies have demonstrated the excellent trapping properties of agar and gellan gum substances, which make them widely used as encapsulating agents, avoiding undesirable degradation of compounds (COSTA *et al*., 2017; LAM *et al*., 2014; LEÓN; ROJAS, 2007; XU *et al*., 2019).

Furthermore, the results show that the use of different hydrocolloids promoted a significant difference in ascorbic acid content before IGD for agar and LA75/HA25 of the mango with caja, being higher than LA100/HA0 and LA50/HA50. In the mango with cashew apple structured, the agar and LA50/HA50 formulations presented higher values than LA100/HA0. However, in the mango with acerola, the sample containing agar (533.20 mg/100 g) had lower ascorbic acid content than LA100/HA0 (569.71 mg/100 g). This can be justified by the polysaccharides behavior in different food matrices and how their components such as pH may affect the gel traits, which require further studies to explain the interactions into these food matrices. In a study with gellan gels and agar, Tiwari, Chakkaravarthi and Bhattacharya (2015) concluded that the addition of ingredients such as mango pulp, $FeSO_4$, sucrose, whey protein concentrates and flax powder differently interfered in the characteristics of the formed gels, improving or impairing their properties.

There was a significant reduction in the amounts of ascorbic acid in all three pulps and structured mixed fruits after IGD (Table 1), in which the values ranged from 15.10% (LA100/HA0 mango with acerola) to 71.18% (LA50/HA50 mango with cashew apple). This reduction was expected, according to bioaccessibility values of other studies, ranging from 10 to 80% em different plant matrices (CARBONELL-CAPELLA *et al*., 2015; COSTA *et al*, 2021; RODRÍGUEZ-ROQUE *et al*., 2015). This can be justified by the low stability of this component in high pH values of the intestinal phase simulation (7.5), causing its loss (CARBONELL-CAPELLA *et al*., 2015).

All mango with acerola structured samples presented lower bioaccessible contents (15.10 a 16.94%), differing significantly from the mixed pulp (18.96%). This is believed to have occurred as a result of chemical reactions between the anthocyanins and acerola ascorbic acid, which may undergo condensation during processing (DE ROSSO; MERCADANTE, 2007). Thus, there were no significant differences between bioaccessible percent values of samples using different hydrocolloids, suggesting similar behavior to agar and gellan gum during the IGD.

The acid ascorbic results, by the spectrophotometric method, before and after the IGD of the pulps and structured fruits samples, as well as the bioaccessibility percentages, are shown in Table 2.

It was not possible to detect ascorbic acid after gastrointestinal digestion of the mango with caja samples by the spectrophotometric method (Table 2). This was probably due to the low content of this component in the digested samples, thus making it impossible to calculate the bioaccessibility. Sanches *et al*. (2018) presented 10.5 mg/100 g as the lowest value of ascorbic acid, at studying seriguela senescence. Nevertheless, this present research had 8.36 mg/100 g as the lowest value, suggesting lower ascorbic acid values after IGD, since, in general, the results in the spectrophotometric method were lower than those obtained in the titrimetric method.

A significant reduction of ascorbic acid was observed after the IGD, yielding from 8.03 (LA50/HA50) to 12.38% (agar) in the mango with cashew apple, and from 17.69 (LA100/HA0) to 23.56% (mixed pulp) in the mango with acerola. These results of ascorbic acid bioaccessibility are coherent as per data previously reported (10 to 80 %) (CARBONELL-CAPELLA *et al*., 2015; COSTA *et al*, 2021; RODRÍGUEZ-ROQUE *et al*., 2015), although mango/cashew apple samples have presented lower results in this method in comparison with titrimetry (30.31 a 38.88%). However, in the Tillmans titration method, several compounds can act as interferents, such as iron, copper and tin ions, pigments, tannins, cysteine, metabisulfite and other reducing substances, which react with 2,6-dichlorophenolindophenol, which can result in values overestimated (BALL, 2006; HOEHNE; MARMITT, 2019).

Bioaccessibility of Total Extractable Polyphenols (TEP)

TEP results of pulps, structured fruits, and their bioaccessibility are presented in Table 3.

