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Bioaccessibility assay, antioxidant activity and consumer-oriented sensory analysis of *Beta vulgaris* by-product encapsulated in Ca(II)-alginate beads for different foods

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ABSTRACT

Bioaccessibility analysis and antioxidant activity along *in vitro* digestion and a consumer-oriented sensory analysis were conducted in three potential functional foods based on Ca(II)-alginate beads containing bioactive compounds extracted from beet stems. Ca(II)-alginate beads *per se*, and two selected products (cookies and turkish delights supplemented with the beads) were prepared. Regarding the beads, among the attributes rated by consumers, visual appreciation predominates, being color in the *just-as-right* (JAR) category and in the *like* preference. Instead, both flavor and sweet taste were attributes highly penalized and should be improved in beads to be accepted as food *per se*. A higher percentage of customers preferred cookies and turkish delights instead of only beads, considering global satisfaction. Regarding *in vitro* digestion, there was a significant content of phenolic compounds in the products with beads, showing a bioaccessibility greater than 80% (for cookies) and 26% (for turkish delights). Also, the antioxidant capacity measured by ABTS ranged between 50 and 109% for cookies and turkish delights, being lower when measured by FRAP (between 20 and 30%, respectively). Thus, including the beads with beet stem extract in both products leads to a significant increase in the content of phenolic compounds and in the antioxidant capacity compared to their counterparts, protecting the compound during oral and gastric phases. These results allow the generation of improved Ca(II)-alginate systems with promising functional properties for the development of ingredients and functional foods.

1. Introduction

The development of functional foods provides an opportunity to improve the quality of nutrients available to consumers and to benefit their health (Lu, Mao, Hou, Miao, & Gao, 2019). In the food industry, functional ingredients have been investigated to enhance technological properties and develop food products with health claims (Galanakis, 2021). Research on functional food ingredients with micro or nano encapsulates offers an opportunity to deliver bioactive materials through daily diet providing an advantage that goes beyond basic nutritional functions to improve health. The encapsulation of bioactive compounds with a protective wall material turns out to be a practical solution, which under certain conditions also enables the controlled release of these bioactive compounds (Wichchukit, Oztop, McCarthy, & McCarthy, 2013). Since the encapsulated bioactives can be protected from moisture, heat, or other conditions, enhancing their stability, and/

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or maintaining viability, (Jimenez, Garcia, & Beristain, 2004) the uses of encapsulation techniques have impressively increased in the food industry. However, despite the advantages of reducing physicochemical changes and the possibility of controlled release, encapsulation matrices often present problems at the sensory analysis level. The evaluation by the consumer is a crucial step in the production and development of functional foods, rendering any development unenforceable if not accepted by the user. Descriptive sensory analysis of food products enables understanding and control of the key attributes for customer satisfaction and market success (Moskowitz, Beckley, & Resurreccion, 2012) and, at the same time, gains importance to test and evaluate the acceptance of new products (Sirangelo, 2019) or even their reformulation (Świąder, Florowska, Konisiewicz, & Chen, 2020).

Among encapsulation materials and techniques, hydrogels are excellent matrices for the encapsulation of bioactive compounds thanks to their bio-similarity, resembling soft matter of biological origin, and their consequent biocompatibility. Moreover, they exhibit a three-dimensional network which offers a suitable aqueous environment with a porous structure able to host the bioactive compounds (Kruif, Anema, Zhu, Havea, & Coker, 2015; Zazzali, Aguirre Calvo, Pizones Ruíz-Henestrosa, Santagapita, & Perullini, 2019) and to protect them from moisture and heat, and masking undesirable odors and/or tastes (Dallabona et al., 2020). When biopolymer-based encapsulation is used for the delivery of functional ingredients, their inclusion within the hydrogel matrix can be achieved through physical entrapment of the bioactives during gel formation or through their complexation with the biopolymer chains (Matalanis, Jones, & McClements, 2011). Hydrogel encapsulation is a technique associated with molecular gastronomy, also known as spherification (Lee & Rogers, 2012). Among the different available hydrogels, alginate-based systems were used to create good quality foods having desirable sensory properties with a high degree of consumer acceptability (Binsi et al., 2019) and satisfaction (Gaikwad, Kulthe, & Suthar, 2019). The widespread use of alginate hydrogel particles has resulted in the proliferation of methods for their tailored generation for applications in a variety of fields such as food, microbiology, biotechnology, pharmacy, and medicine (Salvati, Santagapita, & Perullini, 2022). The different synthesis methods are based on the simple and rapid gel formation induced by the addition of various di- or trivalent cations to alginate chains (Posbeyikian et al., 2021).

In the food industry, there is a strong tradition of food design and often encapsulation is used to design the product or ingredients (Ariceaga et al., 2018). For instance, encapsulation is central to the formation of faux caviar in modernist cuisine (i.e. carp (Binsi et al., 2019), sturgeon (Ji, Cho, Gu, & Kim, 2007) and flying fish roes (Ha, Jo, Cho, & Kim, 2016), ravioli and gnocchi (Lee & Rogers, 2012)). Some sensory studies and analysis of the properties of alginate-based products have been performed, for instance, for edible coatings (Vital et al., 2018), for beads enriched with bioactive compounds (Kaltsa, Alibade, Bozinou, Makris, & Lalas, 2021) and for the incorporation of alginate beads to products (Balabanova, Petkova, Ivanova, & Panayotov, 2020). However, beyond having been good product developments from both sensory and bioactive-protective viewpoints, these studies did not consider the bioaccesibility of the bioactives (i. e. the potential fraction of nutrients released from the food matrix that would be accessible for intestinal absorption in relation to the total initial content (Rodríguez-Roque et al., 2015)) which can be a limiting factor to their potential biological activity, often having a higher relevance than the concentration of bioactives in the food matrix (Tesoriere, Fazzari, Angileri, Gentile, & Livrea, 2008). To mimic the conditions in each stage of gastrointestinal (GIT) digestion of foods, several *in vitro* simulated GIT methodologies have been standardized, such as the well-known international protocol INFOGEST (Minekus et al., 2014).