Before IGD, it was observed that all samples of structured mixed mango with caja (186.35 to 259.06 mg GAE/100 g) and mango with cashew apple (192.75 to 258.08 mg GAE/100 g) had lower polyphenol contents

Table 2 - Bioaccessibility of ascorbic acid content by the spectrophotometric method in mixed fruit pulps and structured mixed fruits with different hydrocolloids

Sample	Ascorbic acid (mg/100 g sample)		Bioaccessibility (%)
	Native	Bioaccessible	
Mango with cashew apple			
Mixed pulp	97.97 ± 3.27 A	11.48 ± 0.99 B	11.70 ± 0.62
Agar	104.01 ± 9.17 aA	12.81 ± 2.12 aB	12.38 ± 2.41 a
LA100/HA0	100.09 ± 5.54 aA	10.89 ± 1.64 aB	10.93 ± 1.99 a
LA75/HA25	102.99 ± 11.12 aA	10.84 ± 0.13 aB	10.60 ± 1.04 a
LA50/HA50	104.80 ± 11.27 aA	8.36 ± 1.85 aB	8.03 ± 1.84 a
Mango with acerola			
Mixed pulp	447.55 ± 22.60 A	105.13 ± 11.30 B	23.56 ± 3.08
Agar	452.06 ± 47.67 aA	83.31 ± 5.54 aB	18.59 ± 2.62 a
LA100/HA0	480.18 ± 39.02 aA	84.05 ± 9.57 aB	17.69 ± 3.49 a
LA75/HA25	421.83 ± 45.00 aA	82.14 ± 1.35 aB	19.65 ± 2.64 a
LA50/HA50	443.01 ± 15.47 aA	85.84 ± 18.11 aB	19.48 ± 4.69 a

Means followed by at least one different lower case letter in the same column in the same flavor, and averages followed by at least one different capital letter on the same line differ significantly at the 5% probability level by the Tukey test ($p \leq 0.05$). * Averages in the same column, in the same flavor, present a significant difference in comparison to the respective mixed pulp at the 5% probability level by the Dunnett test ($p \leq 0.05$)

Table 3 - Bioaccessibility of total extractable polyphenols (TEP) in mixed fruit pulps and structured mixed fruits with different hydrocolloids

Sample	TEP (mg gallic acid/100 g sample)		Bioaccessibility (%)
	Native	Bioaccessible	
Mango with caja			
Mixed pulp	259.06 ± 17.97 A	63.36 ± 2.66 B	24.58 ± 2.65
Agar	196.43 ± 14.60* aA	57.54 ± 3.56 aB	29.45 ± 3.58 a
LA100/HA0	189.62 ± 10.24* aA	52.72 ± 3.43* aB	27.89 ± 2.91 a
LA75/HA25	191.95 ± 13.98* aA	54.65 ± 4.53* aB	28.46 ± 0.74 a
LA50/HA50	186.35 ± 19.34* aA	55.26 ± 4.04 aB	27.98 ± 5.59 a
Mango with cashew apple			
Mixed pulp	258.08 ± 6.62 A	89.57 ± 1.88 B	34.72 ± 1.27
Agar	223.51 ± 16.55* aA	82.80 ± 8.59 aB	36.99 ± 1.11 a
LA100/HA0	202.23 ± 10.71* aA	80.93 ± 11.35 aB	40.13 ± 6.36 a
LA75/HA25	192.75 ± 23.46* aA	88.65 ± 5.65 aB	46.35 ± 4.96* a
LA50/HA50	198.47 ± 5.07* aA	75.44 ± 2.07 aB	38.01 ± 0.37 a
Mango with acerola			
Mixed pulp	745.72 ± 19.63 A	445.95 ± 15.62 B	59.79 ± 0.53
Agar	694.20 ± 55.23 aA	431.15 ± 62.20 aB	62.72 ± 12.75 a
LA100/HA0	709.58 ± 35.22 aA	452.84 ± 2.23 aB	63.92 ± 3.18 a
LA75/HA25	645.64 ± 48.96 aA	469.41 ± 20.91 aB	75.50 ± 2.60 a
LA50/HA50	704.78 ± 63.57 aA	471.18 ± 44.70 aB	67.15 ± 8.00 a

Means followed by at least one different lower case letter in the same column in the same flavor, and averages followed by at least one different capital letter on the same line differ significantly at the 5% probability level by the Tukey test ($p \leq 0.05$). *Averages in the same column, in the same flavor, present a significant difference in comparison to the respective mixed pulp at the 5% probability level by the Dunnett test ($p \leq 0.05$)

than the mixed pulp, indicating a reduction of these components after processing. The reduction of PET may be related to heating application during structured fruits processing (GĄSECKA *et al*., 2020). Also, it is suggested that the fruit pulp polyphenols established connections with the hydrocolloids, thus reducing their extraction capacity. Padayachee *et al*. (2012) observed that phenolic compounds and pectin and cellulose carbohydrates chemically bind through hydrogen bonds and van der Waals forces.