In the present work, beads containing an extract rich in phenolic compounds derived from beet stems have been produced as previously reported (Aguirre-Calvo, Perullini, & Santagapita, 2018; Aguirre-Calvo, Santagapita, & Perullini, 2019). This aqueous extract was obtained from beet by-products and contained significant amounts of betacyanin and phenolic compounds with antioxidant properties, with low contents of both protein and sugar (Aguirre-Calvo et al., 2018). Beads had previously been optimized for encapsulation in alginate systems, highlighting their advantage in terms of loading efficiency of encapsulated bioactive compounds (by adding sugars and hydrocolloids during the formulation, (Aguirre-Calvo et al., 2019) & Aguirre-Calvo, Molino, Perullini, Rufián-Henares, & Santagapita, 2020a), as well as their demonstrated capability as an ingredient to provide functional properties through digestion-fermentation (Aguirre-Calvo et al., 2020a; b). The encapsulated systems showed better global antioxidant response in the digested and fermented phases than the extracts without encapsulation (Aguirre-Calvo, Molino, Perullini, Rufián-Henares, & Santagapita, 2020b), also enhancing the production of short-chain fatty acids (SCFAs), particularly propionic acid, a key metabolite involved in inflammatory processes. Finally, the produced beads have an additional advantage from the structural point of view: microstructural analysis by small X-ray scattering (SAXS) revealed that they slightly changed in oral and gastric fluids and partially softened their structure in intestinal fluid, allowing the absorption of bioactive compounds (Aguirre-Calvo et al., 2020a). Thus, the aim of the present work was to evaluate three potential functional foods based on Ca(II)-alginate beads containing bioactive compounds extracted from beet stems (a by-product) through sensory analysis focused on consumers and by determining the bioaccessibility and the antioxidant activity along *in vitro* digestion following the INFOGEST method including the analysis of each GIT stage. Ca (II)-alginate beads (as molecular caviar), cookies, and turkish delights with beads were selected as products. The latter food matrices were chosen to have distinctive representative products (dough-based and jelly-based systems). Cookies and turkish delight without beads were also compared to products. The term potential functional food is used since bioavailability studies were not performed in the current study.

2. Materials and methods

2.1. Extract production and beads synthesis

The beet stems (*Beta vulgaris* var. *conditiva*, purchased from a local market) were separated from the roots and washed, scalded (10 min at 100 °C) and homogenized as a puree in a blender (model HR 1372, Philips N.V., Amsterdam, Netherlands). Then, 15 g of the stem puree was mixed with 22.5 g of water by magnetic stirring (1500 rpm; 5 min at 20 °C) and then centrifuged (6000 rpm; 30 min). All the reagents used for the generation of the beads were food grade. Ca(II)-alginate beads were generated with the dropping method according to Aguirre-Calvo et al. (2018; 2020a; b). Briefly, a solution containing the stem extract with 1.5 % (w/v) of sodium alginate (TodoDrogas S.R.L, Córdoba, Argentina), 0.25 % (w/v) of guar gum (Xantana S.R.L, Lomas de Zamora, Argentina) and 20 % (w/v) of sucrose (Ledesma S.A., Buenos Aires, Argentina) was prepared. Then, the gelling solution was prepared in mineral water with 2.5 % (w/v) of calcium chloride (TodoDrogas S.R.L, Córdoba, Argentina) and 20 % (w/v) of sucrose, adjusting pH at 5.5 with 0.1 M of lactic acid (Argentina Brew S.A., Don Torcuato, Argentina). The dropping was set by means of a peristaltic pump (model 7518–00, Cole Parmer, Masterflex, Vernon Hills, IL, USA), at an extrusion speed of 9 rpm, with a 6 ± 1 cm distance between the tip (0.45 mm) and the surface of the gelling solution. The beads generated were left for 5 min in the gelling bath. Then, they were washed twice with distilled water, and stored in a conventional fridge at 5 ± 1 °C until further use. Macroscopic characterization and pictures of the beads are shown in Table S1 and Figure S1A of Supplementary File, respectively.

2.2. Preparation of products

All ingredients are commercially available and were acquired in a supermarket.

Preparation of cookies:

The cookie making process consisted of mixing separately dry ingredients (refined wheat flour (5 ½ cup), baking powder (2 tsp) and salt (2 tsp)) with wet ingredients (butter (1 ¼ cup), sugar (2 cups), eggs (4 units) and vanilla extract (2 tsp)), using a mixer (Oster, Neosho, MO, USA) for 5 min. The dough was cooled down, then cut into slices 5 mm thick, from which 50 mm wide circular shapes were obtained. Then, Ca (II)-alginate beads (¼ tsp) were added on top of each cookie. Finally, baking was carried out at 180 °C for 12 min in an electric oven (model 225516, Hendi Co, Amsterdam, Netherlands). After cooling, the cookies were packed and kept at 20 ± 1 °C.

Three samples were prepared: (1) cookies without the addition of Ca (II)-alginate beads (cookies- C), (2) cookies containing Ca(II)-alginate beads with stem extract (cookies-T), and (3) cookies with Ca(II)-alginate beads without extract (cookies-X). The last one was only used for bioaccessibility studies. Cookies-T and Cookies-X are shown in Figure S1B of Supplementary File.

Preparation of turkish delight:

The turkish delight were prepared by mixing: sugar (5 ½ cup), water (2 tsp), cream of tartar (2 tsp) and lemon juice (2 tsp) and brought to a slight boil until reaching 180 °C, adding the cornstarch (5/8 cups) and water (1/2 cups) to the mixture, whisking until it is fully incorporated; then, the mix was cooled down and spread into a mold. Ca(II)-alginate beads were added in this step. After being refrigerated overnight, the turkish delight was sliced in pieces of 2x2x1.5 cm, and finally cornstarch was added to separate them.

Three samples were prepared: (1) turkish delight without the addition of Ca(II)-alginate beads (Turkish-C), (2) turkish delight containing Ca(II)-alginate beads with stem extract (Turkish-T), and (3) turkish delight with Ca(II)-alginate beads without extract (Turkish-X). The last one was only used for bioaccessibility studies. Turkish-T and Turkish-X are shown in Figure S1C of Supplementary File.

2.3. Consumers panel for sensory analysis

For the sensory evaluation, a panel of 100 potential consumers between 18 and 65 years, consisting of students, professors, and staff members from Facultad de Bromatología (UNER, Argentina) were randomly selected.

2.3.1. Descriptive and affective tests

To measure the pleasant or unpleasant sensations evoked by the samples when they are tasted by potential consumers, we implemented the Evaluation of the degree of satisfaction test (Tadesse, Beri, & Abera, 2019). The degree of satisfaction of the different attributes and the global satisfaction of the products were analyzed using 5 and 7-point hedonic scales, respectively (Zhi, Zhao, & Shi, 2016). Fig. 1 shows the different attributes assessed in the products by the consumers.

To evaluate the intensity of each attribute, the scale *Just-about-right* (JAR) was used. Consumers were asked to describe the perceived intensity of each of the previous attributes on a three- point scale: 'too much', 'JAR' or 'too low' (Lawless & Heymann, 2010).

2.3.2. Penalty analysis

Penalty analysis was used to relate the descriptive test of the JAR scales to the global satisfaction test (Stone, Bleibaum, & Thomas, 2020) to know whether an attribute that has been penalized at the consumer global satisfaction is below or above the JAR point. In addition, this analysis is a useful tool to identify directions for product improvement through tracking analysis of the mean drops for each attribute compared to the percentage of consumers who rated the product as "too high" or "too low" on that attribute. The attributes found in the upper right quadrant corresponding to a certain percentage of consumers (~30 %) and a mean drop higher than 1 represent an alert as they have the highest biases and are associated with the largest mean drop. These attributes will be the first choices for optimization, development, or reformulation of the product for consumers' acceptance.

2.4. In vitro digestion

Samples were digested according to the INFOGEST® *in vitro* gastrointestinal methodology (Minekus et al., 2014; Pérez-Burillo, Rufian-Henares, & Pastoriza, 2018) employing Simulated Saliva Fluid (SSF), Simulated Gastric Fluid (SGF), and Simulated Intestinal Fluid (SIF); the process was schematized in Figure S2 of Supplementary File. Briefly, 3.0 g of sample were mixed with 3.0 mL of SSF, 25.0 μL 0.3 M $CaCl_2$ (aqueous solution) and 150 U/mL of α-amylase (Sigma-Aldrich Ref.: A6380, EC 3.2.1.1). The mixture was incubated for 2 min at 37 °C. For the gastric phase, 6.0 mL of SGF with 4000 U/mL of pepsin (Sigma-Aldrich Re.: P700, EC 3.4.23.1) and 5.0 μL of $CaCl_2$ were added to the oral phase, the pH was lowered to 3.0 (with the addition of 1 N HCl), and then samples were incubated at 37 °C for 120 min. Finally, for the intestinal phase, 12.0 mL of SIF with 26.74 mg/mL of pancreatin (Sigma-Aldrich Ref.: P7545), bile salts (20 mM) (Sigma-Aldrich Ref.: B8631)

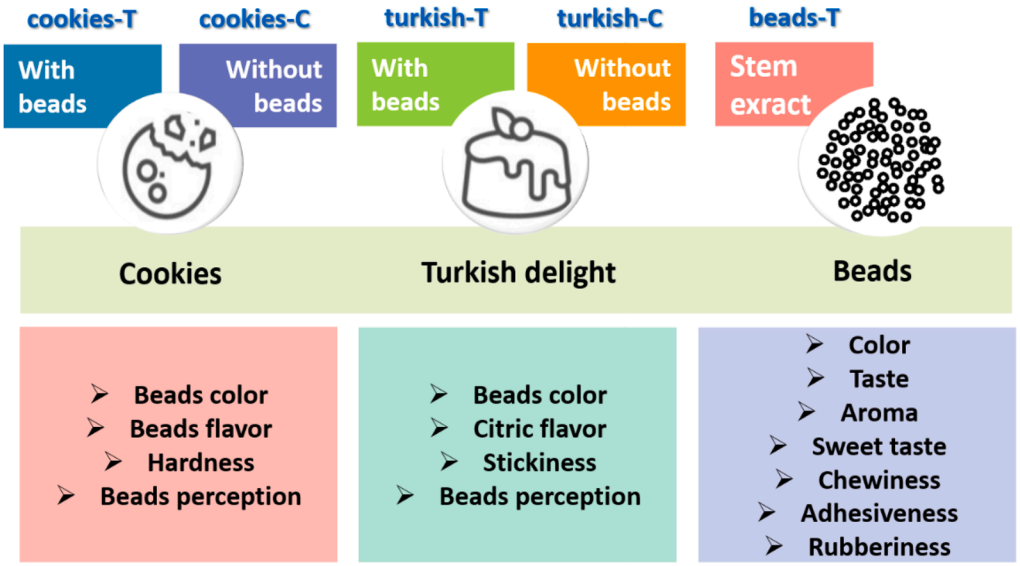

Fig. 1. Attributes analyzed for the products in the sensory evaluation.

and 40.0 μL of $CaCl_2$ were added to the gastric phase; the pH value was adjusted to 7.0 (by adding 1 N NaOH), and then it was incubated at 37 °C for 120 min. All the enzymatic reactions were halted by immersing the tubes in iced water. The samples were then centrifuged (6000 rpm; 10 min at 4 °C) and the supernatants were labeled as intestinal phase (potentially absorbable solution). In particular, the protocol used made it possible to extract aliquots from each of the digestion phases (saliva, gastric and intestinal), monitoring the behavior of bioactive compounds at each phase (Beltrán, Sandoval-Cuellar, Bauer, & Quintanilla-Carvajal, 2019).