This reduction was not perceived in mango/acerola samples due to the structured fruits had PET values statistically equal to mixed pulps (745.72 mg GAE/100 g). This may be justified by the high bioactive potential of acerola, already extensively reported in the literature (CHANG; ALASALVAR; SHAHIDI, 2018; XU *et al*., 2020).

There was a significant reduction in the TEP content after the IGD, with bioaccessibility varying from 24.58 (mixed pulp mango with caja) to 75.50% (LA75/HA25 mango with acerola) (Table 3). Several studies have reported significant reductions in TEP values after the *in vitro* gastrointestinal digestion (BERNARDES *et al*., 2019; COSTA *et al*., 2021; MA *et al*., 2021; SILVA *et al*., 2018). According to Lopes Neto *et al*. (2017), there may be a variation of 30 to 100% in the phenolic bioaccessibility in plant matrices. These reductions in TEP bioaccessibility may be related to several factors, such as these compounds degradation or conversion due to digestive enzymes and pH variation during digestion (GUERGOLETTO *et al*., 2016; LIMA *et al*., 2014; LOPES NETO *et al*., 2017), and TEP interaction with other macromolecules such as hydrocolloides, fibers, celluloses, and other compounds with bondering ability with phenolics (JAKOBEK, 2015).

There was a significant reduction in bioaccessibility, but it is important to emphasize that the mango/acerola formulation had percentages above 50%, both in the pulp and in the structured fruit. Thus, compared to others, the TEP contents remained high, which suggests that these pulps structuring greatly preserved the phenolic composition during *in vitro* digestion.

Thus, it was also observed that there were no significant differences in bioaccessibility between the structured fruit samples, suggesting the agar and gellan gums (LA and HA) did not interfere in TEP bioaccessibility.

Antioxidant activity

Native and bioaccessible results of antioxidant activity are shown in Table 4.

Regarding the results obtained before the IGD, there was a small decrease in the antioxidant activity values after thermal processing. However, this reduction was not significant in most formulations, and the structured fruits were not statistically different from the pulp, except some samples of mango with cashew apple with agar (7.43 µM/g), LA75/HA25 (6.85 µM/g) and LA50/HA50 (7.03 µM/g), in which there was a significant reduction. It is believed that the small decrease in the antioxidant capacity of the structured fruits in relation to pulp, although not significant, occurred due to the decrease in the polyphenol contents in preparing the product, as research shows a high correlation of the antioxidant activity with polyphenols (ALMEIDA *et al*., 2011; MOO-HUCHIN *et al*., 2014).

In comparing the formulations with each other (agar, LA100/HA0, LA75/HA25 and LA50/ A50), all of the mango with caja and mango with acerola were statistically the same. However, LA100/HA0 formulation (8.31 µM/g) of mango with cashew apple showed higher antioxidant activity than LA75/HA25 (6.85 µM/g) and LA50/HA50 (7.03 µM/g).

It was observed that there was a significant reduction in the antioxidant capacity of the samples after the IGD. Therefore, bioaccessibility varied from 21.10 (LA75/HA25) to 35.21% (mixed pulp) in the mango with cajá samples, from 36.57 (LA100/HA0) to 50.08% (LA75/HA25) in mango with cashew apple, and from 40.01 (agar) to 51.05% (LA75/HA25) in mango with acerola. This decrease in the antioxidant capacity is related to interactions between antioxidant compounds and food matrices such as polysaccharides, fibers, cellulose, and conditions during IGD, such as digestive enzymes action and pH changes, resulting in degradation and uncomplete release of phenolic compounds (SCHULZ *et al*., 2017; YÜCETEPE; ALTIN; ÖZÇELIK, 2021).

Table 4 - Bioaccessibility of antioxidant activity in mixed fruit pulps and structured mixed fruit with different hydrocolloids