All the activities for the enzymes were previously determined with several methods to establish the concentration to be used in the digestion protocol (Minekus et al., 2014). All reagents were from Sigma-Aldrich Co., Ltd., Saint Louis, USA.

2.4.1. Evaluation of bioaccessibility of total phenolic compounds

Bioaccessible phenolic compounds (Eq. (1)) were calculated as the ratio between the total phenolic content (TPC) in intestinal aqueous phase (TPC_{IP}) and its respective total phenolic content in the stem extract (TPC_{ext}). This ratio represents the amount of the compounds that are released from the food matrix in the gastrointestinal tract and is available for absorption.

$$Bioacccesibility(\%) = \frac{TPC_{IP}}{TPC_{ext}} * 100 \quad (1)$$

Total phenolic compounds for intestinal phase (IP) were determined according to Singleton, Orthofer, and Lamuela-Raventós (1999). Moreover, oral and gastric phase (OP and GP, respectively) samples of each of the digested samples were also analyzed. Briefly, 50 μL of sample, 125 μL of Na_2CO_3 aqueous solution (200 g/kg), and 800 μL of distilled water were added to 125 μL of the Folin-Ciocalteu reagent (Sigma-Aldrich Co.). After 30 min of reaction at 40 °C in the dark, the absorbance (765 nm) was measured, and based on a calibration curve, the total phenolic compounds (TPC) were expressed as mg of gallic acid/mL or $GEAC_{FOLIN}$ (Gallic acid equivalents antioxidant capacity referred to Folin-Ciocalteu).

The results shown correspond to the values obtained by subtracting the reagent blank (for all samples) and by subtracting the contribution of the products without beads (for the samples with control and with stem extract beads). Thus, only the contribution produced by the addition of the beads (Beltrán et al., 2019) is analyzed.

2.4.2. In vitro evaluation of antioxidant activity of bioaccessible phenolic compounds

The samples measured in each method were the oral, gastric, and intestinal phase aliquots of each of the digested samples.

Briefly, antioxidant activities of the samples before and after encapsulation were investigated by measuring their ability to scavenge the $ABTS^{\bullet+}$ free radical by means of the methodology developed by Re et al. (1999). ABTS (7 mM) (Sigma-Aldrich Co.) reacts with $K_2S_2O_8$ (2.45 mM) after 12–16 h of incubation in the dark. The solution was then diluted with buffer phosphate (0.01 M; pH 7.4) to an absorbance of 0.70 ± 0.01 at 734 nm to form the $ABTS^{\bullet+}$ reagent. Reaction mixtures containing 100 μL of sample and 1.90 mL of reagent were incubated for 30 min at 30 °C. The percentage of inhibition was calculated against a control and compared to a gallic acid standard curve. Results are expressed as mg eq gallic acid (GAE)/ml or $GEAC_{ABTS}$ (Gallic acid equivalents antioxidant capacity referred to ABTS).

The ferric reducing ability of the extract and the alginate beads was determined based on the method developed by Benzie & Strain, 1996, with a slight variation. FRAP reagent was prepared mixing buffer acetate (0.3 M, pH = 3.6), TPTZ (10 mM in 0.04 M HCl) (Sigma-Aldrich Co.) and $FeCl_3 \cdot 6\ H_2O$ (20 mM), in a ratio of 10:1:1 (v:v:v). Then, 40 μL of the extract were mixed with 60 μL of water and 600 μL of freshly prepared reagent and incubated in the dark at 30 °C for 30 min. The ferric reducing ability was calculated based on the absorbance at 563 nm, against a control (water or blank beads), compared to a gallic acid standard curve, and expressed as mg eq gallic acid (GAE)/mL or $GEAC_{FRAP}$ (Gallic acid equivalents antioxidant capacity referred to FRAP). Results are shown by subtracting the reagent blank for all samples and the contribution of the products without beads as previously commented. The antioxidant activity of the bioaccessible phenolic compounds was calculated by using Eq. (1), even though they are not defined as bioaccessibility.

2.5. Statistical analysis

Sensory analysis (n = 100) was performed with contingency tables and data recollection through Excel 365. The analysis of the results of the Descriptive and affective tests was carried out using statistical descriptors (moments of normal) (Montgomery & Runger, 2010) such as:

-Skewness: It allows to identify and describe the way in which data tends to be collected according to the frequency that is found within the distribution (how equal are the mean and median). If the skewness is between −0.50 and 0.50, the data is symmetrical. If the skewness is between −1.00 and −0.50 or between 0.50 and 1.00, the data is moderately skewed (even if negatively or positively skewed, respectively). If the skewness is less than −1.00 or greater than 1.00, the data is highly skewed (even if negatively or positively skewed, respectively).

-Kurtosis as a measure of whether the data are heavy-tailed or light-tailed relative to a normal distribution (Data is shown in Table S2 of Supplementary File).

Total phenolic compounds and antioxidant capacity by $ABTS^{\bullet+}$ for each phase of the digestive process of the products were determined on n = 6 and the analysis of antioxidant capacity by FRAP on n = 4. Bioaccessibility data were analyzed using one-way ANOVA with Tukey's post-test using Prism 6.01 (GraphPad Software Inc., San Diego, CA, USA). When the analysis of variance indicates differences between means, a *t*-test was used to differentiate the means with 95 % confidence ($p < 0.05$).

Table 1
Skewness of the JAR scale data distribution for Ca(II)-alginate beads containing stem extract and for products with the addition of Ca(II)-alginate beads. (N indicates number of responses).

(A)	STEM BEADS	
Attribute	N	Skewness
Color	100	−0.08
Flavor	99	2.36
Aroma	100	4.21
Sweet taste	99	2.89
Chewiness	99	0.39
Adhesiveness	100	1.01
Rubberiness	100	0.34

(B)	COOKIE (C)		COOKIE (T)	
Attribute	N	Skewness	N	Skewness
Bead's color	99	−0.09	99	0.08
Citric flavor	98	−0.09	99	0.68
Hardness	99	−0.04	97	−0.03
Bead's perception	99	1.29	99	0.53

(C)	TURKISH DELIGHT (C)		TURKISH DELIGHT (T)	
Attribute	N	Skewness	N	Skewness
Bead's color	99	0.63	100	0.25
Citric flavor	100	0.42	100	−0.78
Stickiness	99	0.45	100	−0.22
Bead's perception	99	1.44	100	0.96

3. Results & discussion

3.1. Sensory analysis

3.1.1. Descriptive and affective sensory analysis

Table 1A shows the asymmetry in the data distribution for the intensity level of attributes of the beads. It is worth noting that the attributes of color, chewiness and rubberiness were evaluated in JAR point by the consumers (skewness between −0.50 and 0.50), while the attributes of flavor, aroma, sweet taste, and adhesiveness were estimated as too low, being extremely skewed (as shown by the values greater than 1.00). For cookies containing beads, the attributes of color and hardness were evaluated in the JAR point by the consumers (as shown in Table 1B), while the flavor and perception of the beads were considered as too low, being moderately skewed. However, for cookies without beads, the attributes of color, flavor and hardness were considered in the JAR point, while once again the perception of the beads was considered as too low but being extremely skewed. For turkish delights containing beads the attributes color of the beads and stickiness were considered JAR by the consumers (Table 1C), whereas the citrus flavor was considered as too high. In contrast, for turkish delights without beads, the color of the beads was considered too low, and the taste was considered JAR, both at a level below the samples with beads, as shown by the skewness data from Table 1C; instead, stickiness was kept JAR point. On the other hand, the beads perception of turkish delight with or without beads was considered as too low; however, this value was extremely skewed for turkish delight without beads, as previously observed for cookies (Table 1B). Kurtosis (Table S2 of Supplementary File) showed the same qualitative trends described for skewness without further insights.

3.1.2. Evaluation of the degree of satisfaction

Once the degree of intensity of the characteristic attributes of a product has been determined by the consumers, it is fundamental to assess the global satisfaction of the product (Lawless & Heymann, 2010). Fig. 2 shows the results obtained after evaluating the level of global satisfaction for each product and Figures S3-S5 of Supplementary File shows the degree of satisfaction for each attribute, which is correlated with the intensity of each attribute for the products.

Regarding beads, it was observed that 37 % of consumers rated them in dislike categories. When the individual attributes were evaluated, most were rated in the "neither like nor dislike" point (as shown in Figure S3 of the Supplementary File). These ratings were possibly due to the unfamiliar or unknown of the product. Therefore, given that it is an unknown and tasteless product, these results suggest that the beads do not have the potential to constitute a product by itself, unless the attributes are modified for consumer acceptance.

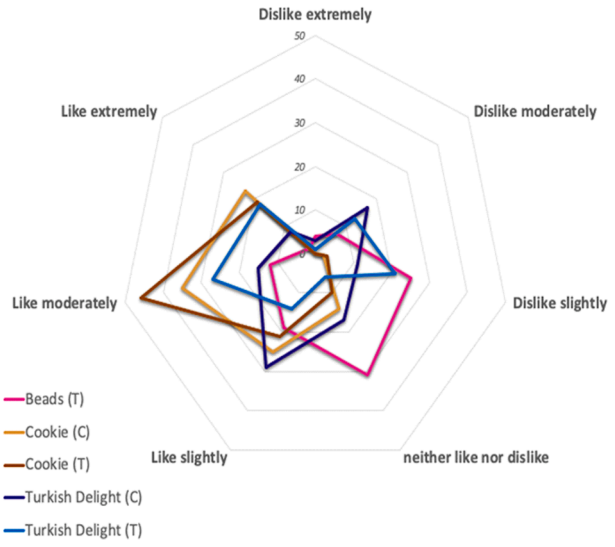

Fig. 2. Global satisfaction of food products: Ca(II)-alginate beads with stem extract, cookies and turkish delight prepared with Ca(II)-alginate beads evaluated with the 7-level hedonic scale. Codification: "Beads (T)" =Ca(II)-alginate bead with stem extracts; "C" = control (without beads); "T" = with Ca(II)-alginate bead with stem extracts.

For cookies prepared with Ca(II)-alginate beads containing stem extract (T) and for the control without beads (C), a similar level of global satisfaction was obtained with respect to consumer perception. It was observed that the percentages of liking (like extremely, like or like slightly) were high and similar in both cases. In contrast to the beads themselves, this product provided more satisfaction, as the responses were mostly rated in the "like" categories. The color attribute in cookies T and C was classified in the "like" category and with a high percentage of consumers at the JAR point, as shown in Figure S4 of the Supplementary File. The taste of the beads showed different and very interesting behaviors: in cookies-T, the preference was rated as "neither like nor dislike"; conversely, cookies-C was rated in the "like" categories by a high percentage of consumers who also rate them at the JAR point. Consumers indicate the hardness of the cookies in the JAR point rating the preference in the "like" category. Perception of the beads in the cookies did not present categories of dislike, being this attribute at the low / JAR level.

Turkish delight with and without beads showed the most different behavior regarding consumer acceptance. Particularly, the ones with beads were more accepted and more likable than those without beads (Fig. 2). Moreover, consumers classified the color of the beads as "like" for turkish-T, while turkish-C was rated as "neither like nor dislike" (as shown in Figure S5 of the Supplementary File). Regarding citrus flavor, consumers selected "like" the turkish-T and perceived it as "too high" in the intensity level. Perhaps, some associations between beads and citrus flavor were made by some consumers, revealing an interesting feature. Concerning stickiness, the product with beads exhibited a level of intensity rated "too high" with the predominant categories rated as "dislike or slightly dislike". The perception of the beads was classified as "neither like nor dislike" in both samples, showing a similar percentage of consumers perceiving them with low intensity.