Sample	Antioxidant activity (µM Trolox/g sample)		Bioaccessibility (%)
	Native	Bioacessible	
Mango with caja			
Mixed pulp	7.42 ± 0.02 A	2.61 ± 0.35 B	35.21 ± 4.71
Agar	7.40 ± 0.34 aA	1.81 ± 0.37* aB	24.59 ± 6.04 a
LA100/HA0	6.56 ± 0.41 aA	1.46 ± 0.29* aB	22.19 ± 3.12* a
LA75/HA25	6.60 ±0.72 aA	1.39 ± 0.29* aB	21.10 ± 3.67* a
LA50/HA50	6.66 ± 1.10 aA	1.82 ± 0.16 aB	25.13 ± 3.73 a
Mango with cashew apple			
Mixed pulp	9.07 ± 0.27 A	3.34 ± 0.48 B	36.93 ± 6.48
Agar	7.43 ± 0.19* abA	2.85 ± 0.46 aB	38.29 ± 5.85 bc
LA100/HA0	8.31 ± 0.87 aA	3.02 ± 0.46 aB	36.57 ± 5.59 c
LA75/HA25	6.85± 0.02* bA	3.43 ± 0.06 aB	50.08 ± 0.73* a
LA50/HA50	7.03 ± 0.35* bA	3.36 ± 0.11 aB	47.88 ± 2.27 ab
Mango with acerola			
Mixed pulp	24.80 ± 1.54 A	12.09 ± 0.99 B	48.74 ± 2.50
Agar	23.13 ± 0.76 aA	9.26 ± 2.06 aB	40.01 ± 8.43 a
LA100/HA0	24.12 ± 0.87 aA	10.57 ±0.15 aB	43.89 ± 1.86 a
LA75/HA25	22.56 ± 1.39 aA	11.86 ± 0.89 aB	51.05 ± 2.15 a
LA50/HA50	22.12 ± 2.25 aA	10.86 ± 1.34 aB	49.08 ± 3.54 a

Means followed by at least one different lower case letter in the same column in the same flavor, and averages followed by at least one different capital letter on the same line differ significantly at the 5% probability level by the Tukey test ($p \leq 0.05$). * Averages in the same column, in the same flavor, present a significant difference in comparison to the respective mixed pulp at the 5% probability level by the Dunnett test ($p \leq 0.05$)

Structured fruit samples presented bioaccessibility percentual similar to pulps, except for LA100/HA0 and LA75/HA25 (results lower than pulps), and LA75/HA25 (results higher than pulps), both from mango/cajá. This implies that different food matrix interactions interfere significatively in antioxidant activity bioaccessibility. In comparing the structured formulations with each other, the hydrocolloids did not interfere this parameter, except in the structured mango cashew apple, the LA75/HA25 formulation differed from agar and LA100/HA0 because of its higher bioaccessibility, and the LA100/HA0 sample had a lower bioaccessible value than LA50/HA50 for this parameter. The higher bioaccessibility of formulations with HA gellan may be related to this hydrocolloid forms weaker gel chains than LA gellan and agar, providing higher antioxidant compounds release after IGD (ZIA *et al*., 2018).

Correlation analysis

Through Pearson's correlation analysis (Table 5) was observed for all studied samples (mixed pulp and structured fruit) a high positive correlation among total antioxidant activity and content of TEP ($r = 0.935$ to 0.973, $p \leq 0.05$) and ascorbic acid by the titrimetric ($r = 0.935$ to 0.973, $p \leq 0.05$) and spectrophotometric methods ($r = 0.942$ to 0.973, $p \leq 0.05$). The results suggest that the bioactive compounds found in the samples are associated with their antioxidant capacity. In a study by Rufino *et al*. (2010) was also observed a high correlation between antioxidant activity by $ABTS^+$ method and ascorbic ($r = 0.70$) and TEP ($r = 0.92$) content in tropical fruits pulp.

Table 5 - Pearson correlation between total antioxidant activity by the $ABTS^+$ method and the bioactive compounds of mixed pulps and structured mixed fruits samples before and after gastrointestinal digestion *in vitro*

Variable	Coefficient of correlation (r)
	ABTS+
Mango with caja	
TEP	0.964*
Ascorbic acid (titrator)	0.969*
Mango with cashew apple	
TEP	0.967*
Ascorbic acid (titrator)	0.935*
Ascorbic acid (spectrophotometry)	0.942*
Mango with acerola	
TEP	0.957*
Ascorbic acid (titrator)	0.973*
Ascorbic acid (spectrophotometry)	0.973*

*Significant at the 5% probability level ($p \leq 0.05$). TEP: total extractable polyphenols

CONCLUSIONS

1. Bioactive compound contents and antioxidant activity of mixed pulps and structured fruits varied after the IGD. On the other hand, the formulations presented bioaccessibility percentual similar to mixed pulps, although there are some variations in the bioaccessible fractions in some samples such as mango/acerola ascorbic acid and mango/cashew apple HA gellan gum, suggesting the food matrix interference in the results. Furthermore, the bioaccessibility reduction in some samples may be related to heating application in the structured fruits processing. Further studies are necessary to confirm the hypothesis and explain mechanisms involved during this product digestion;
2. Thus, the hydrocolloids agar and LA and HA gellan gums were capable of retaining the bioactive compounds during the gastrointestinal *in vitro* digestion, which shows the similarity between structured fruits and fresh pulps, being a great option to diversify this food consumption.

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