To explore consumers' perception of the product, they were asked open-ended questions; then, the answers were organized into a word cloud to analyze the frequency of the words they use to express their perceptions about the products (Figure S6 of the Supplementary File). This information allows knowing the vocabulary of the consumers and gives the opportunity to focus on the attributes that are important to them, without forcing their attention to attributes previously selected by the researchers (Symoneaux & Galmarini, 2014). These perceptions are directly related to the attributes that they rated when testing the products.

3.1.3. Penalty analysis

Fig. 3 shows the penalty analysis that allows to obtain information regarding the influence of attributes on the global acceptance of the product and the extent to which they have been penalized by consumers to identify the changes in direction that an attribute can take to achieve its ideal point.

For the beads (Fig. 3A), the attributes that consumers penalized for global satisfaction were "too low" taste and sweet taste (mean drop greater than 1 and more than 80 % of consumers, pink upper right quadrant). Then, the intensity of the sweet taste and flavor must be increased to achieve acceptance. No parameter was found in the penalty quadrant for cookies-T (Fig. 3B), which means that there is no attribute for this product that needs to be changed. On the other hand, for turkish-T (Fig. 3C), it was observed that the parameter with the high penalty was "too much" stickiness; this attribute should be modified to increase overall acceptance of the product.

It seems that the visual appreciation of the beads by the consumers

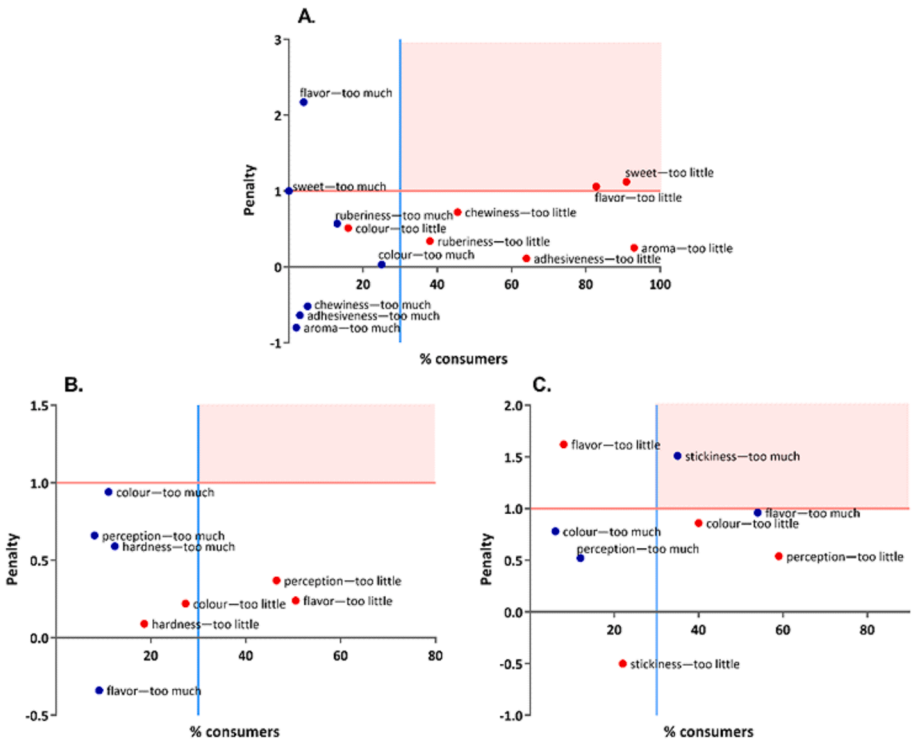

Fig. 3. Penalty analysis based on the percentage of consumers who established a higher (blue dot) or lower (red dot) deviation from the ideal point for A. Ca(II)-alginate beads with stem extract; B. cookies prepared with Ca(II)-alginate beads with stem extract; C. Turkish delight prepared with Ca(II)-alginate beads with stem extract. Controls of B and C are shown in Figure S7 of the Supplementary File. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

determined the preference for them, but the taste perception led to a rejection compared to cookies or turkish delight products, as these were well accepted in terms of their global satisfaction (Fig. 2). In agreement with acceptance, the penalty test showed that no improvement was needed to change the attributes of the cookies (Fig. 3B), probably because they were based on a well- known and common product in their consumption. On the other hand, turkish delights with beads presented some attributes that need to be improved (Fig. 3C) to achieve

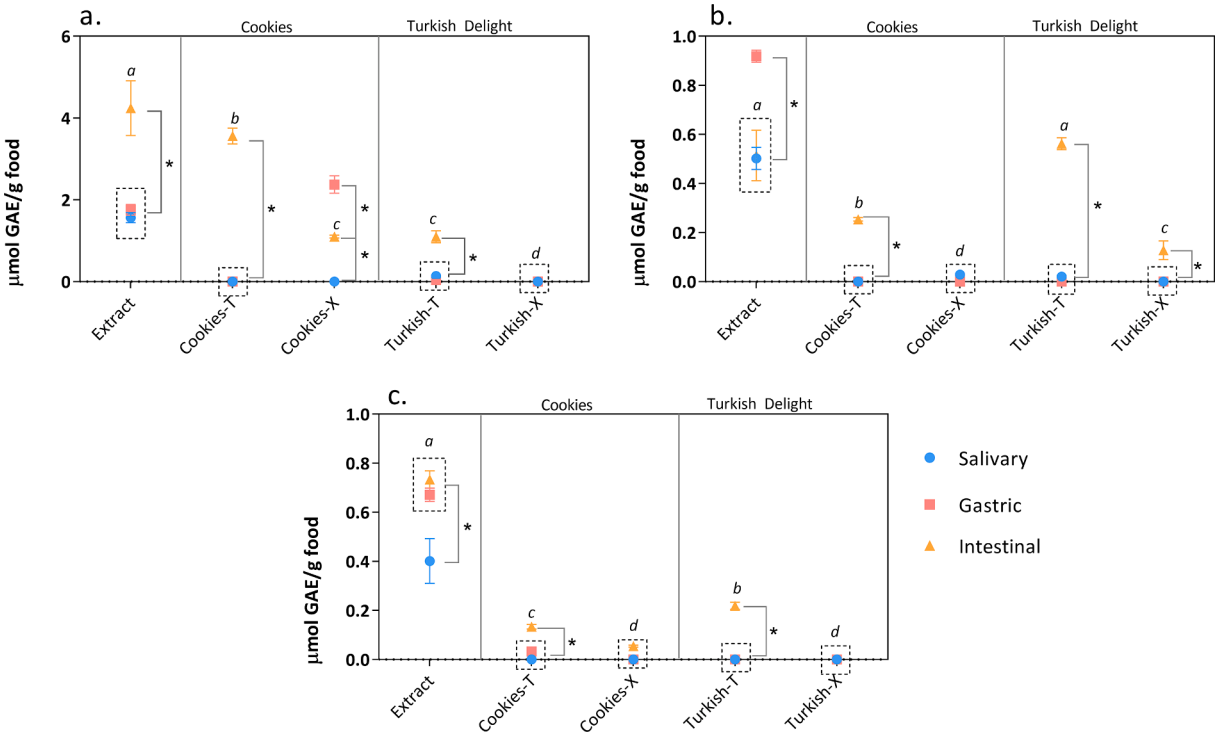

Fig. 4. Digested fractions (salivary, gastric, and intestinal) for (A): Total phenolic compounds by FOLIN (B): Antioxidant capacity measured by ABTS and (C): Antioxidant capacity measured by FRAP of extracts and cookies/turkish delight prepared with stem beads or control beads. The values obtained for the cookies and turkish delight without beads were subtracted. Different letters above the symbols (a-d) indicate significant differences between the intestinal phase of each food product ($p < 0.05$). The dotted lines with * indicate significant differences between the phases of the same food product ($p < 0.05$), while the dotted square indicates that there are no significant differences between the phases ($n = 6$).

acceptance. This behavior is also observed in the consumer's penalty test for the turkish delight without beads. This could be related to that turkish delight are a more innovative and lesser-known food in the Argentine market, as confirmed by the consumers' opinions regarding this product (Figure S6 of the Supplementary File), in which words such as *unknown* and *rare* were included.

3.2. Bioaccessibility

In addition to the sensory analysis, bioaccessibility approach is fundamental to ensure and guarantee the potentiality of a food as functional. Particularly, all the stages of digestion were analyzed in this work. It is important to highlight that Ca(II)-alginate beads were analyzed in previous studies showing a very good behavior both as a carrier and with increasing antioxidant capacity in both digestion and fermentation (Aguirre-Calvo et al., 2020a; b). However, when beads are used as ingredients for functional products, the complexity of the matrix and the possible interactions between components could drastically affect the obtained results.

Total phenolic compounds ($GEAC_{FOLIN}$, Fig. 4A), the antioxidant capacity measured by ABTS ($GEAC_{ABTS}$, Fig. 4B), and by FRAP ($GEAC_{FRAP}$, Fig. 4C) were evaluated. In this case, not only samples with Ca(II)-alginate beads and control without beads were produced (as in previous sections), but also products with Ca(II)-alginate beads without extract (control beads). On the other hand, the measured activity of the products without beads (cookies-C and turkish-C) was subtracted from both those with control beads (cookies-X and turkish-X) and those that contained beads with extract (cookies-T and turkish-T) to analyze the bioactive activity of the beads. Table 2 summarizes the bioaccessibility for both cookies and turkish delight from the data obtained in Fig. 4 and the antioxidant capacities of bioaccessible phenolic compounds.

It is important to emphasize that the absorption of bioactive compounds occurs at the intestinal level (Aguirre-Calvo et al., 2020a); however, it is critical to assess the loss in each step of the digestion. For the phenolic compounds (Fig. 4A), the intestinal phase (IP) showed significant differences between the extract and the products (cookies and turkish delights). The cookies-T contain a high content of phenolic compounds and a high bioaccessibility of them with respect to the extract (more than 80 %, Table 2). A portion of this response is due to the presence of the bead components, as shown in cookies-X, as previously determined; by subtracting it, the bioaccessibility of the phenolic compounds with respect to the extract decreases to 58 %. In contrast to the cookies, there is a low bioaccessibility of bioactive activity in the small intestine (Table 2) for turkish delight. It is important to highlight that the bioaccessibility of phenolic compounds depends on several factors; for instance, their interaction with other components present in the food matrix (Karaś, Jakubczyk, Szymanowska, Złotek, & Zielińska, 2017), which can generate different retentions depending on the composition of the beads. Besides, regarding the different phases for each sample (Fig. 4A), both the extract and the products with beads showed no significant differences between the salivary (OP) and gastric (GP) fraction (being much lower than the IP, which is desirable), while in Cookies-X there were significant differences between the three phases, which also showed that the GP had significantly higher activity than the IP, suggesting that there's a release of phenolic compounds in the gastric phase instead of the intestinal phase (where absorption takes place).

The antioxidant capacity measured by ABTS (Fig. 4B) showed significant differences between the samples for the IP, in line with $GEAC_{FOLIN}$ results (Fig. 4A). However, the percentage of antioxidant activity by ABTS of the bioaccessible phenolic compounds for cookies (Table 2) was about 50 % and was lower than that obtained for $GEAC_{FOLIN}$. On the other hand, due to low activity obtained in cookies-X, there was no decrease in the value (no contribution from the beads without stem extract). Turkish-T showed a high antioxidant capacity in the IP (higher than the extract), resulting in a high percentage (greater than 100 %) of antioxidant activity of the bioaccessible phenolic compounds (Table 2), which can be attributed to the protection afforded by encapsulation. The antioxidant capacity of the extract is reduced when it is not protected in the beads (Fig. 4B and C), in agreement with previous research (Aguirre-Calvo et al., 2020b): the high activity in both the OP and the GP, being even higher than for IP in certain cases, underlines the need for the encapsulation of bioactive compounds in a protective matrix. No significant differences were observed between the OP and GP for both cookies and turkish delights, which were lower than the IP.

Finally, the antioxidant capacity measured by FRAP is shown in Fig. 4C. The general trend of the activity for the IP fraction of cookies agrees with the results presented above ($GEAC_{FOLIN}$ and $GEAC_{ABTS}$, Fig. 4a and b, respectively). As previously observed for $GEAC_{FOLIN}$, the antioxidant capacity of the extract in IP is significantly higher than the products (cookies/turkish). The antioxidant activity by FRAP of the bioaccessible phenolic compounds in the cookies-T was <20 % (Table 2) while for the turkish delight it was around 30 %, underlying the importance of measuring antioxidant capacity by various methods. In addition, no significant differences between OP and GP were observed for either cookie or turkish delight (as for $GEAC_{ABTS}$), while much higher losses were observed for OP and GP phases for the extract, as previously observed, highlighting the importance of encapsulation.

From these results, it can be concluded that the addition of beads with beet stem extract to both food products significantly increased the content of bioaccessible phenolic compounds at the intestinal level and, above all, the antioxidant capacity (especially measured by ABTS) in relation to the food base (without beads).

It is interesting to note that the different matrices give distinct results in terms of bioaccessibility. This could be due to several reasons, such as the interactions between compounds (Zhang, Zhang, & McClements, 2019), the chemical composition and its physical form (e.g. particle size, solubility, association with indigestible complexes; Macfarlane & Macfarlane, 2007), but also food preparation processes should be considered. For instance, during preparation of turkish delight, Ca(II)-alginate beads are included in a high a_w medium (albeit viscous) that could favor the selective diffusion of certain bioactive compounds. This could cause a decrease in the protection (provided by encapsulation), resulting in a lower bioaccessibility of phenolic compounds for the turkish delight compared to the cookies. However, the antioxidant activity of the bioaccessible compounds in turkish delight was higher compared to cookies. Additional experiments are required to clarify which compounds are responsible for the antioxidant properties and how selectively they are lost or retained by the beads or matrix in each product during digestion.

Table 2
Bioaccessibility of cookies and turkish delight prepared with Ca(II)-alginate beads with stem extract for total phenolic content and their antioxidant capacities (ABTS and FRAP). Bioaccessibility and antioxidant activity with the subtraction of the contribution of the beads without extract ($Cookies_{(X)}$ and $Delight_{(X)}$) was also included. Standard deviations are included.

	$Cookie_{(T)}$	$Cookie_{(T)}$-$Cookie_{(X)}$	$Delight_{(T)}$	$Delight_{(T)}$-$Delight_{(X)}$
Bioaccessibility (%) by $GEAC_{FOLIN}$	84 ± 18^{aA}	58 ± 14^{aB}	26 ± 8^{bA}	26 ± 8^{bA}
Antioxidant activity of bioaccessible phenolic compounds by $GEAC_{ABTS}$ (%)	49 ± 11^{bA}	49 ± 11^{aA}	109 ± 27^{aA}	84 ± 46^{aA}
Antioxidant activity of bioaccessible phenolic compounds by $GEAC_{FRAP}$ (%)	18 ± 2^{cA}	11 ± 2^{bB}	30 ± 3^{bA}	30 ± 3^{bA}

Lowercase letters (a-c) indicate significant differences between the bioaccessibility measured by the different methods, while uppercase letters (A-B) indicate significant difference between bioaccessibilities with and without control bead subtraction ($p < 0.05$) for the same method.

4. Conclusion

The application of the designed beads allowed the development of potentially functional food products using sensory analysis with consumers as a selection and optimization tool. It was possible to understand how people express, perceive, accept, and penalize formulated products, which is essential for a development process for food formulation. The main conclusions from the sensory analysis (considering altogether the Descriptive and affective sensory analysis, the Evaluation of the degree of satisfaction and the Penalty analysis) indicates that beads do not have the potential to constitute a product by itself, unless the attributes are modified for consumer acceptance, considering that most individual attributes were rated in the "neither like nor dislike" point. Instead, both products -and cookies in particularly- were accepted by consumers, being color the common attribute evaluated by them as JAR. The perception of the beads was classified as "neither like nor dislike" for both products, even though it was considered as too low for turkish delights. This product with and without beads presented the most different behavior regarding consumer acceptance, indicating that the product with beads was more accepted and more likable than the one without beads. However, this product shows "too much" stickiness in the penalty test, which should be improved for achieving a greater consumer acceptance.

Regarding bioaccesibility and the antioxidant capacity, it was clear that the antioxidant capacity of the extract is reduced when it is not protected within the beads confirming the (expected) success of the encapsulation strategy. The addition of beads with beet stem extract to both food products significantly increased the content of bioaccessible phenolic compounds at the intestinal level and, above all, the antioxidant capacity (especially measured by ABTS) in relation to the food without beads. Moreover, it is interesting to note that the different matrices give distinct results in terms of bioaccessibility, highlighting the complexity of results due to food processing and composition.

Ca(II)-alginate beads are a system known for its versatility, high reproducibility, low-cost generation procedure, biocompatibility, and controlled release properties for various encapsulated compounds. It is important to note that this application requires further studies and analysis in order not only to use the possibilities to further improve the formulation from a sensory point of view, but also to explore its full functionality through bioavailability studies as well as other food formulations with the same beads.

From the obtained results, their use as delivery systems for food applications is supported by their properties, which can be further improved with the addition of excipients to their formulations, favoring the loading efficiency of bioactive compounds, and making them suitable food ingredients.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2022.100140.

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